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THE ROLES OF REPRODUCTIVE FUNCTION, BODY COMPOSITION, AND METABOLIC STATUS IN BONE HEALTH OF YOUNG EXERCISING WOMEN

A Dissertation in
Kinesiology

by

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ABSTRACT

Exercising women frequently present with menstrual dysfunction and low bone mineral density (BMD), consequences of an energy-deficient state in which energy intake is inadequate to compensate for energy expenditure. Understanding the factors contributing to bone health in exercising women with exercise-associated menstrual disturbances (EAMD) as well as the efficacy of non-pharmacological treatment strategies for the prevention or reversal of low BMD is essential for the appropriate management of this health problem. The purpose of this dissertation was to explore the determinants of bone health in exercising women with severe EAMD in an effort to gain insight into effective treatment strategies for the prevention and reversal of low BMD. To this end, this dissertation includes four studies that 1) compare volumetric BMD, bone geometry, and estimated bone strength between amenorrheic and eumenorrheic exercising women, 2) explore the respective roles of reproductive function, metabolic status, and body composition in areal BMD and estimated bone geometry, 3) assess the impact of an intervention of increased caloric intake on bone health and related factors in exercising women with EAMD, and 4) examine the responses of two amenorrheic exercising women to a 12-month intervention involving an increase in energy intake. We observed the following main findings: 1) exercising women with amenorrhea had smaller bone area and, consequently, lower estimated bone strength at the tibia compared to their eumenorrheic counterparts; 2) among exercising women, reproductive function appeared to play a key role in BMD at a site primarily composed of trabecular bone; whereas, lean mass was one of the most influential predictors of BMD and estimated bone geometry at weight-bearing sites; 3) an intervention of increased caloric intake successfully improved the energetic and metabolic environment, resulting in favorable changes in body composition and recovery of menstrual function. Overall, among exercising women with impaired bone health as a consequence of severe EAMD, weight gain and resumption of menses which coincide with increases in fat mass,
lean mass, and estrogen concentrations may be beneficial for bone health. A similar intervention of longer duration with a larger sample is necessary to confirm these findings.
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CHAPTER 1

Introduction

Exercising women who fail to consume adequate energy to compensate for energy expenditure are at risk for developing functional hypothalamic amenorrhea (FHA) secondary to an energy-deficient metabolic environment [1, 2]. Alterations in the nutritional and metabolic environments typical of energy conservation and a suppressed estrogen environment typical of amenorrheic exercising women synergistically contribute to the low bone mineral density (BMD) typically observed among these women [3] (Figure 1.1). BMD, which captures about 70% of bone strength [4], is used as a proxy indicator to estimate fracture risk [5]. The low BMD found in amenorrheic exercising women is concerning because it places them at a greater risk for stress fractures and osteoporosis later in life. Furthermore, bone geometry, an additional and highly

![Figure 1.1. In the face of high energy expenditure combined with low energy intake, adaptations occur to conserve fuel. Estrogen, a known anti-resorptive agent, is suppressed causing an increase in bone resorption. Metabolically, leptin, insulin-like growth factor-1 (IGF-1), and triiodothyronine (T3) are also suppressed, exemplifying metabolic adaptations which have been proposed to contribute to a decrease in bone formation. The elevated bone resorption and suppressed bone formation lead to the bone loss seen in energy- and estrogen-deficient women. Source: De Souza MJ and Toombs RJ. Amenorrhea associated with the female athlete triad: etiology, diagnosis and treatment. In: Santoro NF and Neal-Perry G (Eds.). Amenorrhea: A Case-Based, Clinical Guide. Springer Science + Business Media, 2010. Used with permission from Springer Science+Business Media.](image-url)
influential component of bone strength, may also be compromised in exercising women with FHA due to the impaired ability of the skeletal tissue to respond to mechanical loading, thus further contributing to decreases in bone strength [6].

In order to help exercising women maintain BMD within or above the normal range and achieve optimal bone geometry, it is imperative to understand the mechanisms underlying poor bone health. Body mass index and estrogen concentrations are positively related with BMD [7-10]. Likewise, lean mass and age of menarche are associated with bone area, microarchitecture, and estimated bone strength [6, 11]. Further, longitudinal research has demonstrated that an increase in weight and resumption of menses among amenorrheic women have favorable effects on BMD [12].

Several mechanisms may explain the positive influence of body weight on BMD. Among amenorrheic women, an increase in body weight may be associated with an improvement in the nutritional environment, including the availability of essential nutrients for bone health, such as calcium and Vitamin D. In turn, this improvement in the nutritional environment may lead to improvements in the metabolic and reproductive environments, increasing circulating concentrations of hormones that are important for optimal bone health such as leptin, triiodothyronine (T3), insulin-like growth factor-1 (IGF-1), and estrogen. Secondly, an increase in body weight may have different effects on BMD depending on the composition of the weight that is gained, i.e., its proportion of lean mass and fat mass [12]. These two components of body mass are proposed to have independent effects on bone. Lean mass exerts a positive influence on BMD through muscle forces acting on bone [13]; whereas, adipose tissue may influence bone mass indirectly through the effects of leptin, an adipocyte-secreted hormone that influences
Thus, among amenorrheic exercising women, body composition, namely lean mass and fat mass, may influence bone strength via distinct effects on bone density and geometry. It is therefore important to accurately assess body composition in amenorrheic exercising women and to determine the role that the different components of body composition play in bone health.

The role of estrogen, and therefore reproductive function, in promoting bone health has been well-established [10, 15, 16]. Estrogen exerts a positive influence on BMD via its inhibitory actions on osteoclasts, the bone cells responsible for bone resorption [16]. In estrogen-deficient states, the increased activity of osteoclasts and bone resorption contributes to the loss of BMD or the failure to achieve peak BMD [17]. Thus, the reversal of the estrogen deficiency, achieved by an improvement in the nutritional and energetic status, may be a beneficial component in the recovery of bone mass among amenorrheic women. However, since reproductive function may be inextricably linked to body composition [18], the respective effects of both factors, i.e., reproductive function and body composition, need to be clarified to better understand the underlying mechanisms of poor BMD in amenorrheic women.

Pharmacological treatment strategies for the reversal of amenorrhea, resumption of menses, and subsequent improvement in bone health have been explored in both amenorrheic, exercising women and anorexic women, a population also characterized by a chronic energy deficiency; however, these studies have yielded equivocal or controversial results [19-27]. A non-pharmacological approach that targets the root of the problem, i.e., the energy deficiency, involves an increase in caloric intake and weight gain and has been shown to be a successful and beneficial strategy among women with
anorexia nervosa [12, 28]. Importantly, data from women with anorexia nervosa indicate that the response to a given treatment may vary between individuals [12, 28-30]; therefore, identifying the factors that determine the treatment success for bone health outcomes requires further investigations. Little is known about the effects of reversing energy deficiency in amenorrheic exercising women, although limited anecdotal evidence exists supporting the notion that an increase in caloric intake can lead to resumption of menses and improvements in bone health [31-34].

Accordingly, the overarching purpose of this dissertation is to explore the determinants of bone health in exercising women with severe menstrual cycle disturbances in an effort to gain further insight into effective treatment strategies for the prevention and reversal of low bone mass in this population.
Study One: Small bone size and compromised bone strength characterize the tibia in young amenorrheic exercising women

A known health consequence of menstrual dysfunction among exercising women is low BMD [15, 35]. Such reports, however, have largely been limited to two-dimensional assessments of BMD derived from dual-energy x-ray absorptiometry (DXA), which provide measurements of areal BMD (aBMD) rather than true volumetric BMD (vBMD). In addition, DXA is not capable of assessing true bone geometry, an important component of bone strength. Peripheral quantitative computed tomography (pQCT) is a three-dimensional bone imaging technique that provides vBMD of both trabecular and cortical bone as well as measures of bone geometry at appendicular sites such as the radius and tibia, allowing for the estimation of bone strength via the bone strength index (BSI) and strength strain index (SSI). Thus, information gleaned from pQCT measurements among exercising, amenorrheic women provides valuable information regarding what is occurring within bone that may affect bone strength and contribute to the low BMD in this population.

Other investigators have reported measures of vBMD and bone geometry and estimates of bone strength in amenorrheic exercising adolescents and young women, female military recruits, and retired gymnasts with a history of amenorrhea [11, 36-38]. Currently, however, there are no reports that describe these bone health measures among a sample that consists solely of exercising women with FHA. Therefore, the purpose of this study was to compare vBMD, bone geometry, and estimated bone strength at the tibia between amenorrheic and eumenorrheic exercising women.
Aim 1: To determine if amenorrheic and eumenorrheic exercising women differ with respect to vBMD (total, trabecular, and cortical vBMD), bone geometry (total, trabecular, and cortical area; cortical thickness; periosteal and endosteal circumference), and estimated bone strength as assessed by the bone strength index (BSI) and strength strain index (SSI) at the proximal and distal tibia.

Hypothesis 1a: Amenorrheic exercising women will have a significantly lower vBMD at the tibia than eumenorrheic exercising women.

Hypothesis 1b: There will be no significant difference in bone area (total, trabecular, and cortical) between amenorrheic and eumenorrheic exercising women.

Hypothesis 1c: Amenorrheic exercising women will have a significantly lower BSI but no difference in SSI compared to eumenorrheic exercising women.

Rationale: Although the effects of an energy-deficient and estrogen-deficient environment on aBMD have been well-established [3, 15, 39], the effects of exercise-associated menstrual disturbances on bone strength, a key component of fracture risk, are not as well-understood. Two components of bone strength, i.e., bone mass and bone geometry, are reliably assessed using pQCT, a device that also has the capability to separate bone mass into its cortical and trabecular components. Thus, pQCT provides a more comprehensive view of the components that impact bone mass and, ultimately, bone strength.

To gain further insight into the mechanisms underlying the low BMD consistently observed in amenorrheic exercising women, reports of pQCT measurements in this
population are necessary. Due to the opposing effects of exercise and suppressed estrogen concentrations on bone strength in amenorrheic, exercising women, pQCT measurements are useful for determining if the beneficial effects of exercise on bone are compromised by the harmful effects of amenorrhea. The loads placed on bones via muscle and gravitational forces during physical activity have been demonstrated to stimulate periosteal bone formation and result in increases in bone size, a beneficial adaptation because large bones typically indicate strong bones [40, 41]. Suppressed estrogen concentrations, however, have been linked to bone resorption, subsequently causing a decrease in bone mass [3, 16, 17]. Peripheral QCT has the unique ability to assess both bone geometry and bone mass, providing a more complete image of bone strength and invaluable insight into fracture risk in this population. Among retired elite gymnasts with a history of amenorrhea, Ducher et al. [36] reported that certain pQCT-derived bone health parameters were lower than those in gymnasts without a history of amenorrhea but similar to those in control subjects, indicating that a history of amenorrhea compromised some of the benefits associated with exercise. It is currently unknown, however, if the same is true of recreationally-active amenorrheic women who do not engage in the same unique, dynamic activities as gymnasts.

In addition, the energy-deficient and estrogen-deficient environments have been linked to an uncoupling of bone turnover, characterized by suppressed bone formation and elevated bone resorption [3, 17]. This alteration in bone turnover may have distinct effects on trabecular and cortical bone; trabecular bone has a greater rate of bone remodeling than cortical bone and, therefore, may be more sensitive than cortical bone to changes in bone turnover such as those observed in amenorrheic exercising women [42].
The ability of pQCT to separate bone mass and area into its trabecular and cortical components is another advantage of using this technique to assess bone health in this population.

**Methods:** In this cross-sectional study design, exercising women who were between the ages of 18-35 years and participated in at least 2 hours of physical activity each week were grouped according to self-reported history of menses within the past year. Women who reported no menses in the past 3 months were categorized as amenorrheic; whereas, those who reported at least 10 cycles in the past 12 months were categorized as eumenorrheic. DXA scans of the total body, lumbar spine, and dual femur were used to assess body composition and aBMD. pQCT scans of the distal and proximal tibia were used to determine vBMD, bone geometry, and estimated bone strength at the distal and proximal tibia.

This study merged data from three datasets of exercising women to include 1) baseline data from a randomized controlled trial designed to determine the effects of a 12-month intervention of increased caloric intake on indices of bone health and menstrual status in women with severe exercise-associated menstrual disturbances, 2) a cross-sectional study that assessed bone health in exercising women presenting with and without a bone stress injury, and 3) an observational study of female collegiate cross-country runners.

**Statistical Analysis:** For variables that were normally distributed, independent t-tests were used to compare vBMD, bone area and geometry, BSI, and SSI between amenorrheic and ovulatory women. For variables that did not follow a normal distribution and were unable to be transformed, non-parametric Mann-Whitney U tests
were used to compare these measures between amenorrheic and eumenorrheic exercising women. Analysis of covariance (ANCOVA) was also conducted to determine group differences in vBMD, bone area and geometry, BSI, and SSI after controlling for variables that have been demonstrated to influence bone size and mass. Lastly, correlation analyses were used to determine associations between pQCT-derived measures of bone health and variables indicative of body composition, anthropometrics, exercise habits, and reproductive function.

**Expected Findings:** Investigators have reported that total and cortical vBMD at the tibia is lower in amenorrheic athletes compared to sedentary controls [11]. Therefore, in support of these findings, it is expected that vBMD will also be lower in amenorrheic, exercising women compared to their ovulating counterparts due to the suppressed reproductive and metabolic environments typically experienced by the amenorrheic women. On the contrary, total and trabecular area at the tibia were demonstrated to be greater in amenorrheic athletes or in athletes with a history of amenorrhea compared to sedentary controls, but not different than that of eumenorrheic athletes [11, 36]; thus, it is anticipated that no difference in bone area will be observed between amenorrheic and eumenorrheic exercising women. Finally, it has been reported that BSI at the distal tibia does not differ between retired gymnasts with and without a history of amenorrhea but SSI at the proximal tibia is greater (p<0.09) in retired gymnasts with a history of amenorrhea compared to their counterparts without a history of amenorrhea [36]. These interesting results for SSI may have been observed because this estimate of bone strength is calculated at the proximal region which may be more sensitive to increases in bone area synergistically induced by exercise and low circulating estrogen concentrations.
Because our population of amenorrheic exercising women do not participate in the same type of highly osteogenic exercise as that engaged in by the participants of the Ducher et al. [36] study, it is expected that amenorrheic exercising women will present with a lower BSI at the distal tibia but no difference in SSI at the proximal tibia compared to eumenorrheic exercising women.

**Power Analysis:** Sample size calculations were performed for outcome variables that had both mean and standard deviation (SD) or standard error mean presented in previous publications [36] using pQCT to assess vBMD, bone geometry, and estimated bone strength at the tibia.

*Trabecular vBMD.* For trabecular vBMD at the distal tibia, published data from retired elite gymnasts without a history of amenorrhea and sedentary controls provided a meaningful difference of 29.8 mg/cm³ and a SD of 25.5 mg/cm³ after adjustment for height. Using these data, a sample size calculation revealed that 26 women (13 in each group) provide sufficient power (1-β = 0.80) to detect differences at α=0.05 in distal tibia trabecular vBMD.

*Cortical area.* For cortical area at the proximal tibia, published data from retired elite gymnasts with a history of amenorrhea and sedentary controls provided a meaningful difference of 35.1 mm² and a SD of 32.5 mm² after adjustment for height. A sample size calculation revealed that 30 women (15 per group) provide sufficient power (1-β = 0.80) to detect differences at α=0.05 in proximal tibia cortical area.

*Total area.* For total area at the proximal tibia, published data from retired elite gymnasts with a history of amenorrhea and sedentary controls provided a meaningful difference of 58.3 cm² and a SD of 63.5 cm² after adjustment for height. According to a
sample size calculation, 42 women (21 per group) provide sufficient power (1-\(\beta\) = 0.80) to detect differences at \(\alpha\)=0.05 in proximal tibia total area.

**BSI.** For BSI at the **distal tibia**, published data from retired elite gymnasts with a history of amenorrhea and sedentary controls provided a meaningful difference of 5.2 mg\(^2\)/mm\(^4\) and a SD of 16.9 mg\(^2\)/mm\(^4\) after adjustment for height. A sample size calculation revealed that 36 women (18 per group) provide sufficient power (1-\(\beta\) = 0.80) to detect differences at \(\alpha\)=0.05 in distal tibia BSI.

**SSI.** For SSI at the **proximal tibia**, data from retired elite gymnasts with a history of amenorrhea and sedentary controls provided a meaningful difference of 339.3 mm\(^3\) and a SD of 326.8 mm\(^3\) after adjustment for height. According to a sample size calculation, 34 women (17 per group) provide sufficient power (1-\(\beta\) = 0.8) to detect differences at \(\alpha\)=0.05 in proximal tibia SSI.

Therefore, based on these calculations, a sample size of 42 women should provide adequate power to detect differences in trabecular vBMD, BSI, SSI, and proximal bone area.
Study Two: Body composition and reproductive function exert unique influences on indices of bone health in exercising women

Amenorrheic exercising women typically present with low BMD when compared to their ovulating counterparts [35, 39, 43]. The failure to acquire bone mass or the loss of bone mass in this population occurs due to the uncoupling of bone formation and resorption that is observed in an energy-deficient and estrogen-deficient environment [3, 17]. Therefore, both reproductive and metabolic status appear to influence bone health by unfavorably altering bone turnover which may lead to low bone mass and less-than-optimal bone geometry.

The suppressed estrogen concentrations observed among amenorrheic exercising women lead to elevated bone resorption due to the removal of estrogen’s inhibitory effects on osteoclast action [16]. In addition, exercising women with amenorrhea are likely in an energy deficit, and metabolic alterations associated with an energy deficiency such as suppressed T3 may also have detrimental effects on bone health [44]. In fact, it has been reported that amenorrheic athletes with the lowest concentrations of bone formation markers also demonstrated the lowest circulating concentrations of total T3 [45].
Amenorrheic exercising women typically have a lower percent body fat (%BF) than their ovulatory exercising counterparts, which may have a secondary effect on BMD mediated through the adipocyte-derived hormone leptin [46]. Investigators have reported that leptin exerts both positive and negative influences on bone depending on its mechanism and site of action [47]. On the other hand, it is well-established that lean mass is a strong osteogenic stimulus, serving as a beneficial contributor to bone mass [7, 13] among exercising women who have greater lean mass than sedentary women [7, 48]. Therefore, it is evident that several factors including reproductive and metabolic hormones and body...
composition may play a role in bone health among amenorrheic exercising women; however, the relative contribution of these factors has not been explored (Figure 1.2).

It must be considered, however, that the BMD usually reported is derived from DXA, a technique that is only capable of assessing aBMD rather than true vBMD [49]. Areal BMD tends to underestimate true BMD in small, thin individuals and overestimate true BMD in taller, larger individuals [49]. Therefore, corrections of aBMD that account for bone size are recommended when investigating bone health in populations with specific body size phenotypes, i.e., children, adolescents, anorexics, and lean athletes. Consequently, an algorithm has been developed to estimate vBMD (referred to as bone mineral apparent density or BMAD) at the lumbar spine [49]. In addition, because DXA is limited by its 2-dimensional nature, it is unable to measure bone geometry, i.e., cross-sectional bone size and shape. Bone geometry is an important component of bone strength. For this reason, a method of estimating geometric properties of the femoral neck using DXA has been developed, providing measures of femoral neck cross-sectional moment of inertia (CSMI) and cross-sectional area (CSA). These measures serve as an estimate of femoral neck strength and impart potentially useful information regarding bone health when actual 3-dimensional techniques are not available [50].

Therefore, the purpose of this study was twofold. This study sought 1) to determine if amenorrheic and ovulatory exercising women differ with regard to DXA-derived estimates of volumetric density of the lumbar spine (lumbar spine BMAD) and femoral neck strength (femoral neck CSMI and CSA) and 2) to explore the respective roles of reproductive function, metabolic status, and body composition, i.e., fat mass and lean mass, in aBMD, lumbar spine BMAD and femoral neck CSMI and CSA.
Aim 1: To determine if amenorrheic and ovulatory exercising women differ with respect to estimated volumetric density of the lumbar spine (lumbar spine BMAD) and estimated geometrical properties of the femoral neck (CSMI and CSA) that act as surrogate markers of femoral neck strength.

Hypothesis 1: Amenorrheic exercising women will demonstrate lower lumbar spine BMAD, and lower femoral neck CSMI and CSA compared to ovulatory exercising women.

Aim 2: To explore the roles of reproductive function (estrogen and progesterone exposure, age of menarche), metabolic status (leptin and total T3 concentrations), and body composition (lean mass and fat mass) in measures of bone health among exercising women.

Hypothesis 2: Estrogen and progesterone exposure, age of menarche, leptin and T3, and lean mass and fat mass will be significant predictors of bone health measures (aBMD, lumbar BMAD, femoral neck CSA and CSMI) among exercising women but will exert differing influences depending on the bone site.

Rationale: It has been well-established that amenorrheic women are at risk for low bone mass; however, the mechanisms underlying the low bone mass have yet to be fully elucidated [3, 39, 43, 51]. Although lumbar BMAD has been previously reported to be low among amenorrheic adolescents and women compared to menstruating controls [39, 52, 53], estimates of femoral neck strength among both amenorrheic and ovulatory...
exercising women have not been reported. Furthermore, among this population, there is currently little information regarding the roles of reproductive and metabolic status and body composition in the measures of bone health derived from DXA such as aBMD, lumbar BMAD, and femoral neck CSA and CSMI.

The beneficial effects of an energy replete state and optimal reproductive function on bone health are well-known. Investigators have consistently demonstrated that amenorrheic women with an energy deficiency have lower BMD compared to eumenorrheic women, mediated synergistically by suppressed estrogen concentrations and a poor nutritional environment [3, 39, 43, 51]. However, it is not clear what factors may be most important for promoting a healthy bone mass and geometry, both of which are integral components of bone strength.

Investigators have demonstrated that both estrogen and progesterone are significant predictors of BMD in exercising women [15, 54, 55], which is believed to be due to the inhibition of osteoclasts and stimulation of osteoblasts, respectively [16, 56, 57]. Similarly, the age at which women begin menstruating may also have an influence on BMD due to the beneficial effects of estrogen on bone during the pivotal years of puberty and adolescence when much of the gain in bone mass occurs [36, 44]. Estrogen also inhibits further periosteal expansion in bone, thus age of menarche may influence bone geometry and size [58]. In addition, the osteogenic influence of lean mass on BMD has been well-established. Several investigators have reported that lean mass is an important and significant predictor of both bone health and the improvement in BMD among adolescent girls and women [12, 39]. In fact, muscle forces have been proposed to be the primary contributor to bone mass and strength [13, 40].
On the other hand, the influence of fat mass and the metabolic hormones leptin and T3 on bone density is not as well-understood. Fat mass has been shown to be directly associated with circulating concentrations of leptin, an adipocyte-derived hormone [46]. Leptin has been postulated to be influential in determining BMD, providing a potential, albeit indirect, link between fat mass and BMD [7, 14]. Through central and peripheral mechanisms, leptin exerts both anti-osteogenic and osteogenic effects on bone mass [47]. Leptin receptors are present at the level of the hypothalamus; the binding of leptin to these receptors initiates the cascade that results in an increase in cortical bone but a decrease in cancellous bone [47]. Leptin also directly stimulates bone formation at the level of bone cells by stimulating osteoblastic differentiation via leptin receptors present on osteoblasts [47]. Investigators have shown that leptin is positively associated with BMD and bone microarchitecture among adolescent girls and women and that the administration of recombinant human leptin among amenorrheic women significantly increases markers of bone formation and BMD [7, 10, 20-22]. However, other investigators have demonstrated that leptin is not significantly associated with BMD [14], thereby contributing to a lack of clarity regarding the role of leptin and body fat on bone health. As another marker of metabolic status, total T3 concentrations have been reported to be lowest among female distance runners with the lowest concentrations of bone formation markers [45]. T3 has been reported to increase osteoblast activity and synthesis of the bone matrix in vitro [59]; however, whether this hormone has a direct impact on bone metabolism in vivo is currently unclear.

Although reproductive and metabolic hormones as well as body composition have been observed to be important for bone health, it is currently not clear whether
reproductive function, metabolic hormones, or body composition is a stronger predictor of BMD and bone strength among exercising women. Thus, this study may provide beneficial information regarding whether the focus during clinical intervention for amenorrhea among athletes should be restoration of optimal menstrual function and a healthy metabolic environment or attainment of an “ideal” body composition.

**Methods:** This cross-sectional study included data from the following two studies: 1) an observational study that assessed the impact of menstrual function on cardiovascular and bone health in exercising women, and 2) baseline data from a randomized controlled trial designed to determine the effects of a 12-month intervention of increased caloric intake on indices of bone health and menstrual status in premenopausal women who suffer from severe exercise-associated menstrual disturbances.

Exercising women participating in at least 2 hours of exercise per week were grouped according to menstrual status as assessed by self-report and daily urinary concentrations of reproductive hormone metabolites. Women who reported regular menstrual cycles in the past 6 months and presented with an ovulatory cycle of 26-35 days confirmed by daily urinary concentrations of reproductive hormones collected for one complete cycle were categorized as eumenorrheic and ovulatory. Women who reported no menses for at least 90 days or ≤3 cycles in the past 6 months and demonstrated suppressed urinary concentrations of reproductive hormones for one 28-day monitoring period were categorized as amenorrheic.

During the assessment period which typically lasted between 6-8 weeks, DXA scans of the total body, lumbar spine, and dual femur were performed to assess body
composition, aBMD, and femoral neck CSA and CSMI using the Hip Strength Analysis software option. Lumbar spine BMAD was calculated from lumbar spine bone mineral content and area. A blood sample was collected for the measurement of metabolic hormones. Women also collected daily urine samples each morning for one complete menstrual cycle or 28-day monitoring period to assess estrogen and progesterone exposure.

**Statistical Analysis:** For variables that were normally distributed, independent t-tests were used to compare aBMD, lumbar BMAD, and femoral neck CSA and CSMI between amenorrheic and ovulatory women. For variables that did not follow a normal distribution and were unable to be transformed, non-parametric Mann-Whitney U tests were used to compare these measures between amenorrheic and ovulatory exercising women. Analysis of covariance (ANCOVA) was also conducted to determine differences in these measures between amenorrheic and ovulatory exercising women with body mass index, a marker of body size that is known to influence BMD, as a covariate. Correlation analyses were performed to determine relations between variables. In addition, hierarchical linear regression was used to create models that best predict lumbar spine aBMD and BMAD, femoral neck aBMD, CSMI, and CSA, and total hip aBMD.

**Expected Findings:** Other investigators have reported lower lumbar BMAD among both amenorrheic adolescent athletes and anorexic adolescents [7, 39, 52], and lower femoral neck CSA has been observed in amenorrheic compared to eumenorrheic athletes [60]. Furthermore, lower femoral neck CSA and CSMI have been reported in female adolescent runners with elevated bone turnover compared to runners with normal bone turnover [61]. Therefore, it is expected that lumbar BMAD and femoral neck CSA
and CSMI will be lower in amenorrheic vs. ovulatory women due to the hormonal and nutritional environment of the amenorrheic women.

Reproductive function, metabolic hormones, and body composition are all anticipated to play important roles in bone health among exercising women. However, it is expected that variables indicative of reproductive function, i.e., estrogen and progesterone exposure and age of menarche, will be stronger predictors of lumbar spine aBMD and lumbar BMAD; whereas, body composition will be a stronger predictor of total hip and femoral neck aBMD and femoral neck CSA and CSMI. Metabolic hormones may have an important influence at all three sites.

We have previously reported that estrogen is a significant predictor of lumbar spine aBMD in exercising women [15]. Likewise, T3 may stimulate bone formation [44, 45]; however, results have been inconclusive [62]. The lumbar vertebrae are primarily composed of trabecular bone which is characterized by a higher rate of turnover than cortical bone; therefore, the lumbar vertebrae may be more sensitive to reproductive and metabolic hormonal changes that affect bone turnover compared to bone sites that have more cortical bone such as the hip [42].

Lean mass, on the other hand, exerts a strong osteogenic influence, primarily through the action of muscle forces on bone [13, 40], and body weight is also a significant predictor of BMD [15]. The influence of fat mass on bone health is not as clear; however, leptin, a hormone produced by adipocytes, has been reported to increase BMD in amenorrheic women, suggesting that those with greater fat mass may also have better bone health through the effects of leptin [22]. It is anticipated that these effects
may be most strongly observed at weight-bearing sites such as the total hip and femoral neck due to the greater muscle and gravitational forces acting at these sites.

With respect to the roles and relative contributions of reproductive function, metabolic hormones, and body composition in bone health among exercising women, it is anticipated that lean mass will be the strongest predictor of BMD and bone geometry at weight-bearing sites and that reproductive function, as assessed by estrogen and progesterone exposure and age of menarche, will be the strongest predictor of BMD at non-weight bearing, primarily trabecular sites such as the lumbar spine.

**Power Analysis:** To determine the necessary sample size for adequate power, means and standard deviations from published data from other investigators were used to perform sample size calculations.

For lumbar spine aBMD, published data from 21 amenorrheic athletes and 18 eumenorrheic athletes provided a meaningful difference of 0.090 g/cm² and a standard deviation (SD) of 0.100 g/cm² [39]. A sample size calculation revealed that 22 women per group provide sufficient power (1-β = 0.80) to detect differences at α=0.05 in lumbar spine aBMD.

For lumbar spine BMAD, Christo et al. [39] reported a meaningful difference of 0.011 g/cm³ and a SD of 0.015 g/cm³. A sample size calculation revealed that 36 women per group provide sufficient power (1-β = 0.80) to detect differences at α=0.05 in lumbar spine BMAD.

For total hip aBMD, Christo et al.[39] reported a meaningful difference of 0.110 g/cm² and a SD of 0.100 g/cm². A sample size calculation revealed that 15 women per
group provide sufficient power (1-\(\beta\) = 0.80) to detect differences at \(\alpha\)=0.05 in total hip aBMD.

For femoral neck aBMD, Maimoun et al. [63] reported a meaningful difference of 0.134 g/cm\(^2\) and a SD of 0.128 g/cm\(^2\). A sample size calculation revealed that 16 women per group provide sufficient power (1-\(\beta\) = 0.80) to detect differences at \(\alpha\)=0.05 in femoral neck aBMD.

For femoral neck CSA and CSMI, Gong et al. [64] reported a meaningful difference of 0.120 ± 0.18 cm\(^2\) and 0.088±0.158 cm\(^4\), respectively, in a sample of overweight adolescent girls (n=30) and controls (n=52). Using this data, a sample of 35 and 52 women per group, respectively, provide sufficient power (1-\(\beta\) = 0.80) to detect differences at \(\alpha\)=0.05 in femoral neck CSA and CSMI.

Therefore, a sample size of 52 women per group should provide adequate power to detect differences in aBMD, lumbar BMAD, femoral neck CSA and CSMI. For multivariate regression analysis, a sample of 49 women should provide sufficient power (1-\(\beta\) = 0.80) to detect significant relationships at a large effect size of 0.35 [65].
Study Three: The response of bone mineral density, estimated bone geometry, and bone health-related factors to an intervention of increased caloric intake among young women with exercise-associated menstrual disturbances

Prolonged energy deficiency among exercising women has been linked to detrimental health consequences such as exercise-associated menstrual disturbances (EAMD) and low bone mass [66]. As such, reversal of the energy deficiency via an increase in energy intake, a decrease in energy expenditure, or a combination of both, is imperative for recovery of menstrual function and an improvement in bone health. Evidence of an improved energy status includes increases in circulating concentrations of metabolic hormones such as IGF-1 [67] and leptin [68] and restoration of optimal functioning of the hypothalamic-gonadal-axis, leading to an increase in circulating estrogen. Typically, weight gain also occurs, contributing to increases in both lean mass and fat mass [29]. Reversal of the energy deficiency, resumption of menses, and weight gain have all been demonstrated to have a beneficial impact on BMD [12, 28]. However, the influence of specific indicators of an improvement in energy status, i.e., hormonal and body composition changes, on DXA-derived aBMD and estimated bone geometry is not well-understood.

Therefore, the purpose of this study was to assess the impact of a 12-month intervention of increased caloric intake on bone health and related factors in exercising women with EAMD and to assess the changes in body composition, metabolic hormones, and reproductive function that may be associated with improvements in bone health.
**Aim 1:** To assess the impact of a 12-month intervention of increased caloric intake on DXA-derived aBMD, BMAD and estimated bone geometry (femoral neck CSA and CSMI) among women with EAMD.

**Hypothesis 1:** Lumbar spine, femoral neck, and total hip aBMD will increase in women with EAMD undergoing an intervention of increased caloric intake, but femoral neck CSA and CSMI will not increase.

**Aim 2:** To determine the impact of a 12-month intervention of increased caloric intake on factors known to influence bone health to include estrogen exposure, body composition (fat mass and lean mass), and the metabolic environment (leptin and IGF-1 concentrations) among women with EAMD.

**Hypothesis 2:** Estrogen exposure, fat mass, lean mass, and circulating concentrations of leptin and IGF-1 will increase in conjunction with increases in body weight among exercising women with EAMD participating in an intervention of increased caloric intake.

**Aim 3:** To explore associations between changes in factors known to influence bone health and changes in aBMD and estimated bone geometry during the 12-month intervention.

**Hypothesis 3:** Changes in body weight and its components (i.e., fat mass and lean mass) as well as changes in concentrations of reproductive and metabolic hormones (i.e., estrogen, leptin, and IGF-1) will be positively associated with changes in aBMD during the intervention.
**Rationale:** Much of the literature exploring the impact of increased caloric intake and resumption of menstrual cyclicity on bone health among energy-deficient women focuses on the severe model of energy deficiency, i.e., anorexia nervosa. It has been reported that resumption of menses appears to be important for recovery of lumbar spine BMD; whereas, weight gain is important for total hip BMD [12, 28], suggesting that changes in estrogen may be a primary factor in restoring BMD at the lumbar spine and changes in lean mass and/or fat mass may be primary factors for recovery of BMD at weight-bearing sites. Few studies, however, have examined the effects of increased caloric intake and the associated changes in hormone concentrations and body composition on bone health among exercising, amenorrheic women, a model of energy deficiency that differs slightly from that characterized by a clinical eating disorder. In fact, to date, the only published reports that prospectively assess the effects of increased caloric intake and subsequent weight gain and resumption of menses on bone health in exercising women are in the form of case studies [33, 34] and follow-up investigations [69-71]. Frederickson et al. [33] reported a 25.5% and 19.5% increase in lumbar spine and hip BMD, respectively, over the course of 8 years after a gradual weight gain of 17 kg in an athlete who presented with amenorrhea and very low BMD. Similarly, Zanker et al. [34] observed a 16.9% increase in hip BMD after weight gain of 8 kg over 36 months in an endurance athlete with primary amenorrhea and low BMD. Follow-up investigations of amenorrheic athletes, ranging in duration from 15-24 months, have revealed significant increases in lumbar spine BMD with weight gain and, in some cases, resumption of menses; however, lumbar spine BMD persistently remained below that of eumenorrheic athletes [69, 71]. Although the results from these case studies and follow-up
up investigations are encouraging, reports from controlled interventions that focus on increased caloric intake and weight gain in a larger population of exercising women with menstrual dysfunction are currently lacking and are, therefore, necessary.

It has been reported that resumption of menses is crucial for restoration of bone mass in amenorrheic women [12]. However, because suppression of several different hormones such as estrogen, IGF-1, and leptin are frequently observed among amenorrheic women [1, 15, 46, 52, 67, 72], changes in these hormones likely accompany resumption of menses. It is unknown which of these factors is more strongly associated with change in BMD and, therefore, may be mediating the changes observed with resumption of menses. Serum estradiol concentrations have been reported to be a significant predictor of bone mass [10]; however, these measurements only capture estradiol concentrations at a specific point in time. The analysis of daily urinary excretion of estrogen metabolites to obtain monthly estrogen exposure provides a more comprehensive perspective of the changes that occur within the profile of reproductive hormones over time. In addition, a significant positive relation between leptin and bone density and microarchitecture has been reported [10], and treatment with recombinant human leptin has led to an increase in bone formation [20, 21] and an increase in bone density at the lumbar spine among amenorrheic women [22]. Whether changes in endogenous leptin that occur with an improved energy status will impact BMD in a similar manner is unknown. Likewise, IGF-1 is a metabolic hormone that is believed to influence BMD through its role in stimulating osteoblast activity [73]; however, whether or not it is a primary contributor to bone health among exercising women is unclear.
Finally, there is currently limited information available regarding the impact of the components of weight gain, i.e., fat mass and lean mass, on bone health among exercising women with EAMD. Heer et al. [74] reported that nutritional therapy among anorexic women resulted in increases in body weight, fat mass, and leptin which coincided with increases in bone formation markers. Thus, an increase in fat mass may provide anabolic effects to bone via leptin. Likewise, investigators have reported that changes in lean body mass correlate strongly with changes in BMD among anorexic women [12, 75]. Whether similar results are observed among exercising women undergoing treatment for an energy deficiency has yet to be determined.

In summary, this study longitudinally assessed the impact of increased caloric intake and weight gain on parameters of bone health and bone health-related factors among exercising women with EAMD, and also explored the influence of changes in estrogen exposure, metabolic hormones, and body composition, i.e., lean mass and fat mass, on bone health among exercising women with severe EAMD, a population that lacks information in this area. The results from this study will hopefully provide further clarity regarding the most effective treatment strategies for reversal of bone loss among exercising women with severe menstrual cycle disturbances.

**Methods:** This longitudinal study used data from a randomized controlled trial (RCT) designed to investigate the effects of a 12-month intervention of increased caloric intake on menstrual function and bone health among women with severe EAMD.

For the RCT, exercising women who participated in at least 2 hours of physical activity per week were placed into one of three groups. Women who reported no menses
in the past 3 months or ≤6 cycles in the past 12 months were randomized into either the EAMD+Calories (EAMD+Cal) group which was instructed to increase caloric intake during the intervention or the EAMD Control group which was instructed to maintain habitual eating and exercise behaviors. Confirmation of the presence of a severe menstrual cycle disturbance was obtained from assessment of urinary metabolites of reproductive hormones in daily urine samples collected for one 28-day baseline monitoring period prior to the start of the intervention. The Ovulatory Control group (Ov Control) consisted of women who self-reported regular menstrual cycles in the past 6 months and presented with an ovulatory cycle of 26-35 days confirmed by daily concentrations of urinary metabolites of reproductive hormones collected for one complete cycle during the baseline period of the study.

DXA scans of the total body, lumbar spine, and dual femur were performed at baseline and at multiple time points during the intervention to assess changes in body composition, aBMD, lumbar spine BMAD, and femoral neck CSA and CSMI. Blood samples were collected for the measurement of metabolic hormones (leptin and IGF-1) at baseline and periodically throughout the intervention to monitor changes in metabolic status. The women also collected daily urine samples for the assessment of estrogen exposure and menstrual status for the duration of the intervention if in the EAMD groups or for 2-3 menstrual cycles during the intervention if in the Ov Control group.

**Statistical Analysis:** The data was analyzed statistically using 1) one-way analysis of variance (ANOVA) to determine the difference in baseline characteristics among groups and 2) one within, one between repeated measures ANOVA (RMANOVA) with time and group as the factors. RMANOVA was used to assess
change in aBMD, estimated bone geometry, body composition, metabolic hormones, and estrogen exposure during the intervention. Correlation analyses were also performed to determine associations between change in parameters of bone health and change in body composition, metabolic hormone concentrations, and estrogen exposure during the intervention.

**Expected Findings:** Results from previous studies in amenorrheic athletes who gained weight and demonstrated a significant increase in lumbar spine BMD [69, 71] suggest that lumbar spine aBMD and BMAD may increase as a result of an intervention that leads to weight gain. Furthermore, an increase in hip BMD among anorexic women who gained weight and resumed menses [12] suggest that total hip and femoral neck aBMD may also increase in women with severe EAMD participating in an intervention of increased energy intake. However, it is expected that changes in estimated femoral neck geometry, i.e., CSA and CSMI, will be more difficult to observe since we anticipate that these parameters of bone health may be more sensitive to changes in mechanical loading rather than the metabolic environment.

Coinciding with the anticipated weight gain during the intervention, it is expected that fat mass and lean mass will both increase, although increases in fat mass may exceed that of lean mass as is evident in anorexic women and adolescents undergoing treatment [12, 30, 76]. Furthermore, it is anticipated that improvements in energy status that accompany increased energy intake will be observed through increased circulating concentrations of leptin and IGF-1. Leptin concentrations are correlated with fat mass [72, 77]; therefore, it is anticipated that an increase in leptin concentration will parallel increases in fat mass. In addition, an increase in circulating IGF-1 concentrations have
been observed among anorexic women with improvements in energy status [67]. Due to the permissive role of the metabolic environment on the reproductive axis [77-79], it is also anticipated that estrogen exposure will increase during the intervention.

The metabolic hormones IGF-1 and leptin are believed to exert anabolic effects on bone mass via proliferation of osteoblasts and stimulation of osteoblast activity [73, 80]. Among anorexic girls, both leptin [29] and IGF-1 [67] were associated with markers of bone formation during inpatient dietary or intravenous hyperalimentation treatment. Likewise, recombinant leptin treatment among women with FHA resulted in significant increases in bone formation markers and BMD [21, 22]. Estrogen also impacts bone mass by altering bone turnover, although the primary effects of estrogen inhibit osteoclast activity and bone resorption [16]. Furthermore, circulating concentrations of leptin, IGF-1, and estradiol have demonstrated significant, positive associations with BMD and bone microarchitecture [10]. Due to the greater sensitivity of trabecular sites such as the lumbar spine to changes in bone turnover, it is expected that changes in leptin, IGF-1, and estrogen will be associated with changes in lumbar spine BMD and BMAD during the intervention.

The osteogenic effect of lean mass on bone strength as a result of muscles forces exerted on bone has been well-established [13, 40], and lean mass has been demonstrated to exert a significant influence on bone health among adolescent girls and women [12, 39, 75]. Therefore, it is anticipated that an increase in lean mass will be associated with a positive change in aBMD and estimated bone geometry at weight-bearing sites such as the femoral neck and total hip. In addition, it is expected that change in fat mass will also correlate with change in aBMD at the lumbar spine, total hip, and femoral neck.
Investigators have previously reported strong correlations between fat mass and BMD at various sites [81-83]. Furthermore, weight gain has been demonstrated to be important for improvements in total hip BMD [12]; therefore, an increase in fat mass that accompanies an increase in body mass may also contribute to an increase in BMD.
Study Four: A case report of recovery of menstrual function following a nutritional intervention in two exercising women with amenorrhea of varying duration

It is not uncommon to observe FHA among women participating in habitual exercise. FHA, characterized by the absence of menses for at least 90 days, is the most severe form of menstrual cycle disturbance and is caused by an energy deficiency in which there is inadequate energy intake to compensate for energy expenditure. In an effort to conserve fuel, the body repartitions energy to the functions necessary for survival such as thermoregulation, growth, and cellular maintenance and away from those functions that are not necessary for survival such as reproduction. As a result, metabolic and hormonal alterations occur that ultimately result in the loss of menses and low bone mass.

Ultimately, the optimal treatment approach to FHA is the reversal of the energy deficiency, achieved by an increase in energy intake, a decrease in energy expenditure, or both [66]. Weight gain often occurs and has been observed to be a favorable outcome associated with improved energy status, resumption of menses, and enhanced bone health [9, 28, 32-34]. Because a decrease in exercise volume is often not viewed favorably among exercising women, an increase in caloric intake is a common treatment strategy for women presenting with FHA and has been shown to be effective [32, 84, 85]. Strategies and effectiveness of this non-pharmacological treatment, however, have not been fully explored.

Therefore, the purpose of this case report was to examine the responses of two exercising women with FHA of varying duration (short-term vs. long-term) to a 12-month intervention involving an increase in energy intake designed to meet total energy
expenditure (TEE) needs. Thus, this case report describes the changes in energy status, menstrual function and bone health that accompany increased caloric intake and weight gain by describing in detail two women with amenorrhea of varying duration.

**Aim 1:** To compare and contrast the recovery of two exercising women with current FHA of varying duration (short-term vs. long-term) to a 12-month nutritional intervention involving an increase in energy intake. This case report describes the changes in energy status (resting metabolic rate, metabolic hormones), menstrual function (resumption of menses, ovulation, and cycle regularity) and bone health (bone markers, hip and spine areal BMD) that accompany increased caloric intake and weight gain.

**Rationale:** Functional hypothalamic amenorrhea has detrimental consequences to the health and well-being of exercising women; therefore, the identification of optimal and effective treatment strategies is essential. Women who fail to menstruate for extended periods of time (>90 days) are at risk for low bone mass, attributable to either the failure to achieve peak bone mass or the loss of bone mass [3, 39, 43, 51]. This low bone mass can lead to osteoporotic fractures in the post-menopausal years [86], emphasizing the long-term impact of FHA and highlighting the importance of reversal of the energy deficiency and resumption of menses among women with FHA. Pharmacological strategies to restore menstruation and improve bone health have demonstrated controversial success in amenorrheic women [19]. Administration of recombinant human leptin successfully resulted in resumption of menses and improved bone turnover and BMD among amenorrheic women; however, treatment with leptin resulted in weight loss, an unfavorable outcome among this population of women [20-
Non-pharmacological strategies aimed at increasing caloric intake and weight gain in an effort to resume menstruation and improve bone health have been reported to be successful in anecdotal accounts of exercising women [31-34], but results have been mixed in populations of anorexic women [28, 30].

Few case studies of amenorrheic, exercising women have been reported. Dueck et al. [31] and Kopp-Woodroffe et al. [32] described case studies of five amenorrheic athletes who participated in an intervention of increased caloric intake for 12 to 20 weeks. Weight gain ranged from 1-3 kg, and three of the five women resumed menses during the intervention. Fredericson and Kent [33] reported a case study of an amenorrheic athlete who gained weight over the course of 5 years, resulting in the maintenance of normal menstrual cycles and improved bone health. Similarly, Zanker et al. [34] followed an amenorrheic athlete for 12 years and reported an increase in BMD of the proximal femur with an increase in body mass index. There are currently no published reports of amenorrheic women who have been closely followed for 12 months while increasing caloric intake and undergoing consistent repeated measurements of energy balance, body composition and bone density, nutrient intake, reproductive and metabolic hormones, and psychological state. Furthermore, the impact of duration of amenorrhea on the recovery of menstrual function has not been fully explored. Therefore, this information allows for the assessment of physiological and behavioral change throughout the study, providing markers that may be associated with the successful resumption of menses. In addition, using the case study model to explore resumption of menses among women with exercise-associated amenorrhea provides the
opportunity to gain a more clear understanding about the interplay of many factors that may be involved in the resolution of this complex problem.

**Methods:** For this case report, two exercising amenorrheic women were chosen to demonstrate the impact of increased energy intake on the hormonal aspects of recovery of menstrual function and bone health. These women participated in a 12-month intervention designed to increase caloric intake 20-30% above baseline total energy expenditure needs in an effort to restore optimal menstrual function and improve bone health among women with severe exercise-associated menstrual disturbances.

The primary outcome variables in the 12-month intervention were indices of energy status, bone health and menstrual status. As such, at baseline and periodically throughout the intervention, blood samples were collected for the measurement of metabolic hormones (T3, leptin, and ghrelin) and resting energy expenditure (REE) was assessed to monitor changes in energy status. Likewise, DXA scans of the total body, lumbar spine, and dual femur were performed at baseline and at multiple time points during the intervention to assess changes in body composition and BMD. The women also collected daily urine samples for one 28-day monitoring period during baseline to confirm menstrual status and for the 12 months of the intervention to monitor changes in reproductive hormone concentrations and ovulatory status.

**Statistics:** Due to the nature of this study, formal statistics were not performed. Rather, data analysis focused on a qualitative description of the results.

**Expected Findings:** The design of this study allows for the examination of two exercising women with FHA of varying duration who participated in a 12-month intervention designed to increase caloric intake in an effort to improve energy status,
reproductive function, and bone health. Upon completion of the intervention, both women successfully resumed menses. Because the underlying cause of FHA is an energy deficiency, it is anticipated that increased caloric intake and weight gain will result in improvements in energy status as assessed by increases in REE and changes in metabolic hormones such as increased concentrations of total T3 and leptin and decreased concentrations of ghrelin. It is also expected that improvements in menstrual function will coincide directly with the improvements in energy status that occur subsequent to increased caloric intake and weight gain; however, the time and events required for restoration of optimal menstrual function may differ depending on duration of amenorrhea, exercise volume, and body composition. In addition, the recovery of menstrual function and energy status is expected to be beneficial to bone health, resulting in favorable changes in markers of bone turnover and increased BMD.
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CHAPTER 2: PART 1


Abstract

Although exercise among girls and women is beneficial for overall health and well-being, the development of an energy deficiency as a result of inadequate energy intake to compensate for exercise energy expenditure can lead to menstrual dysfunction. It has been established that it is not the stress of exercise that causes reproductive dysfunction; rather, in an attempt to conserve energy, metabolic adaptations triggered by an energy deficiency alter the normal production and pulsatility of reproductive hormones at all levels of the hypothalamic-pituitary-ovarian (HPO) axis. As such, estrogen and progesterone concentrations decline resulting in a spectrum of exercise-associated menstrual disturbances (EAMD). The spectrum of EAMD includes the severe menstrual disturbances, amenorrhea and oligomenorrhea, which are easily detected by the absence of menses for at least 3 months or long, inconsistent cycles of 36-90 days, respectively. Less severe EAMD include luteal phase defects and anovulation which typically occur within regular intermenstrual intervals, thereby causing these disturbances to often remain undetected. Suppressing follicular growth and oocyte maturation, poor endometrial quality, spontaneous abortion, and infertility are all clinical reproductive consequences of EAMD. However, EAMD can be prevented by maintaining a healthy body weight and an energy replete state. Likewise, effective non-pharmacological treatment of EAMD includes an increase in caloric intake and weight gain to reverse the
energy deficiency and promote recovery of normal menstrual function. Upon recovery and/or maintenance of an energy replete state, regular exercise among girls and women is encouraged.
Introduction

Regular physical activity and exercise among girls and women is beneficial to overall health and well-being; however, increases in exercise energy expenditure that are not compensated for by energy intake can lead to perturbations in the reproductive axis, resulting in infertility and numerous skeletal and cardiovascular health consequences [1-4]. Exercise-associated menstrual disturbances (EAMD) have been commonly reported in exercising women, with prevalence estimates of EAMD approaching 50% [5]. Severe forms of EAMD such as the absence of menses (amenorrhea) or long intermenstrual intervals (oligomenorrhea) are clinically recognizable and typically identified based on menstrual history and self-report. However, other forms of EAMD such as luteal phase defects (LPD) and anovulation are not readily apparent and are often masked within cycles of regular length, yet are arguably the most prevalent [5, 6]. Therefore, these subtle menstrual disturbances in exercising women are not easily diagnosed, silently indicating an energy deficit and potentially contributing to infertility. Due to the frequency and, at times, silent nature of EAMD, awareness of the symptoms and consequences of an energy deficiency in exercising women is vital for the health and well-being of physically active girls and women.

The challenge that is imposed on physiological systems during an energy deficiency causes energy to be repartitioned toward processes that are necessary for survival such as thermoregulation, locomotion, and cellular maintenance, and away from processes that are not critical for survival, i.e. reproduction and growth [7]. As such, an energy deficiency often promotes a cascade of metabolic alterations in an effort to conserve energy, and these energy-conserving mechanisms in turn contribute to
disruptions in the hypothalamic-pituitary-ovarian (HPO) axis [7]. Beginning with the “generator” of reproductive function in the hypothalamus and ending with ovarian steroid secretion by the follicles, alterations in the production and secretion of reproductive hormones occur at each level of the reproductive axis. As such, the purpose of this chapter is to explore the impact of physical activity and exercise on reproductive function and fertility in adolescent girls and women by examining both subtle and severe menstrual disturbances and the changes that occur within the HPO axis to induce reproductive dysfunction. The effects of exercise and, more specifically, an energy deficiency on reproductive function and fertility will be explored by assessing the presence and quality of key reproductive events such as ovulation, the normal cyclicity of reproductive hormone concentrations, and endometrial proliferation.

Normal menstrual cycle and HPO activity

To adequately understand the effects of exercise on reproductive function, it is imperative to begin with a description of a normal menstrual cycle and optimal reproductive function, which serve as the healthy reference point to which the changes that are observed in both subtle and severe menstrual disturbances are compared. The menstrual cycle, which is defined as the period from the onset of menses to the day before the next onset of menses, typically lasts about 28 days and is divided into 2 phases, the follicular phase and the luteal phase [8]. The follicular phase begins at the onset of menses, and the luteal phase begins the day after ovulation occurs (Figure 2.1.1). Therefore, ovulation is a mid-cycle event that separates the follicular and luteal phases.
The cascade of events surrounding the menstrual cycle commences with secretion of gonadotropin-releasing hormone (GnRH) from the arcuate nucleus of the hypothalamus which in turn stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from gonadotroph cells of the anterior pituitary gland, the two gonadotropins that stimulate the production of estrogen and progesterone from the ovaries [9] (Figure 2.1.2). GnRH is known as the “master hormone” of reproduction due to its role as regulator of LH and FSH pulsatility [10]. Evidence from classic experiments conducted in rhesus monkeys [11, 12] demonstrated the rhythmic and acute secretory actions of GnRH. Typically, GnRH release occurs every 60-90 minutes, and
consequently, gonadotropin secretion occurs approximately once per hour from the anterior pituitary, paralleling the release of GnRH [8, 9, 13]. During the follicular phase, GnRH pulsatility and, therefore, LH and FSH pulsatility maintain a relatively high frequency [13]. Near the end of the follicular phase, the frequency and amplitude of GnRH pulses and, subsequently, LH and FSH pulses increase in response to the positive feedback of high estradiol concentrations [13]. However, at the end of the luteal phase, GnRH and LH pulsatility declines in response to negative feedback from progesterone [9, 13]. In turn, LH and FSH bind to receptors on the granulosa and theca cells of the developing ovarian follicle during the follicular phase and luteal cells during the luteal phase to produce estrogens, androgens, and progesterone [14].

Figure 2.1.2. HPO axis sequence of events related to the menstrual cycle. Neurons in the arcuate nucleus of the hypothalamus secrete GnRH which in turn stimulates the release of FSH and LH from the gonadotroph cells of the anterior pituitary gland. FSH and LH increase the production of estrogens and androgens by follicular granulosa and theca cells in the ovaries. During the luteal phase, the corpus luteum formed from the dominant follicle produces progesterone and estrogens. Typically, the ovarian hormones exert negative feedback on the anterior pituitary, causing a decrease in the secretion of the gonadotropins. However, during the late follicular phase, rapidly rising estrogen concentrations exert positive feedback on the anterior pituitary resulting in the LH surge. HPO: hypothalamic-pituitary-ovarian axis; GnRH: gonadotropin-releasing hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone
During the late luteal and early follicular phase, FSH production increases, stimulating follicular growth and recruitment of the dominant follicle within the ovaries [13]. Production of the estrogen, estradiol, by the synergistic efforts of the theca and granulosa cells of the ovarian follicles also increases [13]; thus, during the follicular phase, estradiol concentrations gradually increase, upregulating the number of FSH receptors in the mature follicles and consequently increasing the action of FSH and the production of estradiol [13, 14]. During the mid-follicular phase, negative feedback by estradiol on the anterior pituitary prevents further increases in FSH and LH [9, 15]. Near the late mid-follicular phase to the end of the follicular phase, one follicle has achieved dominance and rapidly increases its production of estradiol while the other less-dominant follicles undergo atresia [13]. The rapidly rising concentrations of estradiol exert positive feedback on the gonadotroph cells of the anterior pituitary, sensitizing the cells to GnRH and stimulating the release of a bolus of LH after plasma estradiol concentrations exceed a threshold for at least 36 hours [9, 11, 13, 15]. Therefore, the LH surge typically occurs 24-36 hours after attainment of peak estradiol secretion and lasts for approximately 24-48 hours [13]. In turn, the LH surge prompts proteolytic enzymes to digest the follicular wall, allowing the release of the oocyte from the dominant follicle and initiating ovulation, the event that separates the follicular and luteal phases [13]. Under the influence of LH, luteinization of the erupted follicle occurs, resulting in the formation of a corpus luteum, consisting of theca-lutein and granulosa-lutein cells [13]. These luteinized cells produce progesterone and, to a lesser extent, estradiol, which inhibit both the release of gonadotropins from the anterior pituitary and subsequent folliculogenesis [13]. In the absence of pregnancy-induced concentrations of human
chorionic gonadotropin (hCG), the corpus luteum degenerates forming the corpus albicans and progesterone production declines at the end of the luteal phase, thereby removing the negative feedback on the anterior pituitary [13]. FSH concentrations begin to increase again, recruiting another cohort of follicles for the subsequent cycle [13].

Therefore, in sum, a normal menstrual cycle demonstrates slowly increasing concentrations of FSH during the luteal-follicular transition and early follicular phase. Rising estradiol concentrations during the follicular phase exert negative feedback on the anterior pituitary, resulting in no further increases of FSH and LH during the mid-follicular phase [9, 13, 15]. The peak in estradiol concentration mid-cycle triggers the LH surge, leading to ovulation and commencement of the luteal phase. The luteal phase is characterized by rising progesterone concentrations that decline near the end of the cycle as the corpus luteum degenerates (Figure 2.1.1).

Within the uterus, the proliferative and secretory phases of the uterine cycle coincide with the follicular and luteal phases of the ovarian cycle. The proliferative phase involves a rebuilding of the functional layer of the endometrium after it has been shed during menses [16]. The cells of the zona basalis, i.e. basal stromal cells, proliferate in response to rising concentrations of estradiol from the developing follicles [13]. During the late proliferative phase, hyperplasia of endometrial cells results in thickening of the endometrial wall, such that the endometrium may increase in thickness from 0.5 mm to 5 mm [13]. Stimulated by progesterone from the corpus luteum, the secretory phase involves the glandular secretion of glycogen and increased vascularization to support the implantation of an embryo in the event that fertilization occurred [13, 16]. Decidual cells formed from stromal cells produce secretions in concert with the
endometrial glands and create the *zona compacta*, the dense layer of upper endometrial cells [13]. The *zona spongiosa*, i.e. the mid-layer of epithelial cells which consists of prominent endometrial glands, also becomes apparent during this phase [13]. In the absence of fertilization, progesterone and estrogen concentrations decline and the endometrium is deprived of hormonal support, causing the spiral arteries to constrict and destruction of the functional layer of the endometrium, i.e. the *zona compacta* and the *zona spongiosa* [13]. Eventually, as the upper two-thirds of the endometrium degenerates, the arteries relax, and menses begins [16]. As such, the menstrual phase of the uterine cycle is characterized by the loss of the functional layer of the endometrium as a result of ischemia and necrosis of endometrial tissues [13]. The innermost layer of the endometrium, the *zona basalis*, is all that remains at the end of the menstrual phase [13].

Thus, it is evident that both the ovarian and uterine cycles rely on proper functioning of the HPO axis and, subsequently, adequate hormonal concentrations, for normal menstrual function. Among exercising women with reproductive disturbances, the metabolic environment alters the HPO axis, leading to disruptions in the menstrual cycle that affect both the ovarian and uterine cycles and, in turn, influence reproductive potential.

**Types of EAMD**

EAMD occur along a spectrum ranging from mild to severe (Figure 2.1.3). The least severe presentations of menstrual dysfunction include subtle menstrual disturbances, also known as subclinical menstrual disturbances, that occur without a change in cycle length and are therefore frequently undetected; these subtle menstrual disturbances include LPD and anovulation. Severe menstrual disturbances, also known as clinical
menstrual disturbances, exist at the pathological endpoint of the continuum and are characterized by long intermenstrual intervals (oligomenorrhea) or the absence of menstruation for more than 90 days which is referred to clinically as functional hypothalamic amenorrhea (FHA).

Subtle menstrual disturbances: LPD. LPD are characterized by adequate ovulatory function despite poor implantation and poor endometrial quality [1, 17, 18]. More specifically, LPD cycles are characterized by ovulatory cycles with normal and repeatable inter-menstrual intervals but luteal phase dysfunction; they are defined by a short luteal phase length of <10 days and/or inadequate progesterone production during the luteal phase [17, 19] (Figure 2.1.4a). It has been suggested that a critical 3- or 5-day sum of mid-luteal progesterone concentrations can be used to identify inadequate progesterone exposure associated with LPD [19, 20]. As such, previous reports have used either a urinary pregnanediol glucuronide (PdG) peak of <5 μg/ml or the sum of a three-day mid-luteal PdG peak of <10 μg/ml as indicators of an inadequate luteal progesterone production [5, 20]. Typically, women with LPD demonstrate a prolonged follicular phase in concert with the shortened luteal phase; thus for example, an
individual with a 28-day cycle and a 7-day luteal phase will have a 21-day follicular phase with an LH peak occurring on day 21 compared to women with normal ovulatory cycles in whom the LH peak and presumably ovulation occurs mid-cycle (day 12-14) for a 28-day cycle [1, 19].

The etiology of LPD has been proposed to be impaired folliculogenesis and oocyte maturation that results from disruptions of the reproductive axis [1, 17, 19]. Estrogen exposure during the follicular phase is suppressed among LPD cycles of exercising women compared to ovulatory cycles with normal luteal function [6]. Likewise, there is a delayed rise in FSH concentrations during the end of the preceding luteal phase, often referred to as the luteal-follicular transition, a critical time period for successful follicle recruitment, among LPD cycles of exercising women [6]. A reduction in the concentration of the LH peak has also been reported in LPD cycles [5, 21]. Each of these hormonal alterations may contribute to abnormal function of the corpus luteum and, subsequently, suppressed progesterone concentrations [5, 6, 17, 21].

The determination of LPD in exercising women relies on the measurement of mid-cycle LH and daily progesterone concentrations in the luteal phase via daily urine or a timed serum sample during a single cycle; however, the monitoring of multiple consecutive cycles is advised for detection of LPD due to the inconsistency with which LPD cycles are observed in exercising women [5, 6]. For example, women may present with a normal, ovulatory cycle one month followed by an LPD or anovulatory cycle the next month. Inconsistent presentations of LPD and anovulation during consecutive cycles may also occur in the same individual. In fact, it has been reported that almost half (46%) of exercising women present with inconsistent menstrual status; therefore,
monitoring only one cycle may underestimate the incidence of menstrual disturbances among exercising women by 38% [6]. Procedures for daily urine or serum sampling are costly and often not feasible; therefore, the detection of LPD in exercising women is difficult and the majority of women with LPD are often unaware of the presence of this subclinical menstrual perturbation. Notably, self-report and/or assessment of menstrual history alone will not detect LPD, thereby further contributing to the underestimation of the prevalence of menstrual disturbances among exercising women.

Figure 2.1.4. Profile of daily urinary excretion of reproductive hormones for subtle menstrual disturbances. a) Representative menstrual cycle with a short and inadequate luteal phase defect. Classic characteristics include a luteal phase <10 days in length and suppressed progesterone production during the luteal phase. b) Representative anovulatory menstrual cycle. Classic characteristics include the lack of a mid-cycle E1G peak and LH surge and the failure of progesterone to rise during the latter part of the cycle, indicating the absence of ovulation. E1G: estrone-1-glucuronide; PdG: pregnanediol glucuronide; LH: luteinizing hormone.
Subtle menstrual disturbances: Anovulation. Anovulation represents a subtle menstrual disturbance that is more severe than LPD. The hallmark characteristic of anovulation is the failure of follicular estrogen to rise concomitant with the lack of the mid-cycle LH surge and the subsequent failure to ovulate [5] (Figure 2.1.4b). Similar to women with LPD, women with anovulatory cycles are, for the most part, experiencing regular inter-menstrual intervals making the identification of anovulation difficult. Due to the absence of ovulation and, consequently, the failure to produce a corpus luteum, progesterone concentrations do not increase during the latter part of the cycle. Therefore, anovulation has also been defined as the lack of an increase in urinary PdG from a 5-day follicular phase baseline or a peak PdG value <2.49 µg/ml [5]. Both estrogen and progesterone concentrations have been reported to be lower in anovulatory cycles of exercising women compared to ovulatory cycles of exercising women, suggesting that disruptions in FSH and LH pulsatility contribute to anovulation [5, 6]. Adequate estrogen concentrations, however, allow for degeneration of the functional layer of the endometrium upon withdrawal of hormonal support at the end of the luteal phase, thereby resulting in normal menses [13].

Severe menstrual disturbances: Oligomenorrhea. Oligomenorrhea represents cycles with long and inconsistent inter-menstrual intervals of 36-90 days that are often accompanied by 3-6 menses events per year [1, 22, 23] (Figure 2.1.5a). This severe menstrual disturbance is perhaps the least understood and most difficult perturbation to interpret due to its inconsistent hormonal characteristics. An oligomenorrheic cycle may be ovulatory or anovulatory, and estrogen concentrations often produce erratic profiles during the extended cycle as follicles seek dominance [1]. The etiology of
Oligomenorrhea in exercising women may or may not be hypothalamic in nature [22]. Oligomenorrhea can be associated with prolactin-secreting tumors, thyroidtoxicosis and other endocrinopathies, but most often, oligomenorrhea is associated with hyperandrogenism [22, 24-27]. Hyperandrogenism is often secondary to polycystic ovarian syndrome (PCOS) [28], which is causally linked to infertility in women [29]. In exercising women, oligomenorrhea has been often associated with hyperandrogenism, but may also occur secondary to an energy deficit. Investigators have observed hyperandrogenism concomitant with elevated LH/FSH ratio and free androgen index, two additional markers of PCOS, among athletes with menstrual dysfunction [24-26, 30]. Rickenlund et al. [24] identified that a distinct group of athletes with menstrual dysfunction presented with hyperandrogenism, and upon comparison of the oligo-amenorrheic athletes with hyperandrogenemia (H-OAM) to oligo-amenorrheic athletes with normal androgen profiles (N-OAM), the H-OAM group demonstrated a higher LH/FSH ratio than the N-OAM group, indicating that the profile of reproductive hormones differed between the two groups. Of interest, however, is that circulating concentrations of triiodothyronine (TT3), a marker of energy deficiency, were significantly lower in both the H-OAM and N-OAM groups compared to a control group of sedentary women, suggesting that both groups may have been in an energy-deficient state [24]. On the other hand, when assessing athletes based on type of menstrual disturbance, Rickenlund et al. [25] observed that 24-hour diurnal secretion of testosterone was significantly elevated among oligomenorrheic athletes compared to amenorrheic and regularly-menstruating athletes. In addition, amenorrheic athletes demonstrated reduced LH pulsatility, a surrogate marker of GnRH inhibition at the hypothalamus, compared to
regularly-menstruating controls; whereas, oligomenorrheic athletes demonstrated an LH pulse pattern similar to that observed in regularly-menstruating controls [25]. Therefore, oligomenorrheic athletes did not display the normal hormonal pattern typical of hypothalamic inhibition due to an energy deficiency as was observed in amenorrheic athletes, suggesting that other factors such as hyperandrogenism could be a mechanism underlying oligomenorrhea in athletes. As such, the etiology of oligomenorrhea among exercising women with hyperandrogenemia is ambiguous, thereby complicating the treatment of menstrual dysfunction among this subgroup of exercising women. Careful screening of oligomenorrheic exercising women is necessary to determine if the long, inconsistent cycles are due to an energy deficit or PCOS [22].

Severe menstrual disturbances: FHA. At the extreme end of the menstrual disturbance continuum is FHA, the most severe menstrual disturbance that is associated with severe estrogen deficiency and typically defined as the absence of menses for at least 90 days [1, 23], although definitions have varied [23, 31]. FHA is typically classified as either primary or secondary in nature [27]. Primary amenorrhea is defined as the failure to menstruate by 15 years of age in girls with secondary sex characteristics [27]; whereas, secondary amenorrhea is the abnormal cessation of the menstrual cycle after menarche [27]. FHA among exercising women refers to menstrual dysfunction that is caused by disruptions in the hypothalamus due to energy conservation and is unrelated to other causes of FHA associated with the four-compartment model [27, 32]. Exercising women with FHA present with chronically suppressed estrogen and progesterone concentrations [5, 33, 34] (Figure 2.1.5b), most likely as a result of impaired GnRH, LH, and FSH pulsatility that are therefore inadequate to stimulate
ovulation from the ovary as well as appropriate proliferation and removal of the functional layer of the endometrium. As such, the ovaries and uterus of amenorrheic women are largely quiescent with minimal production of reproductive hormones. FHA is associated with the most severe clinical sequelae such as low bone mineral density (BMD) [35, 36], poor bone quality [37], and cardiovascular consequences to include a poor lipid profile and endothelial dysfunction [38-40].

**Figure 2.1.5.** Profile of daily urinary excretion of reproductive hormones for severe menstrual disturbances. a) Representative oligomenorrheic, anovulatory menstrual cycle. Classic characteristics include a cycle 36-90 days in length and an erratic hormonal profile. b) Representative amenorrheic 28-day monitoring period. Classic characteristics include chronic suppression of E1G and PdG. E1G: estrone-1-glucuronide; PdG: pregnanediol glucuronide; LH: luteinizing hormone.
Prevalence of EAMD

The prevalence of menstrual disturbances among exercising women has been reported to range from 0-60%, a large range that encompasses the prevalence of both subtle and severe menstrual disturbances [41]. The range in prevalence rates in exercising women is large because of the variations in definitions used and methods of assessment in exercising women [5, 6, 42-53]. The prevalence estimates, however, frequently exceed that observed in the general population of non-athletic women that is as low as 3-5% [5, 54-56].

Prevalence of subtle menstrual disturbances. Due to the burdensome nature of investigating the presence of subtle menstrual disturbances, only a few investigators have reported their prevalence among exercising women [5, 6, 42-44] despite LPD and anovulation together representing the most common menstrual disturbances linked to exercise training [5, 6]. The prevalence of subtle menstrual disturbances is alarmingly high given that these disturbances are masked by regular inter-menstrual intervals. Prevalence estimates range from 5.9-43.0% [5, 6, 42, 44] and 12.0-30.0% [5, 6, 44] for LPD and anovulation, respectively. Indeed, the ideal method of identifying subtle menstrual disturbances requires the measurement of daily urinary excretion of reproductive hormones over multiple consecutive cycles. Based on reports from our lab which has undertaken the task of assessing multiple cycles among exercising women, we observed that 27% and 25% of exercising women with self-reported eumenorrheic cycles (i.e. 26-35 days in length) presented with a luteal phase defect or anovulatory cycle, respectively [5]. Therefore, over half of exercising women presented with a subtle menstrual disturbance, compared to only 5% of sedentary women (Figure 2.1.6).
Similarly, upon evaluation of individual menstrual cycles among exercising women monitored for 1-3 menstrual cycles, 21% and 29% of the cycles demonstrated evidence of a luteal phase defect and anovulation, respectively, representing 50% of the 120 cycles assessed in exercising women [5]. Only 4% of the cycles of sedentary women had a subtle menstrual disturbance, all of which were characterized by a luteal phase defect [5] (Figure 2.1.7). Therefore, these results clearly demonstrate the high prevalence with which these largely under-diagnosed menstrual disturbances occur, most often indicative
of an energy-deficient state and other underlying health concerns. The strength of our study lies in the daily urinary assessment of reproductive hormones, which provides a complete picture of the hormonal fluctuation throughout the cycle. Therefore, this methodology allows for a more accurate estimate of the prevalence of EAMD than can be obtained from relying solely on self-report measures that often underestimate EAMD prevalence. The frequency with which these “hidden” menstrual disturbances present in exercising women is cause for concern due to the negative impact of an energy deficit and menstrual disturbances on health outcomes and lack of symptomatic indicators that such disturbances are present.

Figure 2.1.7. Prevalence of subtle menstrual disturbances among individual cycles of sedentary and exercising women. a) Demonstrates the proportion of cycles displaying subtle menstrual disturbances among sedentary women. b) Illustrates the proportion of cycles displaying subtle menstrual disturbances among exercising women. Exercising women presented with significantly more anovulatory and luteal phase defect cycles and fewer ovulatory cycles compared to the sedentary women. Cycles of exercising women compared to cycles of sedentary women: ‡ indicates p<0.050; § indicates p<0.001. Reprinted with permission from De Souza et al. [5].
Prevalence of severe menstrual disturbances. Several investigators have evaluated the prevalence of the severe menstrual disturbances (FHA and oligomenorrhea) in female athletes, to include both high school [48, 57-59] and adult women [5, 6, 45-47, 60-65]. The earliest prevalence estimates of clinical menstrual disturbances were evaluated in long-distance runners [49-53, 66], dancers [67, 68], and gymnasts [69], and in general, severe menstrual disturbances are documented at much higher rates in premenopausal exercising women than in sedentary women [54-56]. Based on these reports in female athletes and exercising women, the prevalence of primary and secondary amenorrhea ranged from 0-56.0% (determined in 13 studies) [41] and 1-60.0% (determined in 35 studies) [41], respectively; whereas, the range in prevalence of oligomenorrhea was 0.9-52.5% (determined in 23 studies) [41]. In a recent report that assessed menstrual status in recreationally active women based on daily urinary steroid excretion, investigators observed that 7% of exercising women presented with oligomenorrhea; whereas, 37% were amenorrheic [5]. No sedentary women in the sample, however, presented with either oligomenorrhea or amenorrhea [5]. Therefore, menstrual disturbances among exercising women are relatively frequent, highlighting the need for awareness of the problem and its associated consequences among physically active girls and women in an effort to promote healthy exercise habits.

Changes in HPO Activity associated with EAMD

Chronic energy deficiency targets the pulsatile secretion of GnRH from the arcuate nucleus of the hypothalamus. Disruptions in GnRH pulsatility often lead to changes in the frequency and amplitude of LH and FSH pulses, longer cycle length (particularly a longer follicular phase), reductions in average and peak luteal phase
progesterone concentrations, and suppressed estradiol and progesterone [70]. It is believed that GnRH pulsatility, as governed by the pulse generator located in the hypothalamus [8], is sensitive to changes in the metabolic environment that are characteristic of an energy deficiency [7, 71]. (For a detailed description of metabolic adaptations that affect reproductive function, please refer to Chapter 12 of the book *Exercise and Human Reproduction - Induced Fertility Disorders and Possible Therapies*, 2014). Thus, an energy deficit disrupts GnRH pulsatility, beginning the cascade of alterations in FSH and LH secretion, estrogen and progesterone production, and ultimately reproductive dysfunction.

**Effects of Energy Deficiency on LH Secretion.** One of the characteristics of the HPO axis in response to an energy deficit that is created either by energy intake restriction or increased energy expenditure or both is disruption of LH pulsatility. In an environment of chronic energy deficiency, a reduction in LH frequency has been observed [72, 73]. Loucks et al. [72] compared LH secretory dynamics among regularly-menstruating athletes (CA), amenorrheic athletes (AA), and regularly-menstruating sedentary women (CS). The frequency of LH pulses was significantly lower in the AA group compared to both CA and CS groups [72]. In addition, relative quiescence of 24-hour LH pulsatility was observed in AA, as evidenced by no significant changes in the wake and sleep values of LH in AA compared to the slowing of LH pulse frequency and increase of pulse amplitude that were observed during the sleep hours vs. awake hours in both CA and CS women [72]. Likewise, in a similarly-designed study, Veldhuis et al. [73] also observed decreased LH pulse frequency among amenorrheic/oligomenorrheic athletes when compared to sedentary, regularly-
menstruating control women. In response to GnRH administration, however, the amenorrheic/oligomenorrheic athletes demonstrated greater LH secretion than the control women [73], a result that was replicated by Loucks et al. [72]. These results suggest that the reduced LH pulsatility among amenorrheic athletes is likely due to metabolically-induced alterations in GnRH pulsatility rather than diminished pituitary responsiveness to GnRH [72, 73].

Causal evidence of the effect of a chronic energy deficiency derived from either dietary energy restriction or increased energy expenditure or both on LH pulsatility was demonstrated by Scheid et al. [74] in a longitudinal model and by Loucks et al. [75] and Williams et al. [76] in an acute model. Scheid et al. [74] exposed previously sedentary women to a 3-month longitudinal model of energy deficiency that consisted of dietary restriction (-30 to -60% of baseline energy needs) combined with regular aerobic exercise (70-80% of maximum heart rate) performed 5 days per week. Before and after the 3-month intervention, women underwent 24-hour repeated blood sampling to determine the impact of a long-term energy deficiency on LH pulsatility [74]. After completion of the intervention, a significant decline in 24-hour LH pulse frequency was observed among the women exposed to the energy deficit; whereas, women in a control group who neither exercised nor restricted dietary intake demonstrated no change in LH pulse characteristics [74]. This decrease in LH pulse frequency is evident in Figure 2.1.8 which depicts individual 24-hour profiles of LH pulsatility pre- and post-intervention.
Figure 2.1.8. Examples of individual 24-hour profiles of LH pulsatility before (pre) and after (post) a 3-month intervention consisting of dietary restriction and aerobic exercise. a) Subject #1 was in the Control group, lost 0.1 kg body weight, and increased LH pulse frequency by 0.02 pulses/hour. b) Subject #2 was in the Energy Deficit group, lost 3.3 kg body weight, and decreased LH pulse frequency by 0.75 pulses/hour. c) Subject #3 was in the Energy Deficit group, lost 6.3 kg body weight, and decreased LH pulse frequency by 0.89 pulses/hour. *represents LH pulse determined using cluster analysis software. LH: luteinizing hormone. Reprinted with permission from Scheid et al. [74].
An acute energy deficiency has also been demonstrated to impact LH pulsatility [75, 76]. In a classic study, the induction of low energy availability (≤20 kcal/kg LBM/day) for 5 days in sedentary, regularly-menstruating women resulted in suppressed LH pulse frequency and increased LH pulse amplitude compared to a replete energy availability (45 kcal/kg LBM/day) [75]. Similarly, restriction of energy intake by 60% for 7 days concomitant with a short-term increase in exercise training volume for 3 days in premenopausal women resulted in a significant decrease in LH pulse frequency compared to an experimental condition characterized by a 3-day increase in exercise volume with 7 days of eucaloric intake [76]. In fact, LH pulsatility appears to rapidly respond, even within minutes, to shifts in energy availability in the animal model [7]. Taken together, these observations provide evidence that both chronic and acute energy deficits driven either by dietary energy restriction or increased energy expenditure or both lead to disruption of LH secretory patterns, likely due to energetically-driven alterations in GnRH pulsatility.

Energy Deficiency and FSH Secretion. Although it is well-established that energy deficiency alters LH pulsatility, likely via disruptions in GnRH pulsatility, the role of FSH in menstrual disturbances has not been well characterized [75]. It appears, however, that FSH secretion is indeed impacted by an energy deficiency and contributes to menstrual disturbances. Among exercising women with LPD and a low energy availability, De Souza et al. [6] observed significantly lower FSH concentrations during the luteal-follicular transition (last 5 days of the cycles) compared to cycles of sedentary ovulatory women, suggesting that a decline in FSH concentrations may contribute to the suppressed ovarian function that is observed among women with EAMD. This decrease
in FSH secretion during the luteal-follicular transition is believed to impact follicular
recruitment and maturation, thereby contributing to the low estrogen concentrations that
were also observed in this group of exercising women during days 6-12 of the cycle [6].
However, the imposition of low energy availability for 5 days among regularly-
menstruating women did not result in changes in FSH concentrations despite changes in
LH pulsatility and estrogen concentrations compared to an adequate energy availability
[75]. These findings suggest that FSH may respond differently to acute and chronic
energy deficits.

*Energy Deficiency and Ovarian Hormone Production.* Disruptions in LH and
FSH secretion, in turn, lead to suppression of estrogen and progesterone production from
the ovaries, a hallmark characteristic of EAMD. Reductions in FSH during the luteal-
follicular transition may cause delayed follicular maturation and thus a decrease in
estrogen production from the developing follicles during the follicular phase.
Anovulatory cycles of exercising women demonstrated significantly lower estrogen
excretion and area under the curve during the follicular phase compared to ovulatory
cycles of both sedentary and exercising women [5]. Likewise, mean estrogen
concentration and estrogen exposure during a 28-day monitoring period among
amenorrheic exercising women has been observed to be significantly lower than that
observed during a monitored cycle of exercising ovulatory women [34]. Disruption of
LH pulsatility and the LH surge may contribute to lower progesterone production as has
been demonstrated in LPD, anovulatory, and amenorrheic cycles of exercising women
compared to ovulatory cycles of sedentary and exercising women [5, 34]. As such,
urinary and serum measurements of ovarian hormone concentrations in exercising
women with menstrual disturbances indicating a chronic energy deficit or regularly-menstruating women with an acute energy deficit have revealed declines in estrogen and progesterone concentrations [5, 33, 34, 75, 77]. The suppression in ovarian hormone concentrations varies with the severity of the menstrual disturbance, with the degree of suppression increasing from the least severe menstrual disturbance of LPD to the most severe menstrual disturbance of amenorrhea [5]. In fact, a characteristic hormonal profile of amenorrhea is a chronic suppression of estrogen and progesterone, with the normal peaks of these ovarian hormones notably absent (Figure 2.1.5b). Therefore, taken together, menstrual dysfunction associated with exercise, of which the underlying etiology is an energy deficit, is the result of a sequence of reproductive hormone changes, beginning in the hypothalamus and affecting each level of the HPO axis.

**Stress Hypothesis vs. Energy Availability Hypothesis**

Although exercising women frequently present with menstrual dysfunction, it is imperative to highlight that it is not the exercise per se that leads to menstrual dysfunction and the cascade of subsequent health consequences; rather, it is the energy deficiency caused by inadequate caloric intake to compensate for energy expenditure that leads to the altered metabolic and hormonal environment typical of women with FHA. The “exercise stress hypothesis” postulates that the stress of exercise, defined as everything related to exercise except the energy cost, upregulates the hypothalamic-pituitary-adrenal (HPA) axis, disrupting reproductive function at the level of the hypothalamus by altering GnRH secretion [78]. On the other hand, the “energy availability hypothesis” suggests that markers of energy status alter reproductive function by influencing the GnRH pulse generator and subsequently LH pulsatility [78] (please
refer to Chapter 12 for a detailed description of the metabolic markers that alter reproductive function).

These hypotheses were tested by Loucks et al. [78] using a carefully designed experimental protocol that included both exercising and non-exercising treatment groups among sedentary women. For four days on two separate occasions, the exercising treatment group engaged in 30 kcal/kg LBM/day of exercise and were either provided with 1) a 75 kcal/kg LBM/day diet to set energy availability (EA) at 45 kcal/kg LBM/day (balanced) or 2) a diet of 40 kcal/kg LBM/day to set EA at 10 kcal/kg LBM/day (deprived). The non-exercising treatment group did not expend energy via exercise but were provided with either 1) a diet of 45 kcal/kg LBM/day (balanced) or 2) a diet of 10 kcal/kg LBM/day (deprived). It was previously demonstrated that an EA of 30 kcal/kg LBM/day was adequate to maintain optimal TT3 concentrations and LH pulsatility [75, 79]; whereas, at an EA below 25-30 kcal/kg LBM/day, a cascade of negative metabolic and hormonal adaptations occurred, indicating an energy deficient state [75, 79, 80]. In the exercise treatment group, the deprived condition demonstrated a significant reduction in TT3 concentrations, a hormone that is a key marker of energy status and is typically suppressed among amenorrheic exercising women [79], as well as a reduction in LH pulse frequency compared to the balanced condition [78]. Within in the non-exercise treatment, these results were mimicked with the exception of LH pulse frequency which showed a much larger reduction in the deprived condition. However, upon comparison of the exercise treatment group with the non-exercise treatment group, the stress of exercise did not suppress TT3 concentrations or LH pulse frequency [78, 79]. Taken together, these results indicate that exercise itself, apart from its energy cost, does not
disrupt the reproductive axis. On the contrary, alterations in LH pulsatility that translate to EAMD are due to the energetic cost of exercise, in particular, when energy intake is inadequate for energy expenditure, creating an energy deficit.

These findings have been supported by studies in monkeys that demonstrated that the induction of amenorrhea was associated with an increase in exercise energy expenditure that was not combined with compensatory increases in energy intake, thus creating an energy deficit [81]. Among eight monkeys that began exercise training (progressive increase to 12.3±0.9 km/d of running) without changes in energy intake, each monkey developed amenorrhea (defined as absence of menses for at least 100 days) 7-24 months into the intervention, coinciding with suppressed estrogen, progesterone, LH and FSH concentrations [81]. Four of the monkeys were then fed supplemental calories (138-181% of energy intake during amenorrhea) without changes in exercise training [70]. These monkeys resumed normal menstrual cycles in response to the adequate energy intake, corresponding with increases in LH, FSH and estrogen during the follicular phase and increases in progesterone during the luteal phase [70]. Accordingly, the recovery of menses correlated with energy intake during refeeding and an increase in TT3 [70]. As such, these results provide further support for the hypothesis that energy availability rather than the stress of exercise is primarily responsible for regulating the HPO axis in exercising women.

Reproductive Potential and Clinical Relevance

In terms of reproductive and clinical significance, the impact of menstrual dysfunction at any point along the continuum in exercising women has a profound impact on fertility. The acute effects of FHA on reproductive potential are clear in that the
complete absence of the menstrual cycle precludes pregnancy. Likewise, the inconsistent presentation of oligomenorrhea with varied cycle lengths and the occurrence of both ovulatory and anovulatory cycles may create an unstable environment for both unwanted and wanted conception due to the uncertain status of the cycle at any given time. LPD are a unique menstrual disturbance in that, unlike the other disturbances, the irregular cycles are masked in the presence of regular length and ovulation. However, LPD are associated with infertility and spontaneous abortion due to the inadequate progesterone environment [17]; the low progesterone production inhibits proper maturation of the endometrium, leading to poor endometrial quality that cannot support blastocyst implantation and causes embryonic loss [18-20, 82]. Therefore, in sum, women with LPD associated with inadequate progesterone production often experience disruptions in follicular growth, suppressed oocyte maturation, and endometrial dysfunction, which may lead to compromised fecundity, spontaneous abortion, and infertility [17, 19, 20].

An important consideration for reproductive potential among exercising women, particularly those presenting with apparently regular menstrual cycles, is the consistency at which ovulatory and abnormal cycles occur. A greater proportion of sedentary women (Sed) present with consistently ovulatory cycles compared to exercising women (Ex) (Sed: 95% vs. Ex: 32%) [5]; whereas, exercising women present with more consistently abnormal cycles (Ex: 32% vs. Sed: 0%) and more inconsistent cycles than sedentary women (Ex: 36% vs. Sed: 6%) [5]. These findings indicate that exercising women are more likely to experience abnormal cycles over the course of several consecutive cycles [5]. The inconsistent presentations of normal and abnormal cycles in exercising women introduce challenges for exercising women with respect to fertility.
Although the impact of EAMD on reproductive potential is unfavorable, consequences of EAMD on reproductive function appear to be acute and do not cause permanent damage [83, 84]. Upon recovery of an optimal energy status, exercising women with menstrual dysfunction can recover normal menstrual function [83-86], allowing proper follicular, ovarian, and endometrial function. It is believed that as the improvement in energy status progresses, menstrual disturbances may also move along the spectrum from the most severe (i.e. amenorrhea) to the least severe (i.e. LPD) before attaining eumenorrheic, ovulatory cycles [83][unpublished data], thus complete restoration of energy status is essential for optimal reproductive function. For example, an amenorrheic woman who resumes normal menses may still present with anovulatory or LPD cycles, identifying that a small degree of energy deficiency may still be present. Continued improvement in energy status would be necessary to prevent infertility and spontaneous abortion.

Effective non-pharmacological treatment strategies for EAMD include an increase in caloric intake and weight gain. Dueck et al. [85] and Kopp-Woodroffe et al. [86] described case studies of five amenorrheic recreationally-active women and female athletes who participated in a diet and training intervention to reverse amenorrhea. Caloric intake was increased by approximately 360 kcal/day and training was reduced by 1 day/week for 12 to 20 weeks, contributing to a weight gain of 1-3 kg [85, 86]. During the intervention, three of the five women resumed menses [85, 86]. Of the two that did not resume menstruation during the intervention, one woman maintained the increased caloric intake and resumed menses three months after completion of the intervention [85]; whereas, the other woman withdrew from the study to begin oral contraceptives, so
Other case reports in recreationally active women with amenorrhea have revealed that an increase in caloric intake of approximately 300 kcal and a weight gain of 3-4 kg contributes to resumption of menses [unpublished data]. Although the actual time to resumption of menses may vary for women depending on the severity of the energy deficiency and menstrual disturbance, the amount of weight that is typically gained leading to resumption of menses is not exorbitant, thereby alleviating some concerns that exercising women may have about weight gain. In addition, weight gain that leads to resumption of menses is also associated with improvement of other clinical sequelae characteristic of exercise-associated amenorrhea [87]. Miller et al. [87] reported that resumption of menses occurred in anorexic women who gained 4 kg of body mass, on average, and the combined effects of weight gain and resumption of menses contributed to significant improvements in lumbar spine and hip BMD. As such, relatively small increases in body mass can lead to a cascade of beneficial health outcomes among women with menstrual dysfunction associated with an energy deficiency.

**Conclusion**

Adolescent girls and premenopausal women engaging in regular exercise, to include both recreational and competitive physical activity, are at risk for developing a menstrual disturbance if energy intake is inadequate to compensate for energy expenditure, resulting in an energy deficit. EAMD include both clinical presentations, i.e. FHA and oligomenorrhea, and subclinical presentations, i.e. LPD and anovulation; therefore, awareness of the importance of adequate energy intake among exercising girls and women is essential. Each menstrual disturbance is linked to spontaneous abortion or
infertility, thereby having a profound effect on fecundity. However, with the maintenance of a replete energy state, EAMD that is commonly observed among exercising women can be avoided. Non-pharmacological strategies to prevent and reverse EAMD include consuming adequate kilocalories on a daily basis to support healthy body weight and menstrual function. Although energy intake requirements vary among individuals, regular estimations of daily energy expenditure and energy intake and subsequent calculations of energy balance (energy intake-energy expenditure) will promote maintenance of a positive energy balance that should ultimately result in favorable outcomes for both reproductive and overall health.
References


Loucks AB, Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab 2003;88: 297-311.


CHAPTER 2: PART 2

Mallinson RJ, Williams NI, Southmayd ES, and De Souza MJ. Insight into the bone strength of amenorrheic exercising women through the lens of stress fractures and anorexia nervosa.

Abstract

Low bone mineral density (BMD) is frequently observed among exercising women with functional hypothalamic amenorrhea (FHA) as a consequence of an energy deficiency. For the majority of reports describing the impact of energy deficiency on bone health, BMD measurements have been obtained from two-dimensional imaging techniques such as dual-energy x-ray absorptiometry (DXA). Due to its two-dimensional nature, DXA is only capable of providing areal BMD measurements and does not assess bone geometry, thereby providing an incomplete picture of bone strength. On the other hand, three-dimensional imaging techniques such as peripheral quantitative computed tomography (pQCT) provide measurements of volumetric BMD (vBMD), bone geometry, and in the case of high-resolution scanners, bone microarchitecture. Furthermore, pQCT is capable of separately assessing the cortical and trabecular compartments of bone. As such, pQCT is a useful tool for estimating bone strength and the underlying mechanisms of low bone mass in amenorrheic exercising women. Therefore, this paper reviews the current literature regarding vBMD, bone geometry and microarchitecture, and estimated bone strength in amenorrheic athletes, female athletes with stress fracture, and anorexic girls and women in an effort to gain further understanding of 1) the impact of energy deficiency on bone health in amenorrheic athletes and 2) characteristics of bone that may contribute to stress fracture risk among amenorrheic athletes. Both amenorrheic athletes
and anorexic adolescents or women present with decreased trabecular vBMD and a deterioration of the 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 also display reduced trabecular vBMD, suggesting that the amenorrheic athletes may be at increased risk for fracture due to the compromised density of trabecular bone.
**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 physiological adaptations include a decrease in resting metabolic rate [1] and altered concentrations of metabolic hormones, i.e., decreased triiodothyronine (T3) [1], leptin [2], and insulin-like growth factor-1 (IGF-1) [3], and elevated ghrelin [1, 2, 4] and peptide YY [5], all of which influence bone metabolism or bone mineral density (BMD) [6-11]. Perhaps a more overt symptom of an energy deficiency in exercising women and adolescent girls is menstrual dysfunction, such as functional hypothalamic amenorrhea (FHA) [1], which is often accompanied by poor bone health that is characterized by changes in bone size and shape [3], decreased density [3-6], and a greater fracture risk [7]. To date, the relation between FHA and bone mineral density (BMD) in exercising women and adolescent girls with amenorrhea has been extensively investigated using two-dimensional 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 [3, 10, 12-16]. However, three-dimensional 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 three-dimensional 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 [17]. High resolution pQCT (HR-pQCT), flat panel volume CT, and finite element analysis (FEA) are yet 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, three-dimensional 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) [18, 19], which are key components of bone strength, in exercising amenorrheic women and adolescents.

To date, the literature available that has described 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, this paper reviews the current literature regarding vBMD, bone geometry and microarchitecture, and estimated bone strength in amenorrheic athletes in an effort to gain further understanding of bone health in this population that is at risk for low bone mass and fractures. In addition, this paper also serves to review vBMD, bone geometry and microarchitecture, and estimated bone strength in exercising women with stress fractures as well as adolescent girls or women with anorexia nervosa, 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 amenorrheic exercising women.

**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 anorexia nervosa 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 is 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 the tibia.
Currently, five papers have been published that describe vBMD, bone geometry and estimated bone strength in amenorrheic athletes [20-24]. The low aBMD that has been observed among amenorrheic athletes when using DPA and DXA [3, 10, 12-16] is consistent with the results of studies assessing vBMD, bone geometry, and estimated bone strength among a similar population of women and girls [20-24]. 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 [20-24]. 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 [20-22]. Ducher et al. [20] 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 to the retired gymnasts. 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 [20]. At the proximal radius, cortical thickness 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 in cortical thickness between the gymnasts without a history of amenorrhea and control subjects [20]. Ackerman et al. [21, 22] reported slightly different findings in adolescent athletes with amenorrhea, perhaps due to
the differences in sport type between the osteogenic activity of gymnastics [20] and the low-impact loading of running [21, 22] as well as the age difference between populations (i.e., adolescent vs. adult); however, the compromised bone health remained evident in the amenorrheic athletes. At the ultradistal radius, trabecular vBMD was 16% and 12% lower among amenorrheic athletes compared to non-athletic controls and eumenorrheic athletes, respectively, after adjusting for height [21]. Unadjusted results revealed decreased total vBMD at the distal radius and cortical vBMD at the distal tibia by 15% and 3.5%, respectively, among amenorrheic athletes compared to non-athletic controls [21], which coincided with a lower cortical area/total area ratio and a greater cortical porosity at the distal tibia [22]. Moreover, the amenorrheic athletes had significantly lower trabecular number and significantly greater trabecular separation at the distal tibia when compared to both eumenorrheic adolescent athletes and non-athletic controls, indicating the deterioration of the trabecular microarchitecture [21].
Table 2.2.1. Summary of pQCT results from studies assessing vBMD, bone geometry and microarchitecture, and estimated bone strength in amenorrheic athletes

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Tool</th>
<th>Sites</th>
<th>Significant Results</th>
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<tr>
<td>Ackerman et al., 2011 [21]*</td>
<td>16 AA 18 EA 16 NAC</td>
<td>HR-pQCT XtremeCT</td>
<td>Distal Radius</td>
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<td>HR-pQCT</td>
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<td>Ackerman et al., 2012 [22]</td>
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<td>Ducher et al., 2009 [20]**</td>
<td>12 AA 12 EA 26 NAC</td>
<td>pQCT Stratec XCT 3000</td>
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<td>Ruffing et al., 2007 [24]</td>
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<td>pQCT Stratec XCT 2000</td>
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<td>To et al., 2005 [23]</td>
<td>7 AO 28 EA 35 NAC</td>
<td>HR-pQCT Deniscan 2000</td>
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<td>HR-pQCT</td>
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*a p<0.05 AA vs. NAC; b p<0.05 AA vs. EA; c p<0.05 EA vs. NAC; d 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 military recruits/dancers; 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
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 [25]. 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 amenorrheic athletes [3, 10] and the low trabecular vBMD observed at these peripheral distal sites [20, 21]. Hypoestrogenism in amenorrheic athletes is confirmed by detailed reports from our lab of estrogen exposure (area under the curve) over a 28-day period; among amenorrheic athletes, 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 [26]. Figure 2.2.1 depicts estrogen exposure in amenorrheic versus ovulatory athletes.
Figure 2.2.1. 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. Source: Mallinson et al. [26].
Interestingly, however, it has also been observed that adolescent amenorrheic athletes and retired athletes with a history of amenorrhea have a greater total bone area at the tibia and radius compared to non-athletic controls [20, 21], 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. [20] 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 amenorrheic athletes 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 cortical thickness, as is often observed in menstruating female athletes [27, 28], Ducher et al. [20] reported that previously-amenorrheic retired athletes gymnasts presented with thinner cortices at the proximal radius compared to eumenorrheic retired gymnasts (p<0.09) and non-athletic controls (p<0.01). Reduced cortical thickness at the distal tibia was also observed by Ruffing et al. [24] 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 [29-32]. Thus, bones that are subjected to loading or muscle forces typically increase in size [31, 32]. A bone that has its mass distributed farther from the center of the bone is typically a stronger bone [33]; thus, habitual exercise is beneficial for bone health by causing an increase in the size of the bone and, therefore, the strength of bone [31, 32]. 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 cortical thickness [34, 35]. 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 cortical shell 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 [36, 37]. On the other hand, estrogen is also extremely important for optimal bone health due to its role in inhibiting osteoclast action and bone resorption, particularly at trabecular sites that are characterized by rapid turnover [38]. 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 osteoclast action, as evidenced by a greater total 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 [39]. These factors can be used to calculate estimates of bone strength such as the bone strength index (BSI) and the strength strain index (SSI) [20]. 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):

\[ \text{BSI} = \text{ToA} \times \text{ToD}^2 \]

[20] and estimates the strength of the bone against compressive forces. Ducher et al.[20] reported on this parameter, finding that BSI was 17% lower at the distal radius in retired gymnasts with a history of amenorrhea compared to retired gymnasts.
without a history of amenorrhea. In addition, in a study of adolescent dancers, To et al. [23] 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: $SSI = Z \times (CoD/CoD_{\text{max}})$ [20]. Ducher et al. [20] 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 regions of the bone. 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. [20] and Ackerman et al. [21], 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 amenorrheic athletes represent a variety of ages, sport types, and life stages at which amenorrhea occurred. 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. [20] participated in gymnastics during childhood and adolescence. Gymnastics is a very osteogenic sport due to both the magnitude and heterogeneity of the loading forces. 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. [21, 22], however, represented both adolescents and young adults 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 particular type of loading modality. As such, exercise-associated amenorrhea may have different effects on bone health depending on the developmental stage during which the menstrual dysfunction occurs and the activity is performed.

However, the bones of amenorrheic athletes 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 [40] and thin cortical shells [41] 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 [42]. The energy-deficient environment often observed among amenorrheic athletes results in metabolic alterations such as suppressed IGF-1 and leptin and elevated peptide YY and ghrelin [2, 3, 5] that may negatively affect bone formation and negate potential positive changes in bone structure and density that occur with chronic exercise training [3, 8, 10]. 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 in bioavailable IGF-1 that was observed among the recruits who did not fracture [43]. These results suggest that a decrease in IGF-1 as is observed in an energy-deficient state often characterized by amenorrhea [1, 3] may be associated with increased fracture risk. In addition, energy deficiency has been reported to suppress bone formation and increase bone resorption, thereby negatively influencing bone turnover and creating an environment within bone tissue that is more susceptible to fracture [44, 45]. Not all amenorrheic athletes experience a stress fracture; therefore, there may be certain characteristics of bone that indicate a greater susceptibility to fracture.

To date, there are currently no reports on the bone geometry and microarchitecture of amenorrheic athletes 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.

**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 [17, 46] (Table 2.2.2). In fact, to date, only one study has explored bone quality among exercising women with a *current* stress fracture [17], 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 [46]. Results from both studies demonstrated that female athletes with bones characterized by a small cortical area may be at increased risk for stress fracture. Schnackenburg et al. [17] 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 [47], 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 [17]. 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 (6%, p=0.09) and number (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 [17].

Likewise, Popp et al. [46] 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). In this study, 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 [46]. In addition, estimated bone strength was impaired in the SFX group as evidenced by a significantly lower SSI at both the 50% and 66% sites [46]. Taken together, it appears that small bones and less cortical bone may increase the risk for stress fracture among female athletes.

Two primary components of bone strength are bone mass and bone geometry [19], as can be appreciated when calculating BSI and SSI. 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 [20], such that a bone that is larger, denser, and has its mass distributed farther away from the central axis will be 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. [48] 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.

Muscle forces have a large impact on bone size and mass, and, therefore, bone strength [49, 50]. 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 were greater in high-training gymnasts compared to non-gymnasts [51]. These differences disappeared, however, after adjusting for muscle cross-sectional area, suggesting that the difference in bone area and estimated bone strength may be at least partially explained by the differences in muscle size [51]. Similarly, differences in cortical area and SSI in exercising women with stress fracture compared to exercising controls were also no longer significant after controlling for muscle cross-sectional area in the previously-described study of Popp et al. [46]. Taken together, these findings indicate that muscle size may also be a determinant factor for stress fracture risk [46, 51]. During loading, muscles help to dissipate energy that is exerted on bone, thereby relieving some of the load imparted on bone and helping to prevent the accumulation of microdamage and subsequent fracture [52]. Thus, less lean mass may be a risk factor for stress fractures.
Table 2.2.2. Summary of pQCT results from studies assessing vBMD, bone geometry and microarchitecture, and estimated bone strength in women with stress fractures

<table>
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<td>Schnackenburg et al,</td>
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<td>HR-pQCT XtremeCT</td>
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<td>Ultradistal</td>
<td>Piano Tibia</td>
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<td>Distal</td>
<td>Piano Tibia</td>
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*a p≤0.05 SF vs. NSF
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
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 [17, 46, 52]. Thus, according to the findings of Popp et al. [46], female athletes with a smaller muscle size may have smaller bones and less distribution of bone mass to areas of high stress such as the posterior region of the tibia simply due to less muscle force. In turn, these women may therefore be at a greater risk for stress fracture.

Although Schnackenburg et al. [17] did observe a decrease in trabecular vBMD and a trend (p=0.08) toward reduced trabecular number in the distal tibia in accordance with the findings of bone density and microarchitecture in amenorrheic athletes [20, 21], the primary outcomes of pQCT measurements of amenorrheic athletes and athletes with a stress fracture (or athletes with a history of either) display stark contrasts. While amenorrheic adolescent athletes 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 [21], athletes with stress fractures displayed a smaller total area and cortical area compared to athletes without a stress fracture [17]. 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 [20]; whereas, athletes with a history of stress fracture demonstrated lower SSI results compared to athletes without a history of stress fracture [46] (Figure 2.2.2). These findings may provide an explanation for the observation that all amenorrheic athletes do NOT experience stress fractures and that other factors likely play a role in the occurrence of these fractures. Interestingly,
amenorrheic athletes 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 density and deterioration of the trabecular microarchitecture. Thus, weight training may be an effective technique for stress fracture risk reduction among amenorrheic athletes with a small frame.

**Figure 2.2.2.** 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.
Notably, the majority of the 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 amenorrheic athletes 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 [3, 44, 45]. To determine the influence of an energy deficiency on parameters of bone strength in amenorrheic athletes, 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 anorexia nervosa.

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

Bone mass among women and adolescents with anorexia nervosa, 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 [53-58]. In fact, Grinspoon et al. [53] observed that 92% and 38% of anorexic women were osteopenic and osteoporotic, respectively. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), the diagnostic criterion for anorexia includes refusal to maintain a body weight that is >85% of that expected for age and height, the intense fear of gaining weight, distorted body image, and amenorrhea [59]. Therefore, due to the presence of amenorrhea which is characterized
by chronic hypoestrogenism, one would expect that anorexic women may present with similar bone deficiencies as amenorrheic athletes, but perhaps to a greater severity due to the additional factors of 1) low body weight and potentially long duration of amenorrhea, which are direct consequences of the severe energy deficiency, and 2) 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 [21, 44, 60, 61]. 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 anorexia nervosa have illustrated compromised bone geometry, density and microarchitecture in this population [55, 56, 60-66] (Table 2.2.3, Figure 2.2.3). Thus, because anorexic women and girls represent a similar phenotype to amenorrheic athletes minus the osteogenic loading component of exercise and with the additional influence of a more severe energy deficiency, the impact of the energy deficiency independent of any effects of exercise on bone quality may be elucidated.

To date, information about bone geometry and microarchitecture in anorexic adolescents [61, 66] and women [55, 56, 60, 62-65] is limited to the distal radius; however, these reports among various investigators have demonstrated strong agreement, providing conclusive evidence for the detrimental effects of severe energy deficiency on bone health. In essence, these data indicate that total, trabecular, and cortical bone quantity and/or quality are compromised among women and adolescent girls with anorexia [55, 56, 60-62, 64, 66] (Table 2.2.3). For example, using traditional pQCT, Resch et al. [62] observed significantly lower total vBMD of 18% at the distal radius
among anorexic women compared to age-matched controls, although no differences were observed for trabecular vBMD between groups. On the other hand, the z-score for trabecular vBMD at the distal radius was markedly low (-0.9) among women who had been admitted to the hospital as a result of anorexia at least 3 years previously [64]. About half of the sample were considered to be recovered from anorexia at the time of follow-up; however, z-score remained low (-0.7) among the recovered group [64]. Likewise, investigators that utilized 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 [56, 66]. Furthermore, trabecular microarchitecture was also compromised as evidenced by significantly lower trabecular number (5%), greater trabecular spacing (7%), and a lower ratio of trabecular bone volume to total bone volume (BV/TV) (13%) compared to the control group [56]. These findings were confirmed by several investigators who used flat panel volume CT to assess trabecular microarchitecture in both anorexic women [55, 60] and adolescents [61]. Trabecular BV/TV, trabecular number, and trabecular thickness were lower and trabecular spacing was greater among the anorexic patients compared to controls, highlighting the serious consequences that severe energy deficiency has on bone strength, in particular the integrity of trabecular bone [55, 60, 61].
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<td>Bredella et al., 2008 [61]</td>
<td>10 AN</td>
<td>10 HC</td>
<td>Flat-panel volume CT</td>
<td>Ultradistal Radius</td>
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<td>Walsh et al., 2010 [60]</td>
<td>8 AN</td>
<td>6 HC</td>
<td>Flat-panel volume CT</td>
<td>Ultradistal Radius</td>
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<tr>
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<td>11 HC</td>
<td>Flat-panel volume CT</td>
<td>Ultradistal Radius</td>
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<td>Faje et al., 2013 [66]</td>
<td>21 AN</td>
<td>23 HC</td>
<td>HR-pQCT</td>
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<td>Milos et al., 2005 [56]*</td>
<td>36 AN</td>
<td>30 HC</td>
<td>HR-pQCT</td>
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<td>Resch et al., 2000 [62]</td>
<td>20 AN</td>
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<td>pQCT Stratec XCT 1400</td>
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<td>Milos et al., 2007 [63]**</td>
<td>15 AN-R</td>
<td>9 AN-NR</td>
<td>HR-pQCT</td>
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<td>Fricke et al., 2010 [65]</td>
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<td>pQCT Stratec XCT 900</td>
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*十足維的*: AN vs. HC, **充分**: 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
*a* adjusted for age, **reflects annual change

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Figure 2.2.3. Bone structure and microarchitecture in anorexia nervosa. Flat-panel volume computed tomography (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 et al. [60].
An energy deficiency such as the starvation observed in anorexic girls and women is characterized by a decline in circulating concentrations of certain metabolic hormones known to have anabolic effects, specifically IGF-1 and leptin [67-69]. IGF-1 binds to receptors on skeletal tissue, thereby stimulating the proliferation and action of osteoblasts and leading to bone formation [70, 71]. Women with an energy deficiency present with suppressed IGF-1 production, resulting from nutritionally-induced growth hormone resistance (GH) that is caused by reduced GH receptor number and function and subsequent loss of negative feedback from IGF-1 as circulating IGF-1 concentrations decline [72-74]. Consequently, the low IGF-1 concentrations observed in women with an energy deficit contribute to suppression of bone formation [75, 76]. Likewise, in an animal model, leptin has also been observed to stimulate osteoblast proliferation and modulate osteoclast action [77, 78]; however, whether leptin exerts this same direct influence on skeletal tissue in humans is unclear [77]. Positive associations between leptin and aBMD have been observed in women [79-81] and children [82]. However, after adjusting for fat mass in women [81] and age, lean body mass, fat mass, IGF-1, and estradiol concentrations in children and adolescents [82], leptin was no longer a significant predictor of aBMD. As such, the influence of leptin on bone metabolism in humans may be mediated through fat mass, IGF-1, or estradiol [77]. Regardless, anorexic women and girls typically present with low fat mass [55, 60, 67, 83] which may contribute to the suppressed concentrations of leptin and estradiol due to the role of adipose tissue in the production of leptin [84] and the aromatization of estrogens [85]. In women with hypothalamic amenorrhea, which is indicative of an energy deficit [1], treatment with recombinant human leptin has been observed to restore menstruation [86,
increase circulating concentrations of IGF-1 [8, 86, 87] and markers of bone formation [86, 87], decrease bone resorption [8], and improve aBMD [8]. As such, the suppressed leptin concentrations in energy deficient women appear to impact bone turnover, albeit perhaps through indirect mechanisms. In addition, anorexic women are, by definition, amenorrheic and therefore likely to present with hypoestrogenism, which is, as previously described, associated with an increase in osteoclast action and therefore bone resorption [44]. As such, the hormonal environment characteristic of anorexia nervosa 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 [25, 33], it follows that an energetic environment that encourages unfavorable alterations in bone metabolism, such as that seen in anorexia nervosa, will be accompanied by profound and relatively consistent detriments in trabecular bone.

In addition to the detriments observed in trabecular bone as a consequence of anorexia [55, 56, 60-62, 66], cortical bone has also been observed to be impacted by this disorder. Similar to the decreased cortical thickness observed in the distal radius of amenorrheic athletes [21, 22], the cortical shell of the ultradistal radius has been reported to be thinner in women and adolescents with anorexia compared to healthy controls [56, 66]. 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 [46]. In anorexic women, therefore, the
decreased cortical thickness and resultant decreased cortical area could contribute to a greater risk for stress fractures and other fractures [88-90]. It is important to note, however, that the cortical compartment of the \textit{tibia} was assessed among women with stress fractures [17, 46, 48]; whereas, the \textit{radius} was evaluated among anorexic women and girls [55, 56, 60-65]. 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 [58]. In a prospective study of anorexic women participating in a treatment program, Milos et al. [63] compared vBMD and bone structure between anorexic women who increased body mass index (BMI) during the two-year treatment and those whose BMI remained unchanged or decreased. The “BMI unchanged or decreased” group had a significantly longer duration of illness as well as longer duration of being underweight (BMI<17.5) compared to the “BMI increased” group [63]. In turn, 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, trabecular number, and cortical thickness than the group that increased BMI and demonstrated a shorter duration of anorexia [63]. Interestingly, the “BMI unchanged or decreased” group also presented with a significantly lower minimum and maximum lifetime BMI compared to the group that increased BMI [63]. 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 cortical thickness 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 [63]. Likewise, results from a two-year prospective study that assessed changes in BMD after inpatient refeeding and weight rehabilitation among adolescent girls with anorexia nervosa demonstrated that low bone mass persists even two years after weight rehabilitation, as evidenced by no improvement in lumbar spine and femoral neck BMD from baseline to the two-year follow-up [69]. 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 periosteal and endosteal circumference at the distal radius were observed between those who presented with a persistent eating disorder and those who were recovered [65]. Therefore, the impact of an energy deficit on bone health among anorexic women may also persist for years following recovery despite the increases in body weight that accompany an improved nutritional environment [69].

Mechanical properties of bone, which serve as indicators of bone strength [91] have also been reported to be affected by the energy-deficient and estrogen-deficient environment characteristic of anorexia [60]. Among women with anorexia nervosa, Walsh et al. [60] reported that stiffness and failure load as assessed by FEA of flat panel CT images was significantly lower (-27% and -29%, respectively) compared to healthy controls. Therefore, not only does an energy deficit appear to negatively affect bone
mass and microarchitecture, it also contributes to detriments in the ability of bone to withstand loading, thereby increasing the risk for fracture [60].

Anorexic women and adolescents present with bone health characteristics that are strikingly similar to what is observed in amenorrheic athletes. Both the anorexic population and the amenorrheic athlete population demonstrate a reduction in vBMD at trabecular sites as well as compromised trabecular microarchitecture characterized by a decrease in trabecular number and an increase in trabecular spacing [20, 21, 55, 56, 60-62, 66]. In addition, cortical thickness was reported to be significantly lower in both groups [21, 56, 66]. Finally, estimated bone strength was notably decreased in both anorexic women and adolescents and amenorrheic athletes as evidenced by significant decreases in stiffness and failure load measurements and BSI calculations, respectively, compared to healthy control women and adolescents [20, 60, 66] (Figure 2.2.4). Thus, it appears that an energy deficiency may largely affect both bone mass and structure, primarily of trabecular bone, thereby contributing to decreases in bone strength.
Figure 2.2.4. 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.
Lessons from Stress Fractures and Anorexia Nervosa

Bone strength among amenorrheic athletes is notably compromised as a result of the synergistic actions of both an energy and estrogen deficiency, thereby placing these women and girls at increased risk for fracture [20-22]. On the other hand, the loading achieved through chronic exercise training may help to preserve some components of bone strength among this population [20, 21]. For example, athletes with amenorrhea or a history of amenorrhea presented with greater total bone area at the tibia and radius compared to non-athletic controls [20, 21], suggesting that the involvement in habitual exercise training contributed to increases in bone size despite the presence of amenorrhea. Because the components of bone strength include both bone geometry and bone mass, these increases in bone size may slightly offset the detrimental impact of amenorrhea on bone strength among female athletes. 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 the total area) [20-22]. This deterioration of bone density and structure is strikingly similar to what is observed among anorexic adolescent girls and women, a population that represents the influence of a severe energy deficiency often coupled with an estrogen deficiency on components of bone strength [55, 56, 60-62, 66]. Adolescent girls and women with anorexia nervosa were observed to have significantly lower total and trabecular vBMD, thinner cortical shells, and degradation of the trabecular microarchitecture as evidenced by decreases in trabecular number and thickness and an increase in trabecular spacing compared to healthy girls and women [55, 56, 60-62, 66].
The similarity of these findings within the trabecular bone compartment of amenorrheic athletes and anorexic girls and women indicates that energy and estrogen deficiency 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 trabecular number compared to athletes without a history of stress fracture [17, 46], which are characteristics that also described the bone density and microarchitecture of amenorrheic athletes (Figure 2.2.2). Thus, these similarities suggest that amenorrheic athletes 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 [52]. 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 [52]. Additionally, and perhaps more importantly, microdamage also plays a physiologic role, stimulating the remodeling process and therefore contributing to the normal and healthy renewal of the bone matrix [52]. In the case of a stress fracture, however, microdamage accumulates to a critical limit due to overuse without adequate repair, and it is has been suggested that this inadequate repair that leads to stress fracture is due to the suppression of remodeling [52]. Therefore, the uncoupling of bone turnover that is observed among amenorrheic athletes as a result of the energy deficiency 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 microarchitecture and structure).
Conclusions

Among amenorrheic athletes, bone strength as estimated by three-dimensional imaging techniques is compromised. Striking similarities are observed when comparing the bone characteristics of amenorrheic athletes with those of athletes with stress fractures and adolescent girls and women with anorexia. The data from athletes with stress fractures inform us that bones with a small total and cortical area as well as low trabecular vBMD are at increased risk for fracture; whereas, the data from the anorexic population inform us that an energy deficiency typically combined with hypoestrogenism primarily contributes 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 amenorrheic athletes 1) is due to an energy and estrogen deficiency and 2) may cause the amenorrheic athletes to be at increased risk for stress fracture. When evaluating bone strength, both bone geometry and bone mass must be considered. Although adolescent amenorrheic athletes demonstrate increased total bone area compared to non-athletic controls, bone quantity (i.e., BMD) and structure (i.e., bone geometry and microarchitecture) are also key determinants of bone strength. Among amenorrheic athletes, 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 risk for fracture is 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 [63, 69], regular monitoring of bone
health among these girls and women as well as encouragement to maintain a healthy body weight, adequate energy intake, and regular menstrual function is essential. Participation in exercise such as resistance training may also help to improve the bone health of amenorrheic athletes and decrease the risk for fracture. Muscle forces exerted on the bones via lean mass not only stimulate an increase in the size of the bone, thereby increasing bone strength, but also serve to dissipate energy from loading, therefore aiding the prevention of microdamage accumulation that leads to a stress fracture [52]. As such, targeting both adequate energy intake and increased lean mass will address both bone density and bone geometry, ultimately leading to optimal bone quantity and quality with the hope of reducing the risk for fracture.
References


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CHAPTER 2: PART 3


Abstract

The introduction of dual-energy x-ray absorptiometry (DXA) in the 1980s for the assessment of areal bone mineral density (BMD) greatly benefited the field of bone imaging and the ability to diagnose and monitor osteoporosis. The additional capability of DXA to differentiate between bone mineral, fat tissue, and lean tissue has contributed to its emergence as a popular tool to assess body composition. Throughout the past two decades, technological advancements such as the transition from the original pencil-beam densitometers to the most recent narrow fan-beam densitometers have allowed for faster scan times and better resolution. The majority of reports that have compared DXA-derived body composition measurements to the gold standard method of body composition appraisal, the four-compartment model, have observed significant differences with this criterion method; however, the extent to which the technological advancements of the DXA have impacted its ability to accurately assess body composition remains unclear. Thus, this paper reviews the evidence regarding the trueness and precision of DXA body composition measurements from the pencil-beam to the narrow fan-beam densitometers.
**Introduction**

Since the introduction of dual-energy X-ray absorptiometry (DXA) into the clinical scene in the late 1980s, several technological advances have occurred that have greatly impacted the field of bone and soft tissue imaging. The primary application of DXA is the measurement of areal bone mineral density (BMD) to diagnose osteoporosis and other bone diseases. This technique provides improved trueness and precision over previous methods as well as the ability to measure areal BMD at the preferential sites of osteoporotic fractures (hip, spine and distal forearm) [1-3].

A secondary - although non negligible - application of DXA is the assessment of body composition, i.e., the measurement of fat and bone-free lean mass [4]. The major technological developments of DXA systems over the years were essentially driven by the clinical use of DXA to diagnose osteoporosis. The progressive replacement of the original pencil-beam densitometers by fan-beam devices in the early 1990s allowed for better resolution and faster scan times, thus easing the burden of use for both patient and clinic [5, 6]. The most recent advancement in DXA technology was the introduction of scanners that provided better resolution and image quality [7-9], as well as a wider bed platform and higher weight limit to accommodate the scanning of obese individuals [8-10]. Whether or not these technological advances have affected the ability of DXA to assess body composition with accuracy, i.e., with trueness and precision [11], remains unclear.

As such, the purpose of this paper is to review the evidence regarding the trueness and precision of DXA technology to measure body composition, from the pencil-beam densitometers introduced in the 1980s to the most recent fan-beam densitometers.
Basic Principles of DXA

The fundamental principle of DXA is the measurement of the transmission of X-rays through the body at high and low energies (Figure 2.3.1) [12]. The X-ray source generates a beam of X-rays, which consists of photon particles carried through electromagnetic energy. As photons traverse the subject’s tissues, physical interactions take place that reduce beam intensity [13]. The attenuation depends on the energy of the photons and the density and thickness of the human tissues through which they pass [14]. The measurement of areal BMD by DXA is based on the assumption that the body is a two-compartment model: bone mineral and soft tissue (muscle, fat, skin and water), which has a lower density. In order to differentiate bone and soft tissue, the X-ray source generates an X-ray beam with two distinct photon energies. Attenuation of the X-ray beam decreases as photon energy increases [13]. Low density material (i.e. soft tissues) allows more photons to pass through, thus attenuating the X-ray beam less than high-density material such as bone [13]. In order to determine the amount of fat and fat-free soft tissue in the body, DXA measures the ratio of attenuation of the two photon energies at anatomical sites that do not contain any bone (typically the pure soft tissue that is adjacent to bone). When no bone is present, the ratio of the attenuation of the two photon energies is linearly related to the proportion of fat in the soft tissue [3]. After the attenuation of the X-ray beam has been analyzed in regions with soft tissue and bone, as well as regions with soft tissue only, fat mass, lean tissue mass and bone mineral mass can be discriminated.
Major technological advances have taken place with the progressive transition from pencil-beam densitometers to fan-beam densitometers (Figure 2.3.2). Pencil-beam densitometers use a highly collimated pencil beam of X-rays with a single detector. Because the anatomical site is scanned in a rectilinear fashion, scanning times are relatively long (5-10 minutes per site, 10-20 minutes for total body). In contrast, fan-
beam densitometers use a fan-beam X-ray source and a set of detectors. Measurement of the whole body can be made with a single sweep of the X-ray arm (Figure 2.3.2). This technological advancement, which was first experienced in 1993 with the introduction of the Hologic QDR-2000, allowed for better resolution (0.8-2 mm for fan-beam densitometers vs. 1.5-2.5 mm for pencil-beam densitometers) [15-17] and faster scan times (~1 min per site, ~5 min for total body) [5, 6]. Disadvantages of fan-beam densitometers include a higher radiation dose [14] and inherent magnification of scanned structures as the distance from the X-ray source decreases [5, 18, 19]. This effect, which has been shown to significantly affect bone mineral content and bone area measurements [20], has been corrected with the introduction of narrow fan-beam densitometers, the first of which was the GE Lunar Prodigy. Narrow fan-beam densitometers offer a compromise between older technologies: they scan in a rectilinear fashion with a fan beam that is wider than the original pencil beam (thus gaining time) but still narrower than the first fan beams (thus reducing magnification effects) [14, 17, 21]. Additionally, each pass of the narrow fan beam across the body overlaps the previous one (Figure 2.3.2). These overlapping images are matched and reconstructed, resulting in a more accurate estimation of the depth of the bone and reducing the magnification effect [22]. The most recent advancement in DXA technology was the introduction of the GE Lunar iDXA, a narrow-angle fan-beam densitometer with a greater number of detectors, providing improved resolution (1.05 mm longitudinally, 0.6 mm laterally) and image quality [7-9]. The enhanced resolution allows for better bone edge detection and, subsequently, the development of superior algorithms for body composition assessment compared to earlier densitometers.
Is DXA a true method for the assessment of body composition?

Trueness (formerly denoted as accuracy) is defined as the closeness of agreement between the result of a measurement and a true value of the measure [11]. Trueness is expressed in terms of bias (formerly denoted as accuracy error): the greater the bias, the larger the difference between the measurement and the true value. In the field of body composition assessment, the bias of DXA would refer to the difference between fat mass and bone-free lean mass as measured by DXA and “true” fat mass and bone-free lean
mass, which can be directly measured on cadavers. Interestingly, the trueness of DXA
for the assessment of body composition has not been tested on human cadavers, although
studies were performed on animal cadavers such as pigs [23-26]. In an effort to validate
DXA as a reliable and valid method of body composition assessment in humans, it has
been evaluated against the 4-compartment (4-C) model, the tool that is currently
considered the gold standard method of body composition appraisal. The 4-C model
divides the body into four compartments – fat mass, bone mineral, total body water, and
other (i.e. protein, non-bone minerals, and glycogen) – thus eliminating the assumptions
about the relative components of fat free mass that are inherent to two-compartment
(underwater weighing) and three-compartment (DXA) models and providing a more
complete picture of body composition (Figure 2.3.3). Methodologically, the different
compartments of the 4-C model are measured using hydrodensitometry or air
displacement plethysmography to determine fat mass and fat free mass, isotope dilution
to determine total body water, and DXA to measure bone mineral. Total body water and
bone mineral are subtracted from fat free mass to obtain the residual or “other”
compartment (i.e. protein, non-bone minerals, and glycogen). The proportion of each
compartment relative to body mass and their assumed densities can then be used to
calculate percent body fat [27].
Heymsfield et al. [28] compared the 4-C model to neutron-activation analysis, the most accurate although less accessible in vivo method of body composition, and reported that the 4-C model demonstrates good trueness for an in vivo method. In this study, mean percent body fat derived from the 4-C model using DXA was about 1.4% lower than that derived from the neutron-activation model, and the measurement error for estimated percent body fat was ~1.6% for the 4-C model versus <1% for neutron activation analysis [28]. Additionally, in a paper exploring the measurement error associated with two-, three-, and four-compartment models, Withers et al. [27] demonstrated that the addition
of variables to the body fat equation for the 4-C model, a characteristic inherent to the model due to the greater number of compartments measured, does not increase the measurement error.

The results of studies that compared percent body fat between DXA and the 4-C model in populations of weight-stable, healthy adults are summarized in Table 2.3.1. The majority of studies reported a mean underestimation of percent body fat by DXA when compared to a 4-C model [29-34]. Interestingly, several studies have observed that this underestimation is accentuated in leaner individuals [31, 32, 34]. For example, Van der Ploeg et al.[34] reported an average 1.8% lower percent body fat when measured by DXA compared to the 4-C model. However, the magnitude of the error appeared to be dependent on percent body fat level: the leaner the individual, the greater the underestimation by DXA [34]. Similarly, Arngrimsson et al.[32] reported a 3-4% underestimation of DXA-derived percent body fat when compared to that from a 4-C model in a sample of male and female distance runners. In fact, Arngrimsson et al.[32] compared various two- and three- compartment models of body composition to the reference 4-C model and observed that percent body fat from DXA displayed more deviation from the criterion method than any of the other techniques, including two-compartment models (underwater weighing; deuterium dilution) and other three-
Table 2.3.1. Comparison of percent body fat (%BF) measured by dual-energy x-ray absorptiometry (DXA) and the four-compartment model (4-C)

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>%BF (from 4C)</th>
<th>Athletes?</th>
<th>DXA system</th>
<th>Type of X-ray beam</th>
<th>Mean Difference in %BF vs. 4-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuller et al. [29]</td>
<td>16 M</td>
<td>18-59</td>
<td>12.0–25.0</td>
<td>No</td>
<td>GE Lunar DPX</td>
<td>Pencil</td>
<td></td>
<td>-1.4% (nr)</td>
</tr>
<tr>
<td></td>
<td>12 F</td>
<td>19.6-38.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bergsma-Kadijk et al.[30]</td>
<td>20 F</td>
<td>19-27</td>
<td>29.4±3.2</td>
<td>No</td>
<td>GE Lunar DPX</td>
<td>Pencil</td>
<td></td>
<td>-5.3%*</td>
</tr>
<tr>
<td></td>
<td>18 F</td>
<td>65-78</td>
<td>38.8±5.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior et al. [38]</td>
<td>91 M</td>
<td>21±2</td>
<td>12.5±5.9</td>
<td>111 Athletes</td>
<td>Hologic QDR 1000W</td>
<td>Pencil</td>
<td></td>
<td>0.6%</td>
</tr>
<tr>
<td></td>
<td>81 F</td>
<td></td>
<td>22.3±7.6</td>
<td>61 Non-athletes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Withers et al. [31]</td>
<td>24 M</td>
<td>18-36</td>
<td>Athletes: 12.1±2.8</td>
<td>24 Athletes</td>
<td>GE Lunar DPX-L</td>
<td>Pencil</td>
<td></td>
<td>Athletes: -3.5%* Non-athletes: -1.3%*</td>
</tr>
<tr>
<td></td>
<td>24 F</td>
<td></td>
<td>Non-athletes: 21.8±8.2</td>
<td>24 Non-athletes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modlesky et al.[39]</td>
<td>14 M</td>
<td>27±6</td>
<td>20.7±10.2</td>
<td>No</td>
<td>Hologic QDR 1000W</td>
<td>Pencil</td>
<td></td>
<td>-2.1%</td>
</tr>
<tr>
<td></td>
<td>10 F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arngrimsson et al.[32]</td>
<td>22 M</td>
<td>21±3</td>
<td>Athletes: 9.5±2.5</td>
<td>22 Athletes</td>
<td>Hologic QDR 1000W</td>
<td>Pencil</td>
<td></td>
<td>Athletes: -2.9%* Non-athletes: -2.9%*</td>
</tr>
<tr>
<td></td>
<td>22 F</td>
<td></td>
<td>Non-athletes: 17.2±4.6</td>
<td>22 Non-athletes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deurenberg-Yap et al.[33]</td>
<td>144 M</td>
<td>18-75</td>
<td>26.2±6.5</td>
<td>No</td>
<td>Hologic QDR 4500</td>
<td>Fan</td>
<td></td>
<td>-3.8%*</td>
</tr>
<tr>
<td></td>
<td>147 F</td>
<td></td>
<td>36.2±7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van der Ploeg et al.[34]</td>
<td>118 M</td>
<td>18-59</td>
<td>6.5-36.6</td>
<td>No</td>
<td>GE Lunar DPX-L</td>
<td>Pencil</td>
<td></td>
<td>-1.9% (nr)</td>
</tr>
<tr>
<td></td>
<td>34 F</td>
<td></td>
<td>12.3-37.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.7% (nr)</td>
</tr>
<tr>
<td>van marken Lichtenbelt et al.[40]</td>
<td>27 M</td>
<td>19-44</td>
<td>7.5-23.2</td>
<td>Yes</td>
<td>GE Lunar DPX-L</td>
<td>Pencil</td>
<td></td>
<td>0.9%</td>
</tr>
<tr>
<td>Williams et al.[36]</td>
<td>26 M</td>
<td>19-21</td>
<td>15.6±6.3</td>
<td>No</td>
<td>GE Lunar Prodigy</td>
<td>Narrow Fan</td>
<td></td>
<td>1.7%*</td>
</tr>
<tr>
<td></td>
<td>44 F</td>
<td></td>
<td>29.9±6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.0%*</td>
</tr>
<tr>
<td>Santos et al.[37]</td>
<td>24 M</td>
<td>22±3</td>
<td>9.2±4.1</td>
<td>Yes</td>
<td>Hologic QDR 4500A</td>
<td>Fan</td>
<td></td>
<td>2.9%*</td>
</tr>
</tbody>
</table>

Includes studies with weight-stable healthy adults (studies with populations composed primarily of children or overweight/obese adults and/or studies that did not report percent body fat were not included); Percent body fat using the four compartment model was determined using underwater weighing or BodPod, DXA and deuterium dilution; *Statistically significant difference between DXA and 4-C model (p<0.05); (nr) Statistical significance not reported
compartment models (underwater weighing + deuterium dilution; underwater weighing + DXA). Similar results have been reported by Withers et al. [31] in a sample comprised of trained athletes and sedentary adults. Additionally, in a large meta-analysis of data sets that compared body composition estimates obtained on a Hologic QDR 4500A to either a two-compartment (underwater weighing or total body water) or 4-C model, Schoeller et al. [35] observed that in all data sets DXA consistently underestimated fat mass compared to the criterion method.

However, this underestimation of fat mass by DXA has not been consistently observed by investigators comparing DXA-derived body composition outcomes to those obtained from the 4-C model. Studies by two research teams demonstrated an overestimation of percent body fat by DXA [36, 37]. Williams et al. [36] compared body composition from the GE Lunar Prodigy to a 4-C model and reported a mean overestimation of fat mass and percent body fat by DXA among non-obese adults. Likewise, Santos et al. [37] observed that the percent body fat of elite judo athletes was, on average, overestimated by ~3% when measured by a fan-beam DXA (Hologic 4500A) compared to the 4-C model. Other investigators observed no significant differences when comparing two pencil-beam densitometers (Hologic QDR 1000W and a Lunar DPXL) to a 4-C model [38-40].

It is also important to note that several investigators reported large individual differences as well as inconsistent error between body composition measures from DXA and the 4-C. For example, Prior et al. [38] observed that the differences between a Hologic QDR-1000W and the 4-C model ranged from -9.9 to 7.5% body fat. Additionally, both Van der Ploeg et al. [34] and Arngrimsson et al. [32] reported a large
range of individual differences (-2.6 to 7.3% and -2.3 to 10%, respectively) with DXA underestimating the percent body fat of leaner individuals and overestimating it among individuals with a higher percent body fat.

Results from longitudinal research exploring the ability of DXA to accurately measure changes in body composition are encouraging. Among studies designed to track body composition changes during weight loss or preparation for athletic competition, several investigators observed small, non-significant mean differences between percent body fat changes measured via DXA and those obtained from the 4-C model [37, 40-42]. In agreement with these studies, Fogelholm et al. [43] also reported that the loss of fat mass estimated by DXA during a weight loss intervention was similar to that calculated from the 4-C model when considering the mean difference between methods. Table 2.3.2 provides a summary of these studies.

Although few significant mean differences were observed between measurements from DXA and the 4-C model assessing body composition change, it must be noted, however, that large individual biases were still present as indicated by wide limits of agreement [37, 41, 42]. For example, Santos et al. [37] reported that the 95% limits of agreement between DXA and the 4-C model when tracking percent body fat changes was -3.7 to 5.3%, with DXA overestimating the percent fat losses and underestimating the percent fat gains. Likewise, both Mahon et al. [41] and Evans et al. [42] observed a similar trend, possibly explained by the DXA’s inherent assumption that the hydration of the fat free tissue remains constant. Thus, on a group basis, DXA appears to perform
Table 2.3.2. Comparison of percent body fat (%BF) changes measured by dual-energy x-ray absorptiometry (DXA) and the four-compartment model (4-C)

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>%BF (from 4C)</th>
<th>Athletes</th>
<th>Weight Loss</th>
<th>DXA system</th>
<th>Type of X-ray beam</th>
<th>Mean Difference in %BF change vs. 4-C</th>
<th>95% Limits of Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans et al. [42]</td>
<td></td>
<td>27</td>
<td>F</td>
<td>32.0±6.4</td>
<td>42.5±4.0</td>
<td>✔</td>
<td>Hologic QDR-1000W</td>
<td>Pencil</td>
<td>-1.3</td>
<td>-4.0, 4.6b</td>
</tr>
<tr>
<td>Van Marken Lichtenbelt et al. [40]</td>
<td></td>
<td>15</td>
<td>M</td>
<td>31.5</td>
<td>15.9±4.4</td>
<td>✔</td>
<td>GE Lunar DPX-L</td>
<td>Pencil</td>
<td>-0.21</td>
<td>NR</td>
</tr>
<tr>
<td>Mahon et al. [41]</td>
<td></td>
<td>27</td>
<td>F</td>
<td>59±8</td>
<td>41.5±5.4</td>
<td>✔</td>
<td>GE Lunar Prodigy</td>
<td>Fan</td>
<td>0.6</td>
<td>-8.3, 9.4</td>
</tr>
<tr>
<td>Minderico et al. [72]</td>
<td></td>
<td>48</td>
<td>F</td>
<td>39.8±5.8</td>
<td>41.1±5.2</td>
<td>✔</td>
<td>Hologic QDR-1500</td>
<td>Pencil</td>
<td>-1.7a</td>
<td>-5.7, 2.3</td>
</tr>
<tr>
<td>Santos et al. [37]</td>
<td></td>
<td>27</td>
<td>M</td>
<td>22.2±2.8</td>
<td>9.2±4.1</td>
<td>✔</td>
<td>Hologic QDR-4500A</td>
<td>Fan</td>
<td>0.81</td>
<td>-3.7, 5.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD
Percent body fat using the four compartment model was determined using underwater weighing or BodPod, DXA and deuterium dilution.

aStatistically significant difference between DXA and 4-C model (p<0.05)

bRange reported rather than 95% limits of agreement
Studies that did not report percent body fat were not included.
NR = not reported
well when measuring body composition changes longitudinally; however, when using
DXA to estimate individual changes in body composition, the results should be
interpreted with caution.

Discrepancies in body composition outcomes have also been reported between
DXA machines, both within the same manufacturer and across manufacturers. Table
2.3.3 provides a brief summary of the differences that have been observed by
investigators comparing body composition between DXA systems. These inconsistent
findings that have been observed both when comparing DXA-derived body composition
outcomes to a 4-C model and when comparing body composition measurements between
DXA systems are most likely due to discrepancies in DXA hardware and software, and
serve to illustrate the questionable validity of body composition assessment by DXA
[34].

The Lunar iDXA, which received FDA approval for the assessment of areal BMD
and the diagnosis of osteoporosis in 2005, is the most recent addition to the DXA field; it
is a narrow fan-beam densitometer that uses a high-definition cadmium zinc telluride
staggered array detector and a more powerful X-ray tube than previous densitometers,
thereby enhancing image resolution. Better image quality, added to the capacity to scan
people weighing up to 450 lbs, makes this DXA machine an attractive device. However,
these technological developments may also cause further discrepancies between DXA
machines when assessing body composition.

Our research team recently conducted a cross calibration study between the Lunar
iDXA and Lunar Prodigy for the assessment of body composition. In this pilot study
Table 2.3.3. Differences of body composition between various dual-energy x-ray absorptiometry (DXA) systems according to the type of X-ray beam

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>DXA systems</th>
<th>Differences in body composition between the 2 systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lean mass (kg)</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>Age (yrs)</td>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Tothill et al. [50]</td>
<td>5 M</td>
<td>19 - 28</td>
<td>3.7a</td>
</tr>
<tr>
<td></td>
<td>6 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modlesky et al. [73]</td>
<td>13 M</td>
<td>22.2±3.6</td>
<td>nr</td>
</tr>
<tr>
<td></td>
<td>14 M</td>
<td>26.7±6.0</td>
<td>24.4±3.5</td>
</tr>
<tr>
<td></td>
<td>10 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hull et al. [10]</td>
<td>47 M</td>
<td>18-81</td>
<td>18.1 - 46.4</td>
</tr>
<tr>
<td></td>
<td>52 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genton et al. [51]</td>
<td>36 F</td>
<td>nr</td>
<td>3.4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overweight</td>
<td></td>
</tr>
<tr>
<td>Hull et al. [10]</td>
<td>47 M</td>
<td>18-81</td>
<td>18.1 - 46.4</td>
</tr>
<tr>
<td></td>
<td>52 F</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Pencil Beam vs Pencil Beam**

**Pencil Beam vs Fan Beam**

**Fan Beam vs Fan Beam**

M = male; F = female; BMI = body mass index; %BF = percent body fat

Nr: not reported; aStatistically significant difference (p<0.05); bNPM = normal power mode; HPM = high power mode
conducted in 13 women aged 20-46 years, we found not only large individual differences between machines but also discovered that the error was inconsistent and varied with percent body fat. As displayed in Figure 2.3.4, the difference in percent body fat between the machines ranged from -6.4 to 5.1% (mean difference: 0.7%; 95% CI: -2.5, +1.1). Our results indicated that fat mass and percent body fat were greater on the iDXA than on the Prodigy, particularly when fat mass values were below 20 kg and percent body fat was less than 30%, as measured on the Prodigy. The opposite outcome was observed, however, for fat mass and percent body fat values greater than 20 kg and 30%, respectively. For example, we observed that an individual with 8.2 kg of fat mass and a percent body fat of 19.2% as measured on the Prodigy had a higher fat mass (10.7 kg) and percent body fat (24.7%) on the iDXA. In contrast, another individual with 37.6 kg of fat mass and a percent body fat of 46.3% as measured on the Prodigy had lower values when scanned on the iDXA (32.9 kg fat mass and 40.0% body fat).

Due to the recent introduction of the iDXA, studies that have compared this densitometer with older models of DXA are scarce, particularly with regard to the assessment of body composition. Hull et al.[10] compared total body and regional lean mass and fat mass between the iDXA and two earlier Lunar densitometers, the DPXL and the Prodigy. The differences observed between the iDXA and the other two densitometers were statistically significant for total body lean mass and fat mass in both genders (Table 2.3.3). The direction of these deviations, however, appeared to differ between genders, suggesting that gender may have an impact on body composition results obtained from DXA [10]. Data presented at the International Society for Clinical Densitometry Annual Meeting in 2008 also demonstrated large differences in body composition...
composition parameters between the Lunar iDXA and a densitometer of a previous generation, the Hologic QDR 1000W (pencil beam), reporting that fat mass values from the iDXA were 22% higher than those generated by the Hologic machine [44]. A possible reason for these differences may stem from the improved resolution of the iDXA; however, more research comparing the iDXA to other methods of body composition assessment is necessary.

**Figure 2.3.4.** Bland-Altman graph of the differences between iDXA- and Prodigy-derived percent body fat (%BF).

![Bland-Altman graph](image-url)
The analysis of the trueness of DXA to assess body composition through the generations of DXA systems from pencil-beam to narrow fan-beam technology did not reveal any striking changes in the agreement of measurements with the gold standard 4-C model, indicating that the technological improvements have successfully reduced scan time and maintained a low radiation dose without compromising the trueness of the outcome. Table 2.3.4 provides a summary of scan time, radiation dose, trueness and precision of DXA systems from each generation of advancement in X-ray beam technology. Evidence for the most recently introduced DXA system, however, is still lacking. Very few reports have compared iDXA-derived body composition with outcomes from other densitometers. More importantly, the ability of the Lunar iDXA to accurately assess body composition has not yet been tested against the gold standard 4-C model. Further research in this area is essential due to the use of DXA for clinical management of patients who are under- or over-weight.

Is DXA a precise method for the assessment of body composition?

Precision is defined as the closeness of agreement between independent results of measurement obtained under stipulated conditions [11]. Precision is therefore independent from trueness. Short-term precision refers to the precision obtained with a short time interval between tests (typically minutes or hours) whereas long-term precision refers to time intervals similar to clinical follow-ups, typically weeks or years [45]. The imprecision, or precision error, is usually calculated as standard deviation or coefficient of variation of the measurement results. The short-term precision of DXA body
Table 2.3.4. Technical specifications, precision and trueness (vs. four compartment model, 4-C) of different dual-energy x-ray absorptiometry (DXA) systems

<table>
<thead>
<tr>
<th>Type of X-ray beam</th>
<th>DXA system</th>
<th>Scan Time (min)</th>
<th>Effective Radiation Dose (μSv)(^a)</th>
<th>In Vivo Precision (%CV)</th>
<th>Trueness (Mean Difference in %BF DXA vs.4-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Pencil</td>
<td>Hologic QDR 1000W [32,38,39,50,74]</td>
<td>17</td>
<td>4.6</td>
<td>0.6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Lunar DPX [29-31,34,39,40,48,50,52,75]</td>
<td>~10</td>
<td>--</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Norland XR 26 Mark II HS [50,76]</td>
<td>13</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Fan</td>
<td>Hologic QDR 4500W [33,37,49,75,77,78]</td>
<td>~7</td>
<td>3-3.4</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>Narrow Fan</td>
<td>Lunar Prodigy [22,36,47,51,75,79]</td>
<td>4.5</td>
<td>0.37-0.6</td>
<td>1(^b)</td>
<td>2(^b)</td>
</tr>
<tr>
<td></td>
<td>Lunar iDXA [46,53,80,81]</td>
<td>4</td>
<td>4.7</td>
<td>0.4(^b)</td>
<td>1(^b)</td>
</tr>
</tbody>
</table>

Technical specifications, precision and trueness are for the assessment of soft tissue and percent body fat (%BF) on a whole body scan.

\(^a\) For comparison purposes, the world average for daily natural background radiation dose is 6.6 μSv (2,400 μSv per year) (82)

\(^b\) Root-mean-square coefficient of variation

Precision and trueness data is given as range (min and max).
composition measurements varies slightly by type of soft tissue, with lean mass demonstrating better precision than fat mass. **Table 2.3.5** provides an overview of the precision for body composition measurements obtained on different DXA systems. The coefficient of variation for both phantom and *in vivo* precision of whole-body lean mass measurements has been reported to be approximately 1.0%; whereas, that for fat mass and percent body fat ranges between 0.8 and 2.7% *in vivo* and between 2.8 and 4.4% for a phantom [46-52]. The precision of regional body composition measurements has been reported to be poorer than whole-body measurements, particularly for fat mass and percent fat of the trunk which demonstrated a coefficient of variation between 0.8 and 4.8% greater than that of the total body [46-50, 52].

Similar to the issue of the trueness of the iDXA to assess body composition, the precision of this device has yet to be established. In our pilot study, we scanned 13 women three times on both the Lunar Prodigy and the Lunar iDXA. The root-mean-square coefficient of variation values (RMS CV) for the iDXA were 0.4%, 1.0%, and 0.9% for lean mass, fat mass, and percent body fat, respectively. Corresponding RMS CV values for the Lunar Prodigy were 1.2% for lean mass, 1.8% for fat mass, and 1.8% for percent body fat. Based on this data, the precision of the iDXA appears to be better than that of the Prodigy, which may be attributable to the improved resolution of the iDXA. Our results are consistent with those reported by Hind et al.[46] and Rezzi et al.[53] who demonstrated precision of body composition measurements on the Lunar iDXA to be 0.4-0.5% for lean mass, 0.7-0.8% for fat mass, and 0.6-0.9% for percent body fat. Therefore, preliminary evidence suggests that the Lunar iDXA shows excellent
Table 2.3.5. Precision for body composition obtained with different dual-energy x-ray absorptiometry (DXA) systems in adults

<table>
<thead>
<tr>
<th>Study</th>
<th>DXA System</th>
<th>Type of X-ray Beam</th>
<th>Phantom Or In Vivo</th>
<th>Sample</th>
<th>Coefficient of variation a</th>
<th>Lean Mass</th>
<th>Fat Mass</th>
<th>% Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson and Dawson-Hughes [52]</td>
<td>GE Lunar DPX</td>
<td>Pencil</td>
<td>In Vivo</td>
<td>5 F</td>
<td>1.1% 2.7% nr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tothill et al.[50]</td>
<td>Hologic QDR-1000W</td>
<td>Pencil</td>
<td>Phantom</td>
<td>1</td>
<td>0.6% 3.2% 2.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norland XR 26</td>
<td>Pencil</td>
<td>Phantom</td>
<td>19</td>
<td>0.6% 1.9% 1.9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mark II HS</td>
<td></td>
<td>In Vivo</td>
<td>9</td>
<td>0.9% 3.0% 3.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GE Lunar DPX</td>
<td>Pencil</td>
<td>Phantom</td>
<td>1</td>
<td>1.1% 2.6% 2.7%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiebzak et al.[48]</td>
<td>GE Lunar DPX-L</td>
<td>Pencil</td>
<td>In Vivo</td>
<td>10 M, 10 F</td>
<td>1.1% 2.0% 1.9%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cordero-MacIntyre et al.[49]</td>
<td>Hologic QDR-4500A</td>
<td>Fan</td>
<td>In Vivo</td>
<td>9 F</td>
<td>1.3% 1.3% nr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chen et al.[47]</td>
<td>GE Lunar Prodigy</td>
<td>Narrow Fan</td>
<td>In Vivo</td>
<td>10</td>
<td>0.7% 1.2% 1.2%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genton et al.[51]</td>
<td>GE Lunar Prodigy</td>
<td>Narrow Fan</td>
<td>In Vivo</td>
<td>14 F</td>
<td>1.0% 1.2% nr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rezzi et al.[53]</td>
<td>GE Lunar iDXA</td>
<td>Narrow Fan</td>
<td>In Vivo</td>
<td>8 M, 16 F</td>
<td>0.4% 0.7% 0.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind et al.[46]</td>
<td>GE Lunar iDXA</td>
<td>Narrow Fan</td>
<td>In Vivo</td>
<td>52</td>
<td>0.5% 0.8% 0.9%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Coefficient of variation was calculated as follows: Coefficient of variation for individuals $j$: $CV = \frac{\sum (SD/\text{mean}_j)}{n}$, with $n$ being the number of individuals measured; Except for Kiebzak et al. and Genton et al.: Root-mean-square coefficient of variation (RMS CV) = RMS SD/mean, with RMS SD = $\sqrt{\left(\sum \text{SD}^2\right)/m}$; nr: not reported
precision for body composition measurements and slightly better precision than a
densitometer of a previous generation. However, further investigations using larger
samples are necessary to confirm these findings.

The similar precision values that have been reported for pencil-beam and fan-
beam densitometers indicate that the reduced scan time that accompanies fan-beam
technology does not sacrifice the precision of the measurements, thus providing further
evidence for the beneficial impact of fan-beam technology on the DXA field (see Table
2.3.4). In fact, from the limited studies that have reported precision of the Lunar iDXA,
it appears that this most recent technological advancement improves the precision of
DXA body composition measurements, an advantage that has important clinical benefits
due to the importance of precision in determining treatment success.

Clinical implications and concerns

Because of its convenience relative to other methods of body composition
assessment and its ability to not only divide the body into fat and lean compartments but
also to evaluate the regional distribution of lean and adipose tissues, DXA may be a
useful clinical tool to assess body composition, particularly among those who may be at
risk for exhibiting body composition phenotypes at either the obese or anorexic end of the
spectrum.

Obesity associated with the metabolic syndrome is often described as central
obesity, or visceral adiposity, because of the link between waist circumference and both
metabolic abnormalities and cardiovascular disease [54-56]. Typically, waist
circumference has been the method by which visceral adiposity is assessed, and body
mass index (BMI) cutoffs have been used to diagnose obesity [55, 57]. Although
guidelines have been published that provide percent body fat ranges and the
Corresponding BMI values, BMI is not a completely accurate measure of percent body fat
[58]. The advantage of DXA, however, is that it is able to assess this regional
distribution of fat and lean mass in the visceral compartment, rather than relying on
anthropometric measurements, thereby serving as a useful tool to assess the degree of
Adiposity and risk for obesity-related health consequences as well as monitor change in
visceral adiposity during the course of a treatment and weight loss [57].

On the other end of the spectrum, those with anorexia nervosa typically present
with a low body weight and BMI due to the loss of both fat mass and lean mass [57, 59].
Typical outcomes of interest during treatment include an increase in body weight and
BMI until healthy values have been achieved. Several investigators, however, have
demonstrated that BMI and body weight do not adequately describe the change that
occurs with fat mass during treatment and, additionally, are poor indicators of recovery
[59-61]. It has been observed that weight gain among anorexics during recovery is
characterized by a greater increase in fat mass than lean mass, with the majority of the
increase in fat mass being centrally distributed [62]. Unlike anthropometric indices,
DXA has the capability to monitor these changes in the regional distribution of fat mass
and lean mass throughout the course of the illness and treatment.

For these reasons, DXA is an attractive alternative to BMI, a commonly-used
indicator of underweight and obese phenotypes. Several studies, however, have used
DXA as a criterion method to either determine body fat cutoffs for the diagnosis of
obesity, particularly among the pediatric population [63, 64], or to assess the accuracy of
other body composition techniques [65-69]. Percent body fat measurements from DXA may also become more widely used clinically to assess the obese or anorexic as a result of the recent publication of NHANES body composition data, considered a reference standard for the US population [58]. Due to the significant differences that have been reported between DXA systems as well as between DXA and the current criterion method of body composition assessment, utilizing DXA in this way, as a gold standard, should be undertaken with caution. Because changes in body composition may be primary outcome variables of treatment among both the lean and the obese, the accuracy of the measurements is essential. In fact, a recent study that used DXA to assess body composition in premenopausal obese and anorexic women reported that DXA underestimated fat mass compared to computed tomography (CT), a bias that increased with increasing weight, thereby questioning the use of DXA to accurately assess body composition among the obese population [70]. Similarly, in a study of obese adults, LaForgia et al. [71] demonstrated that DXA underestimated percent body fat compared to the 4-C model as anterior-posterior thickness, waist girth, and BMI increased. Therefore, in light of the potential of DXA to become more widespread as a clinical tool to assess body composition among a wide range of populations as well as the importance of accurate body composition measurements for appropriate treatment, the ambiguity that exists for accuracy within DXA warrants concern and holds important clinical implications.
Conclusion

Due to its good precision, large availability and low radiation dose, DXA is a convenient and useful diagnostic tool for body composition assessment. The past two decades in the history of DXA have been characterized by technological advances which have allowed for a time-efficient and minimal-risk method of assessing both bone mineral density and body composition. The development of the field, leading to the introduction of fan-beam densitometers, has allowed for a reduction in scan time without compromising accuracy and without increasing radiation dose substantially. The rapid introduction of improved machines has led to a plethora of studies comparing densitometers to determine the agreement between machines. Likewise, the ability of DXA to measure regional compartments has made it an attractive method for body composition assessment, thus instigating questions about the validity of body composition measurements by DXA and leading to studies comparing DXA to the in vivo gold standard for body composition appraisal, the 4-C model. Many of these studies have reported significant mean differences as well as large individual differences and inconsistent errors between DXA systems and between DXA and the 4-C model [32, 37, 72]. Thus, it may be prudent at this time to exercise caution when using DXA to improve the clinical management of body composition outcomes among populations that represent the extremes along the spectrum of fat mass. The DXA technology can be improved if the uncertainties associated with the trueness of DXA body composition measurements are addressed. The trueness of DXA for the assessment of body composition has not been tested on human cadavers, and not all DXA systems have been compared to more
accurate *in vivo* methods such as neutron activation analysis and the 4-C model; thus, studies that address these issues are needed. The improvement of bone edge detection techniques and algorithms based on studies using cadavers and phantoms of varying body composition may also help to address the uncertainty.
References


CHAPTER 3: STUDY 1

Mallinson RJ, Williams NI, Schiferl D, Southmayd ES, and De Souza MJ. Small bone size and compromised bone strength characterize the tibia in young amenorrheic exercising women.

Abstract

An energy- and estrogen-deficient environment contributes to low bone mineral density (BMD) in amenorrheic athletes; however, habitual exercise is beneficial for bone strength. To determine the opposing effects of menstrual dysfunction and exercise on bone health in amenorrheic athletes, it is essential to assess both bone mass and geometry. The purpose of this study was to determine if amenorrheic and eumenorrheic exercising women differ with respect to volumetric BMD (vBMD), bone geometry, and estimated bone strength at the tibia. Exercising women were categorized as amenorrheic (ExAmen) if they reported no menses for the past 3 months or eumenorrheic (ExEumen) if they reported ≥10 menstrual cycles in the past 12 months. Body composition and areal BMD were assessed by dual-energy x-ray absorptiometry. Volumetric BMD, bone geometry, and muscle area at the tibia were measured by peripheral quantitative computed tomography, and the bone strength index (BSI) and strength strain index (SSI) were calculated at the distal and proximal tibia, respectively. ExAmen (n=13) and ExEumen (n=12) women aged 20.6 years did not differ in height, body mass index (BMI), age of menarche, or hours of habitual physical activity. Body mass (p=0.023) and lean mass (p=0.014) were lower in ExAmen women compared to ExEumen women, but fat mass was similar between groups. Duration of amenorrhea in ExAmen women was 343 days; 69% and 33% of ExAmen and ExEumen women, respectively, reported a
history of amenorrhea. No differences in vBMD, bone area, or BSI were observed between groups at the distal tibia. At the proximal tibia, ExAmen women had smaller total bone area (p=0.005), periosteal circumference (p=0.005), SSI (p=0.010) and muscle area (p=0.016) compared to ExEumen women but greater cortical vBMD (p=0.048). Lean mass (p<0.001) and habitual physical activity (p<0.03) were positively correlated with total area, periosteal circumference, and SSI at the proximal tibia; whereas, fat mass and BMI were positively correlated with vBMD and BSI at the distal tibia (p<0.05). Compromised bone size and estimated strength at the proximal tibia of ExAmen women may indicate that the normal response of bone to mechanical loading is attenuated in an energy- and estrogen-deficient environment that persists into adulthood; however, the smaller amount of lean mass in the ExAmen group may also explain the reduced bone size and estimated bone strength at the proximal tibia in this group compared to the ExEumen women. Furthermore, it appears that lean mass may strongly influence bone strength at proximal sites; whereas, proxy indicators of body size and body fat may be more influential at distal sites.
Introduction

Exercising women with functional hypothalamic amenorrhea (FHA), a severe menstrual disturbance that is characterized by the absence of menses for at least 90 days and is causally linked to an energy deficiency, present with a unique environment for bone health. On the one hand, the metabolic and hormonal adaptations that occur in response to an energy deficiency contribute to an uncoupling of bone turnover and the subsequently low bone mineral density (BMD) that has been frequently reported among amenorrheic athletes [1-7]. Yet, on the other hand, the osteogenic influence of mechanical loading and muscle forces imposed on bone during regular exercise may serve to enhance BMD and bone size, thereby improving bone strength [8-12].

The majority of reports to date that describe bone health in athletes with FHA have used dual-energy x-ray absorptiometry (DXA) [1, 5-7], a two-dimensional imaging technique that assesses areal BMD (aBMD) but does not have the capability to measure true volumetric BMD (vBMD) or bone size. Areal BMD measurements tend to overestimate true BMD in tall, large individuals but underestimate true BMD in lean, small individuals [13]. Furthermore, both bone mass and bone geometry influence bone strength [14]; therefore, DXA is capable of capturing only one element of bone strength. As such, the advent of three-dimensional imaging techniques such as peripheral quantitative computed tomography (pQCT) allowed for the acquisition of a more complete picture of bone health by providing measures of both vBMD and bone geometry, thus allowing for the estimation of bone strength. Additionally, pQCT has the capability of assessing cortical and trabecular compartments of bone separately, thus
providing valuable information about what is occurring at the level of skeletal tissue that may contribute to the low BMD observed in amenorrheic athletes.

FHA in exercising women is typically attributable to an energy deficiency created when energy intake is inadequate to compensate for energy expenditure [15, 16]. In response to an energy deficiency, physiological alterations in circulating concentrations of metabolic hormones and growth factors occur that influence reproductive function [17-19] and BMD [5, 7, 20, 21] through both direct and indirect mechanisms. The adaptive metabolic response is proposed to contribute to the low BMD in amenorrheic athletes via suppression of bone formation [5, 22]. Furthermore, exercising women with FHA present with chronically suppressed concentrations of estradiol [16, 23], thereby contributing to an increase in bone resorption [22]. As such, the energy and estrogen deficit characteristic of amenorrheic athletes is believed to be the cause of low BMD in this population, as the deficiency of energy and estrogen work synergistically to uncouple bone remodeling.

On the other hand, mechanical loading of the skeleton is known to improve bone health, through increases in bone mass and improved bone geometry [24]. Loading of bone by gravitational [25] and muscle forces [9] lead to an increase in bone formation at the site of stress [26]. In fact, during childhood, a period when skeletal tissue is most responsive to mechanical loading, robust periosteal expansion typically occurs [27]. Consequently, bones exposed to regular loading increase in size and density [11, 12] which synergistically contributes to an increase in bone strength. Therefore, although the bone health of exercising women and girls with FHA is compromised by the energy- and
estrogen-deficient environment, participation in habitual exercise has the potential to provide a protective benefit to bone strength.

To determine the seemingly opposing effects of FHA and exercise on bone health in amenorrheic athletes, it is essential to assess both bone mass and bone geometry. Although DXA is a rapid and precise method of clinically assessing BMD and relating it to a reference population, pQCT provides an estimation of bone strength and its individual components. Gaining a better understanding of the mechanisms underlying the low BMD in amenorrheic exercising women will allow for the design and implementation of effective treatments among this population to improve bone health and prevent increased risk of osteoporosis and fractures later in life. As such, a closer look at the bone geometry of exercising women with FHA that goes beyond measures of areal BMD is warranted.

To date, there have been only five published reports examining estimated bone strength via pQCT in amenorrheic athletes [28-32]. Three of these reports were in amenorrheic adolescent and young adult athletes [29-31], one report was in young military recruits [32], and one report was in retired elite gymnasts with a history of amenorrhea [28]. These investigators reported compromised vBMD [28-30] and trabecular microarchitecture [29] among amenorrheic athletes but a larger bone area [28-30] compared to sedentary controls and eumenorrheic athletes, suggesting a protective effect of exercise on bone health. However, there are currently no reports of vBMD, bone geometry, and estimated bone strength in a sample consisting solely of exercising women with current amenorrhea. As such, the purpose of this paper was to determine if amenorrheic and eumenorrheic exercising women differ with respect to vBMD (total,
trabecular, and cortical vBMD), bone geometry (total, trabecular, and cortical area; cortical thickness; periosteal and endosteal circumference), and estimated bone strength as assessed by the bone strength index (BSI) and strength strain index (SSI) at the proximal and distal tibia. It was hypothesized that 1) amenorrheic exercising women would have a significantly lower vBMD at the tibia than eumenorrheic exercising women, 2) there would be no significant difference in bone area (total, trabecular, and cortical) between amenorrheic and eumenorrheic exercising women, and 3) amenorrheic exercising women would have a significantly lower BSI but no difference in SSI compared to eumenorrheic exercising women.

**Methods**

**Study Design**

This study was a cross-sectional analysis comparing eumenorrheic exercising women (ExEumen, n=14) and exercising women with amenorrhea (ExAmen, n=13). Women were considered exercising if they participated in at least 2 hours of purposeful physical activity per week. Menstrual status was assessed by self-reported history of menses within the past year. The current study merged data from three datasets of exercising women to include 1) baseline data from a randomized controlled trial designed to determine the effects of a 12-month intervention of increased caloric intake on indices of bone health and menstrual status in women who have severe exercise-associated menstrual disturbances (EAMD), 2) a cross-sectional study that assessed bone health in exercising women presenting with and without a bone stress injury, and 3) an observational study of female collegiate cross-country runners.
Participants

Participants were recruited by newspaper advertisements, fliers, and classroom and team announcements targeting physically active women and cross-country runners. The women with a bone stress injury (defined as a stress reaction, medial tibial stress syndrome, or stress fracture in the lower extremity) were recruited from the local sports medicine clinic. Inclusion criteria for this study were: 1) age 18-35 years; 2) good health and no history of any serious medical conditions; 3) no current clinical diagnosis of an eating disorder; 4) not taking any form of hormonal therapy for at least 6 months; 5) ≥ 2 hrs/wk aerobic exercise; 6) reporting at least 10 cycles in the past 12 months if regularly menstruating; 7) no menses for at least 90 days if amenorrheic; 8) not pregnant or lactating, and 9) non-smoking.

Sport Categorization based on Type of Loading

To describe the type of mechanical loading that the participants were habitually exposed to, the primary sport of each participant was grouped based on loading type according to a classification system by Nikander et al. [33, 34]. The primary exercise mode of each participant was assigned to one of five loading modalities as follows: high impact; odd impact; high magnitude; repetitive, low impact; and repetitive, non-impact. The high-impact loading group consisted of sports such as volleyball and hurdling that included maximal jumping and leaping. The odd-impact loading group included activities with rapidly accelerating and decelerating movements that often occur in directions that are not customary to activities of daily living. The high-magnitude loading group consisted of activities such as weight-lifting that involved high muscle force production. Weight-bearing endurance sports such as distance running were
classified as repetitive, low-impact activities; whereas, non-weight bearing endurance sports such as swimming and cycling were grouped as repetitive, non-impact activities.

Study Procedures

During an initial visit, participants were informed of the purpose, procedures, and potential risks of participation in the study before signing an informed consent approved by the Biomedical Institutional Review Board at the Pennsylvania State University. Once consent was obtained, height and weight were measured, and participants completed questionnaires to assess demographics, medical history, exercise history, menstrual history, eating behaviors [35, 36], and bone health. To assess exercise history, the type and volume of habitual physical activity performed during the past 6 months were reported. Eating behaviors were assessed using the Three-Factor Eating Questionnaire [35] and the Eating Disorder Inventory-2 [36], and bone health was evaluated using questions about prior stress fractures and family history of osteoporosis. Resting energy expenditure (REE) was assessed, and a blood sample was collected for the measurement of the metabolic hormone triiodothyronine (T3). DXA scans were performed to assess body composition and aBMD, and pQCT scans were also conducted to measure vBMD and bone geometry and to estimate bone strength.

Menstrual Status Classification

Upon study entry, classification of menstrual status was based on self-reported menstrual histories. Participants reported the number of menses that they had experienced in the past 3, 6, 9, and 12 months. Those reporting at least 10 cycles in the past 12 months were categorized as eumenorrheic (ExEumen). Those reporting no menses in the past 3 months were categorized as amenorrheic (ExAmen). Current
duration of amenorrhea was estimated based on self-report of the number of months/years since the last menstrual period or the specific date of the last menstrual period if able to be recalled. Details regarding previous history of amenorrhea were also collected.

Anthropometrics & Body Composition

Total body weight was measured by a digital scale in the laboratory, and height was measured without shoes. Body mass index (BMI) was calculated as a ratio of weight to height (kg/m²). Body weight and BMI were the average of 1 to 5 measurements depending on the specific study protocols and the amount of time elapsing between DXA and pQCT scans. Fat mass, lean mass, and percent body fat of the total body were measured by DXA (Lunar iDXA, GE Lunar Corporation, Madison, WI, enCORE 2002 software, version 6.50.069).

Resting Energy Expenditure

REE was determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA, USA) and methods previously published in detail [37]. REE was adjusted for lean body mass (REE/kg LBM) using lean mass measurements from the total body DXA scan. Predicted REE (pREE) was also calculated using the Harris Benedict equation [38]. We compared the lab-assessed REE to the predicted REE (REE/pREE) to estimate how much the measured REE deviated from the predicted REE. We have previously published data using a ratio of REE/pREE <0.90 as the operational definition of an energy deficiency [16, 22, 39, 40].
**Blood Sampling and Serum Hormone Measurement**

Total T3 was measured from fasting blood samples that were collected between 0700 and 1000h. Samples were collected 1-2 times, and repeated measurements were pooled for hormone analysis. After collection, samples were allowed to clot for at least 30 minutes at room temperature. Samples were then spun in a centrifuge at 4° Celsius for 15 minutes at 3225.6 g-force (3000 rpm) after which serum was transferred into appropriately labeled 1.5 mL microtubules and stored at -80° Celsius until analysis.

Serum total T3 concentrations were measured using previously published methods [37]. All samples from a given participant were analyzed in duplicate.

**Estimation of Bone Strength**

DXA scans of the total body, lumbar spine, and dual femur were performed to assess aBMD of the total body, anteroposterior L1-L4 spine, femoral neck, and total hip (Lunar iDXA, GE Lunar Corporation, Madison, WI, enCORE 2002 software, version 6.50.069).

Peripheral QCT scans of the dominant distal (4%) and proximal (66%) tibia were performed to assess vBMD (total, trabecular, cortical vBMD) and bone geometry (total, trabecular, and cortical area; cortical thickness, endosteal and periosteal circumference) (Stratec XCT-3000, software version 6.00B, Stratec Medical, Pforzheim, Germany). If a participant had a history of fracture in the tibia, the opposite limb was scanned. The 4% analysis was performed using contour mode 3 and a threshold of 169 mg/cm³ to find total bone. Next, peel mode 4 with a threshold of 650 mg/cm³ 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% tibia density and area analysis was
performed using cortical mode 2 and a threshold of 710 mg/cm³. For the 66% SSI
analysis, cortical mode 2 at a threshold of 480 mg/cm³ 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 density²) [28]. SSI was calculated at
the proximal site using the equation: SSI=section modulus*(cortical density/max cortical
density) [28]. 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 40 mg/cm³, and
710 mg/cm³ were used to separate fat and bone from the muscle area, respectively.

Prior to scanning, tibia length was measured with a measuring tape. The tibia was
measured from the tibial plateau to the base of the medial malleolus. pQCT scans were
performed at 4% and 66% of the tibial length, proximal to the tibial endplate. A scout
view scan was performed prior to each scan to position the reference line in the tibial
endplate according to manufacturer guidelines.

Two tibia scans of women in the ExEumen group were excluded from analysis
due to movement artifacts. As such, complete data for tibia measurements are reported
for 12 ExEumen and 13 ExAmen women.

Statistics and Data Analysis

Prior to analysis, the data were screened for outliers, normality, and homogeneity
of variance within each group. The Shapiro-Wilk test and Levene’s test were performed
to assess normality and homogeneity of variance, respectively. One very tall ExEumen

165
woman (184.9 cm) whose total bone area was identified as an outlier (2.7-3.0 standard deviations above group mean) was excluded from analysis to better meet the assumptions of the analyses used and improve the homogeneity of the samples and validity of the statistical analyses. Independent t-tests were performed to determine unadjusted group differences for variables that were normally distributed and displayed homogeneity of variance. For variables that displayed non-normal distribution and/or non-homogeneity of variance, non-parametric Mann-Whitney U tests were performed to determine unadjusted group differences for these variables. Group differences among pQCT measurements were also assessed with analysis of covariance (ANCOVA), using height and body mass index (BMI) as covariates to remove the effects of body size on the dependent variables. To meet the assumptions of the ANCOVA analyses, the models were screened for independence of the covariate and independent variable, homogeneity of variance using Levene’s test, and homogeneity of regression slopes [41]. For models with a significant result for Levene’s test suggesting heterogeneity of variance, the ratio of group variances was calculated, and a ratio <3.5 was deemed suitable for analysis [41]. Results for analyses that violated the ANCOVA assumptions were not reported [41, 42].

Correlation analyses were performed to determine the associations between pQCT measurements and anthropometrics (height, body mass, and BMI), body composition (lean mass, fat mass, muscle area), habitual physical activity (self-reported 6-month history of habitual exercise), and age of menarche. Pearson correlation coefficients were reported for normally-distributed variables; whereas, Spearman correlation coefficients were reported for variables that did not display a normal distribution (fat mass and habitual exercise).
A p-value <0.05 was considered statistically significant. Analyses were performed using SPSS software (version 19.0; Chicago, IL), and data were reported as mean ± standard error mean (SEM). Our sample size of 12 ExEumen and 13 ExAmen women provided a statistical power (1-β) of 0.84 to detect group differences in proximal tibia total area and periosteal circumference and a statistical power (1-β) of 0.76 to detect group differences in SSI.

**Results**

**Demographics and Body Composition**

Demographic characteristics of the ExEumen (n=12) and ExAmen (n=13) women are presented in Table 3.1. ExEumen and ExAmen women did not differ in age, height, BMI, or age of menarche. Both groups of exercising women reported engaging in a similar volume of exercise each week. ExAmen women had a lower body weight (p=0.023) than the ExEumen women. The two groups did not differ in percent body fat and fat mass; however, lean mass was lower (p=0.014) in the ExAmen group compared to the ExEumen group.

One woman in the ExAmen group presented with primary amenorrhea; whereas, the remainder of the women in the ExAmen group presented with secondary amenorrhea that ranged in duration from approximately 160 to 1,095 days. Sixty-nine percent of women (9/13) in the ExAmen group reported previous episodes of amenorrhea between the ages of 14-19 years. In the ExEumen group, 33% of women (4/12) reported prior amenorrhea between the ages of 12-18 years; three of these women experienced secondary amenorrhea that ranged in duration from 3-8 months, and one woman had a history of primary amenorrhea with an age of menarche of 15 years.
Table 3.1. Demographic and body composition characteristics of the exercising eumenorrheic (ExEumen) and exercising amenorrheic (ExAmen) women.

<table>
<thead>
<tr>
<th></th>
<th>ExEumen (n=12)</th>
<th>ExAmen (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>20.8±0.5</td>
<td>20.4±0.7</td>
<td>0.285</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.6±1.4</td>
<td>164.1±1.5</td>
<td>0.244</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.0±1.9</td>
<td>53.8±1.7</td>
<td>0.023</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.6±0.5</td>
<td>20.0±0.7</td>
<td>0.091</td>
</tr>
<tr>
<td>Age of menarche (yrs)</td>
<td>13.3±0.4</td>
<td>13.6±0.4</td>
<td>0.547</td>
</tr>
<tr>
<td>Duration of amenorrhea (days)</td>
<td>--</td>
<td>343±76.5</td>
<td>--</td>
</tr>
<tr>
<td>Physical activity (min/wk)</td>
<td>651.5±97.5</td>
<td>711.8±308.6</td>
<td>0.082</td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Body Fat</td>
<td>24.6±1.4</td>
<td>24.0±1.6</td>
<td>0.785</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>14.8±1.1</td>
<td>13.1±1.2</td>
<td>0.174</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>42.4±1.2</td>
<td>38.3±1.0</td>
<td>0.014</td>
</tr>
</tbody>
</table>

* n=12 ExAmen
b Self-reported 6-month history of physical activity
BMI: body mass index
Data are mean±SEM

Table 3.2 depicts the proportion of ExEumen and ExAmen women participating in each loading modality. Repetitive, low-impact loading was the primary loading modality for both groups of exercising women with 67% of ExEumen women and 77% of ExAmen women habitually participating in this type of exercise. Among the ExEumen women, 25% and 8% participated in odd impact and high impact loading activities, respectively; however, none of the ExAmen women participated in this type of
loading as their primary mode of exercise. Conversely, 15% and 8% of ExAmen women primarily engaged in non-impact and high magnitude loading activities, respectively; whereas, no ExEumen women regularly participated in these types of activities. Of the two women who primarily engaged in non-weight bearing exercise, both also participated in regular low-impact activities such as running and gym-related cardio exercises, thereby providing a weight-bearing component to their habitual exercise routine. Women reported engaging in their primary sport at the current exercise volume for the past 3.3 years (range 6 months to 10 years); however, the majority of women in both groups began participating in habitual physical activity during the childhood or adolescent years.

Table 3.2. Type of weight-bearing exercise of the eumenorrheic (ExEumen) and amenorrheic (ExAmen) women.

<table>
<thead>
<tr>
<th>Type of Loading</th>
<th>ExEumen (n=12)</th>
<th>ExAmen (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High impact</td>
<td>8.3% (1/12)</td>
<td>0% (0/13)</td>
</tr>
<tr>
<td>Odd impact</td>
<td>25.0% (3/12)</td>
<td>0% (0/13)</td>
</tr>
<tr>
<td>High magnitude</td>
<td>0% (0/12)</td>
<td>7.7% (1/13)</td>
</tr>
<tr>
<td>Repetitive, low impact</td>
<td>66.7% (8/12)</td>
<td>76.9% (10/12)</td>
</tr>
<tr>
<td>Repetitive, non-impact</td>
<td>0% (0/12)</td>
<td>15.4% (2/12)</td>
</tr>
</tbody>
</table>

High Impact: volleyball
Odd impact: softball, field hockey, dance
High magnitude: strength training
Low impact: Running, cardio exercises at gym
Non-impact: cycling, swimming
Metabolic Characteristics

Markers of metabolic status are presented in Table 3.3. The ExAmen women had a lower REE (p=0.003) and REE:pREE (p=0.003) compared to the ExEumen women. When adjusted for lean body mass, REE did not differ significantly between groups. In addition, the ExEumen and ExAmen women did not differ in circulating concentrations of total T3.

Table 3.3. Metabolic characteristics of the exercising eumenorrheic (ExEumen) and exercising amenorrheic (ExAmen) women.

<table>
<thead>
<tr>
<th></th>
<th>ExEumen (n=12)</th>
<th>ExAmen (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resting Energy Expenditure</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE (kcal/day)</td>
<td>1,247.3±32.8</td>
<td>1,071.7±42.0</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>REE/LBM (kcal/day/kg LBM)</td>
<td>29.6±0.6</td>
<td>27.9±0.8</td>
<td>0.109</td>
</tr>
<tr>
<td>REE/pREE</td>
<td>0.87±0.02</td>
<td>0.78±0.02</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td><strong>Metabolic Hormone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total T3 (ng/dl)</td>
<td>81.3±5.9</td>
<td>67.6±4.5</td>
<td>0.103</td>
</tr>
</tbody>
</table>

<sup>a</sup> n= 12 ExAmen due to unreliable REE (kcal/kg LBM/day) and REE:pREE ratio >2.5 SD above mean
REE: resting energy expenditure; LBM: lean body mass; pREE: predicted REE; T3: triiodothyronine
Data are mean±SEM

Areal BMD from DXA

Results of the aBMD measurements are presented in Table 3.4. No differences were observed between ExEumen and ExAmen women for both aBMD absolute values and Z-scores at the total body, lumbar spine, or femoral neck. However, ExAmen women had lower (p=0.042) total hip aBMD than the ExEumen women.
Table 3.4. Areal BMD characteristics of the exercising eumenorrheic (ExEumen) and exercising amenorrheic (ExAmen) women assessed by DXA.

<table>
<thead>
<tr>
<th>Bone Mineral Density</th>
<th>ExEumen (n=12)</th>
<th>ExAmen (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body aBMD (g/cm²)</td>
<td>1.157±0.028</td>
<td>1.095±0.027</td>
<td>0.120</td>
</tr>
<tr>
<td>Total Body aBMD Z-score</td>
<td>0.92±0.30</td>
<td>0.32±0.31</td>
<td>0.172</td>
</tr>
<tr>
<td>Lumbar spine (L1-L4) aBMD (g/cm²)</td>
<td>1.170±0.033</td>
<td>1.079±0.038</td>
<td>0.086</td>
</tr>
<tr>
<td>Lumbar spine (L1-L4) aBMD Z-score</td>
<td>0.01±0.28</td>
<td>-0.68±0.30</td>
<td>0.113</td>
</tr>
<tr>
<td>Femoral Neck aBMD (g/cm²)</td>
<td>1.135±0.045</td>
<td>1.030±0.040</td>
<td>0.093</td>
</tr>
<tr>
<td>Femoral Neck aBMD Z-score a</td>
<td>1.02±0.42</td>
<td>0.34±0.46</td>
<td>0.294</td>
</tr>
<tr>
<td>Total Hip aBMD (g/cm²)</td>
<td>1.150±0.042</td>
<td>1.025±0.041</td>
<td><strong>0.042</strong></td>
</tr>
<tr>
<td>Total Hip aBMD Z-score a</td>
<td>1.09±0.41</td>
<td>0.37±0.45</td>
<td>0.259</td>
</tr>
</tbody>
</table>

a n=9 ExEumen and 7 ExAmen
aBMD: areal bone mineral density
Data are mean±SEM

Volumetric BMD and Bone Geometry from pQCT

Results from pQCT measurements at the tibia are displayed in Table 3.5. At the distal tibia, no significant differences in vBMD, bone area, or BSI were observed between the ExEumen and ExAmen women even after adjusting for height and BMI. At the proximal tibia, the ExEumen women had greater total bone area (p=0.005) and periosteal circumference (p=0.005) compared to the ExAmen women, a finding that remained significant after controlling for height. Furthermore, the ExEumen women demonstrated significantly greater SSI than the ExAmen women (p=0.010), a difference which remained significant after controlling for height and BMI. Interestingly, cortical vBMD was significantly greater in ExAmen women compared to ExEumen women (p=0.048). After adjusting for BMI, this difference remained significant. Muscle area at
the proximal tibia was also greater (p=0.016) in the ExEumen women compared to the ExAmen women.

Table 3.5. Bone mineral density and bone geometry characteristics of the tibia as assessed by pQCT.

<table>
<thead>
<tr>
<th></th>
<th>ExEumen (n=12)</th>
<th>ExAmen (n=13)</th>
<th>P-value</th>
<th>P*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4% Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToD (mg/cm³)</td>
<td>315.8±10.8</td>
<td>312.6±13.8</td>
<td>0.857</td>
<td>0.808</td>
<td>0.440</td>
</tr>
<tr>
<td>ToA (mm²)</td>
<td>998.0±17.2</td>
<td>945.7±29.1</td>
<td>0.143</td>
<td>0.220</td>
<td>0.155</td>
</tr>
<tr>
<td>TrD (mg/cm³)</td>
<td>266.9±9.6</td>
<td>262.6±10.9</td>
<td>0.772</td>
<td>0.664</td>
<td>0.592</td>
</tr>
<tr>
<td>TrA (mm²)</td>
<td>805.1±14.9</td>
<td>760.2±26.8</td>
<td>0.160</td>
<td>0.242</td>
<td>0.133</td>
</tr>
<tr>
<td>CoD (mg/cm³)</td>
<td>518.5±14.3</td>
<td>511.7±21.5</td>
<td>0.799</td>
<td>0.889</td>
<td>0.460</td>
</tr>
<tr>
<td>CoA (mm²)</td>
<td>192.9±4.4</td>
<td>185.5±4.9</td>
<td>0.274</td>
<td>0.408</td>
<td>0.716</td>
</tr>
<tr>
<td>BSI (mg²/mm⁴)</td>
<td>100.9±7.1</td>
<td>93.2±7.2</td>
<td>0.453</td>
<td>0.478</td>
<td>0.862</td>
</tr>
<tr>
<td><strong>66% Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToD (mg/cm³)</td>
<td>662.5±20.5</td>
<td>700.2±23.2</td>
<td>0.238</td>
<td>0.347</td>
<td>--</td>
</tr>
<tr>
<td>ToA (mm²)</td>
<td>553.2±14.3</td>
<td>492.3±13.5</td>
<td>0.005</td>
<td>0.010</td>
<td>--</td>
</tr>
<tr>
<td>CoD (mg/cm³)</td>
<td>1110.0±4.3</td>
<td>1124.2±5.2</td>
<td>0.048</td>
<td>--</td>
<td>0.043</td>
</tr>
<tr>
<td>CoA (mm²)</td>
<td>291.6±9.7</td>
<td>275.0±7.9</td>
<td>0.198</td>
<td>0.367</td>
<td>0.606</td>
</tr>
<tr>
<td>CoTh (mm)</td>
<td>4.2±0.2</td>
<td>4.3±0.2</td>
<td>0.751</td>
<td>0.755</td>
<td>0.266</td>
</tr>
<tr>
<td>PCircum (mm)</td>
<td>83.3±1.1</td>
<td>78.6±1.1</td>
<td>0.005</td>
<td>0.010</td>
<td>--</td>
</tr>
<tr>
<td>ECircum. (mm)</td>
<td>57.1±1.7</td>
<td>51.8±1.9</td>
<td>0.053</td>
<td>0.113</td>
<td>--</td>
</tr>
<tr>
<td>SSI (mm³)</td>
<td>2330.8±84.6</td>
<td>2041.6±61.4</td>
<td>0.010</td>
<td>0.021</td>
<td>0.043</td>
</tr>
<tr>
<td>MuA (mm²)</td>
<td>6865.5±224.0</td>
<td>6057.4±215.7</td>
<td>0.016</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*ANCOVA adjusted for height
**ANCOVA adjusted for BMI
Dashes indicate where assumptions of statistical analysis were violated
n/a = not applicable
Data are mean±SEM
ToD: total density; ToA: total area; TrD: trabecular density; TrA: trabecular area; CoD: cortical density; CoA: cortical area; BSI: bone strength index; CoTh: cortical thickness; PCircum: periosteal circumference; ECircum: endosteal circumference; SSI: strength strain index; MuA: muscle area; ExEumen: exercising eumenorrheic women; ExAmen: exercising amenorrheic women

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Correlation Analysis

Correlation coefficients between pQCT measurements and anthropometric, body composition, and habitual exercise variables are displayed in Table 3.6. Height was not associated with any bone parameters at the distal tibia but demonstrated a significant positive correlation with total area, periosteal and endosteal circumference and SSI at the proximal tibia. Body mass and BMI were positively correlated with total vBMD, cortical vBMD, and BSI at the distal tibia, SSI at the proximal tibia, and cortical area at both the distal and proximal tibia (Figure 3.1). In addition, body mass was positively associated with total area and periosteal circumference at the proximal tibia; whereas, BMI was associated with trabecular vBMD at the distal site. Fat mass was positively correlated with total and cortical vBMD and BSI at the distal tibia as well as cortical area at both proximal and distal sites. Interestingly, lean mass was not correlated with any bone parameters at the distal tibia but demonstrated a significant positive correlation with total and cortical area, periosteal circumference, and SSI at the proximal tibia (Figure 3.2). Likewise, muscle area at the proximal tibia was associated with cortical area and SSI, and habitual physical activity demonstrated significant positive correlations with total area, periosteal and endosteal circumference, and SSI at the proximal site but a negative correlation with total and cortical vBMD. Age of menarche was not significantly correlated with any bone parameter measured by pQCT.
Table 3.6. Correlations between anthropometric and body composition variables and indices of bone health in exercising women

<table>
<thead>
<tr>
<th></th>
<th>Height</th>
<th>Body Mass</th>
<th>BMI</th>
<th>Fat Mass</th>
<th>Lean Mass</th>
<th>Muscle Area</th>
<th>Physical Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( r^a )</td>
<td>( P^c )</td>
<td>( r )</td>
<td>( P )</td>
<td>( \rho^b )</td>
<td>( P )</td>
<td>( r )</td>
</tr>
<tr>
<td><strong>4% Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToD</td>
<td>-0.05</td>
<td>0.800</td>
<td>0.43</td>
<td>0.034</td>
<td>0.50</td>
<td>0.011</td>
<td>0.41</td>
</tr>
<tr>
<td>ToA</td>
<td>0.23</td>
<td>0.261</td>
<td>0.18</td>
<td>0.388</td>
<td>0.06</td>
<td>0.771</td>
<td>0.20</td>
</tr>
<tr>
<td>TrD</td>
<td>-0.12</td>
<td>0.568</td>
<td>0.35</td>
<td>0.086</td>
<td><strong>0.46</strong></td>
<td><strong>0.022</strong></td>
<td>0.36</td>
</tr>
<tr>
<td>TrA</td>
<td>0.21</td>
<td>0.326</td>
<td>0.08</td>
<td>0.697</td>
<td>-0.03</td>
<td>0.884</td>
<td>0.12</td>
</tr>
<tr>
<td>CoD</td>
<td>0.10</td>
<td>0.628</td>
<td><strong>0.53</strong></td>
<td>0.006</td>
<td><strong>0.52</strong></td>
<td><strong>0.007</strong></td>
<td><strong>0.47</strong></td>
</tr>
<tr>
<td>CoA</td>
<td>0.25</td>
<td>0.221</td>
<td><strong>0.56</strong></td>
<td>0.004</td>
<td>0.47</td>
<td>0.018</td>
<td>0.42</td>
</tr>
<tr>
<td>BSI</td>
<td>0.04</td>
<td>0.852</td>
<td><strong>0.51</strong></td>
<td>0.009</td>
<td>0.54</td>
<td>0.005</td>
<td><strong>0.48</strong></td>
</tr>
<tr>
<td><strong>66% Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ToD</td>
<td>-0.23</td>
<td>0.271</td>
<td>0.12</td>
<td>0.573</td>
<td>0.27</td>
<td>0.189</td>
<td>0.14</td>
</tr>
<tr>
<td>ToA</td>
<td><strong>0.61</strong></td>
<td><strong>0.001</strong></td>
<td><strong>0.55</strong></td>
<td><strong>0.005</strong></td>
<td>0.23</td>
<td>0.260</td>
<td>0.16</td>
</tr>
<tr>
<td>CoD</td>
<td>-0.40</td>
<td>0.050</td>
<td>-0.23</td>
<td>0.274</td>
<td>-0.02</td>
<td>0.911</td>
<td>-0.15</td>
</tr>
<tr>
<td>CoA</td>
<td>0.39</td>
<td>0.054</td>
<td><strong>0.68</strong></td>
<td>&lt;0.001</td>
<td><strong>0.51</strong></td>
<td><strong>0.009</strong></td>
<td><strong>0.40</strong></td>
</tr>
<tr>
<td>CoTh</td>
<td>-0.01</td>
<td>0.974</td>
<td>0.35</td>
<td>0.085</td>
<td>0.40</td>
<td>0.050</td>
<td>0.25</td>
</tr>
<tr>
<td>PCircum</td>
<td><strong>0.61</strong></td>
<td><strong>0.001</strong></td>
<td><strong>0.54</strong></td>
<td><strong>0.005</strong></td>
<td>0.22</td>
<td>0.282</td>
<td>0.16</td>
</tr>
<tr>
<td>ECircum</td>
<td><strong>0.40</strong></td>
<td><strong>0.047</strong></td>
<td>0.15</td>
<td>0.467</td>
<td>-0.08</td>
<td>0.711</td>
<td>-0.02</td>
</tr>
<tr>
<td>SSI</td>
<td><strong>0.64</strong></td>
<td><strong>0.001</strong></td>
<td><strong>0.76</strong></td>
<td>&lt;0.001</td>
<td><strong>0.44</strong></td>
<td><strong>0.026</strong></td>
<td><strong>0.31</strong></td>
</tr>
</tbody>
</table>

\(^a r \) indicates Pearson correlation between the variables.

\(^b \rho \) indicates Spearman correlation between variables.

\(^c P \) indicates the p-value of the correlation.

\(^d \)Self-reported 6-month history of regular exercise (min/wk).

ToD: total density; ToA: total area; TrD: trabecular density; TrA: trabecular area; CoD: cortical density; CoA: cortical area; BSI: bone strength index; CoTh: cortical thickness; PCircum: periosteal circumference; ECircum: endosteal circumference; SSI: strength strain index; BMI: body mass index
Figure 3.1. Relation between body mass index and the following pQCT-derived parameters of bone health at the distal tibia: a) bone strength index and b) total vBMD. Squares represent exercising amenorrheic women (ExAmen) and triangles represent exercising eumenorrheic women (ExEumen).
Figure 3.2. Relation between lean mass and the following pQCT-derived parameters of bone health at the proximal tibia: a) strength strain index and b) total bone area. Squares represent exercising amenorrheic women (ExAmen) and triangles represent exercising eumenorrheic women (ExEumen).
Discussion

This study explored vBMD, bone geometry, and estimated bone strength among exercising women currently presenting with amenorrhea and compared their bone health to that of eumenorrheic exercising women. Contrary to our hypotheses, amenorrheic exercising women did not present with lower vBMD and BSI at the tibia but demonstrated a smaller bone area and lower SSI at the proximal tibia compared to the eumenorrheic exercising women. Such findings suggest that among young adult exercising women with amenorrhea, the energetic and hormonal environments may synergistically impair the typical osteogenic response of bone to mechanical loading.

Investigators have reported that amenorrheic athletes, to include those with either current amenorrhea or a history of amenorrhea, have lower trabecular [28] and cortical [29] vBMD and greater total [28], cortical [28], and trabecular [29] area at the tibia compared to sedentary controls [28, 29]. Reports of vBMD, bone geometry, and bone strength estimates from 3-dimensional imaging among amenorrheic athletes are limited to adolescents and young women ranging in age from 14-21 years [29-31], young military recruits [32] and retired adult gymnasts who were physically active and amenorrheic during their adolescent years but sedentary and presumably regularly-menstruating at the time of evaluation [28]. However, bone strength estimates from pQCT have not been previously reported among a sample consisting solely of young adult women who are currently exercising and amenorrheic. At the proximal tibia, our findings differed from those of other investigators [28] in that bone area and periosteal circumference were smaller and cortical vBMD was surprisingly greater in the amenorrheic women compared to the eumenorrheic women. At the distal tibia, however, the lack of a significant
difference in pQCT measurements between amenorrheic and eumenorrheic exercising women was in agreement with that observed among exercising amenorrheic adolescents and young women [29, 30] and retired young adult gymnasts with a history of amenorrhea during adolescence [28] when also compared to their eumenorrheic counterparts. These findings at the distal tibia suggest that amenorrheic women may still be gaining at least some of the skeletal benefits of exercise-induced mechanical loading, but those benefits may be site-specific as suspected by the smaller bone size and lower estimated bone strength observed at the proximal tibia in the ExAmen compared to ExEumen women. It is also notable that despite the small sample size in our study, the sample sizes of the amenorrheic and eumenorrheic athlete groups in these studies of other investigators [28-30] were comparable to that reported herein.

Ducher et al. [28] consistently observed a greater total area at both distal and proximal sites of the tibia among retired gymnasts with a history of amenorrhea compared to non-athletic controls but no significant differences were observed at either tibial site between retired gymnasts with and without a history of amenorrhea during adolescence. Likewise, among adolescent and young adult amenorrheic athletes, total area and trabecular area at the distal tibia were greater compared to non-athletic controls but no different compared to eumenorrheic athletes [29, 30]. The contradictory nature of our findings with respect to bone size and estimated strength at the proximal tibia may be explained by the different populations that were assessed. It must be noted that the women in the study by Ducher et al. [28] participated in a highly osteogenic sport, gymnastics, and reported a high training volume during the childhood and adolescent years. Because the loading imposed on the skeleton with gymnastics is starkly different
to the loading characteristic of the low-impact modality such as running that represents the majority of participants in our study, comparisons between our study and that of Ducher et al. [28] must be made with caution. Further, childhood and adolescence are critical periods of growth during which skeletal tissue is very sensitive to both the sex steroid environment and mechanical loading. The studies by Ducher et al. [28] and Ackerman et al. [29, 30] focused on amenorrhea and habitual physical activity that occurred during the adolescent years; whereas, the current study focuses on amenorrhea among young women. However, the majority of the amenorrheic women in our study reported episodes of amenorrhea during adolescence, and furthermore, most of the women began participating in physical activity during childhood or adolescence. As such, the results reported herein represent the skeletal response not only to the reproductive environment and mechanical loading of young adulthood, but also to the environment during the critical growth period.

It is well-established that rising estrogen concentrations are responsible for the inhibition of periosteal expansion and stimulation of endosteal apposition during puberty in girls [27]. Furthermore, the pre-pubertal years just prior to the drastic puberty-related increases in estrogen concentrations have been observed to be a window of time during which skeletal tissue readily responds to mechanical loading, leading to increases in bone strength [12, 43]. For this reason, adolescent athletes with amenorrhea may demonstrate greater increases in bone size compared to sedentary controls and no difference in bone area when compared to their eumenorrheic counterparts as a result of 1) periosteal expansion induced by mechanical loading and 2) suppressed estrogen concentrations which create an environment similar to the pre-pubertal years when the skeletal tissue
most readily responds to mechanical loading and periosteal expansion is without the inhibitory effects of estrogen. In an environment of suppressed estrogen concentrations, it may be surmised that amenorrheic adolescent athletes would present with greater bone area than eumenorrheic adolescent athletes who are presumably estrogen replete; however, the energy deficiency that is likely underlying the amenorrhea as previously reported [16, 17, 44] may inhibit bone's full response to mechanical loading but perhaps to a lesser extent than that observed among exercising women with amenorrhea that has persisted into adulthood.

The influence of mechanical loading and circulating estrogen concentrations on skeletal tissue may be different during adulthood compared to adolescence. During adulthood, the skeleton may demonstrate reduced mechanosensitivity to loading compared to childhood and adolescence [45-47]. As such, among amenorrheic exercising women, the nutritional component underlying reproductive dysfunction, i.e. the energy deficiency, may play a more significant role in bone health than it does in the adolescent population in which the bone’s ready response to loading and sex steroids may mask the effects of nutrition. Amenorrheic athletes present with metabolic suppression as a result of the energy deficiency, indicated by suppressed resting energy expenditure [16], elevated concentrations of ghrelin [16, 17, 44], reduced circulating concentrations of total T3 [37] and leptin [17], and decreased bioavailability of IGF-1 [48]. Leptin and IGF-1 are two hormones known to stimulate bone formation [18, 20, 49, 50]. As such, suppressed concentrations of these metabolic hormones may hinder adequate bone remodeling, thereby impairing exercise-induced gains in bone mass and geometry. Nutrition may also influence the mechanostat, playing a permissive role for bone
adaptation to loading such that an energy deficiency and poor nutrition may prevent full attainment of the exercise-induced gains in bone strength [45, 51].

Estrogen is believed to not only inhibit periosteal expansion and stimulate endosteal apposition but also to decrease the mechanostat minimum effective strain threshold, thereby causing skeletal tissue to be more sensitive to mechanical load and to more readily appropriately adapt to loading forces [52]. In this case, eumenorrheic women who have greater circulating estrogen concentrations than their amenorrheic counterparts would demonstrate greater mechanosensitivity to exercise, thereby increasing bone size as a result of loading and muscle forces. However, more recently, it has been proposed that estrogen exerts these threshold-lowering effects only at the endosteal surface, not the periosteal surface as was previously believed [52, 53]. In essence, estrogen appears to have opposing actions on the periosteal and endosteal bone surfaces. Via estrogen receptor β (ERβ) signaling, estrogen inhibits periosteal bone formation thereby hindering exercise-induced increases in bone size; whereas, binding of estrogen to estrogen receptor α (ERα) on the endosteal surface stimulates bone formation, ultimately leading to endosteal apposition and greater cortical thickness [52]. Based on this current understanding of the interaction between estrogen and the mechanostat, it would be expected that amenorrheic athletes would demonstrate greater bone size with reduced cortical thickness compared to eumenorrheic athletes and sedentary controls. This skeletal response to mechanical loading, however, was not observed in our population of adult amenorrheic athletes when compared to eumenorrheic athletes. Although it is not clear why our results did not agree with the current hypothesis of the effects of estrogen on the mechanostat, it is surmised that the nutritional inadequacies and
subsequent energy deficiency of the amenorrheic athletes that began during adolescence and persisted into adulthood for the majority of the women may play a role in hindering the expected periosteal expansion. The amenorrheic exercising women in the current study presented with a lower ratio of measured to predicted REE, suggesting that conservation of energy via suppression of REE may have been occurring to a greater extent in the amenorrheic women compared to the eumenorrheic women as a result of an energy deficit.

Contrary to the findings of other investigators who observed no significant difference in vBMD at the tibia between amenorrheic and eumenorrheic athletes [28-30], we surprisingly observed greater cortical vBMD at the proximal tibia in the amenorrheic exercising women compared to their eumenorrheic counterparts. This finding is unexpected due to the known antiresorptive effects of estrogen [54]. Ackerman et al. [29, 30] reported lower cortical vBMD at the distal tibia among adolescent and young adult amenorrheic athletes compared to sedentary adolescents and young women. Likewise, Ducher et al. [28] reported lower trabecular vBMD at the distal tibia among retired gymnasts with a history of amenorrhea compared to non-gymnast women. Although no differences in vBMD were observed at the distal tibia site between amenorrheic and eumenorrheic athletes in our study, similar to that observed by Ackerman et al. [29, 30], our findings differ in that vBMD at the proximal tibia was actually greater rather than not different in the amenorrheic compared to eumenorrheic athletes as Ducher et al. [28] observed. It is not suggested based on our findings, however, that the detrimental effects of an energy- and estrogen-deficient environment on bone mass is negated. Rather, because BMD is defined as the amount of bone mineral in
a given area, the smaller bone area of the amenorrheic exercising women may have consequently caused vBMD to be greater in the amenorrheic women. Total and cortical bone mineral content (BMC) at the proximal tibia were not different between groups (data not shown); therefore, similar BMC combined with significant differences in bone area most likely contributed to the difference observed in cortical vBMD between groups.

The lower SSI that was observed in the amenorrheic compared to eumenorrheic exercising women indicates that estrogen deficiency among exercising women may contribute to compromised bone strength and therefore presumably increased fracture risk. Contrary to our findings, Ducher et al. [28] reported that retired gymnasts with a history of amenorrhea presented with a trend (p<0.09) toward greater SSI at the proximal tibia compared to retired gymnasts without a history of amenorrhea and significantly greater (p<0.05) SSI than sedentary controls. These inconsistent findings, however, may be explained by the differences in bone area. Total area and cortical area were significantly greater at the proximal tibia in retired gymnasts with a history of amenorrhea compared to sedentary controls and were similar to that in retired athletes without a history of amenorrhea; whereas, our sample of exercising women with current amenorrhea demonstrated a smaller total area at the proximal tibia compared to the eumenorrheic women. Because the geometrical radius of the bone cross-section is included in the SSI calculation, SSI is dependent on the size of the bone such that a larger bone will have a greater SSI and, ultimately, be a stronger bone [55]. Therefore, by nature of the smaller total area and periosteal circumference of the proximal tibia in amenorrheic exercising women, resistance to torsional forces as indicated by SSI is also lower in the amenorrheic women compared to the eumenorrheic women.
The correlations observed between anthropometric and body composition variables and pQCT measurements confirmed previous findings of the osteogenic influence of lean mass [29, 30], muscle size [11], and habitual physical activity [11], particularly at the periosteal surface thus contributing to increases in bone size and strength. Neither lean mass nor history of habitual physical activity were correlated with bone strength estimates at the distal tibia; however, both demonstrated moderate to strong correlations with bone area, periosteal circumference, and SSI at the proximal tibia. Given these findings, the significantly greater lean mass and muscle area at the proximal tibia of the eumenorrheic exercising women may have contributed to the greater total area, periosteal circumference, and SSI among the eumenorrheic compared to amenorrheic exercising women. Interestingly, fat mass and BMI demonstrated moderate but significant positive correlations with vBMD, cortical area, and BSI at the distal site yet demonstrated few associations with measurements at the proximal site, suggesting that proxy indicators of body size and body fat may play an important role in bone strength at distal sites that are primarily comprised of trabecular bone and possibly experience less mechanical forces from muscles during physical activity than the proximal sites.

Limitations of this cross-sectional study include the small sample size and the use of self-reported menstrual history rather than daily urinary reproductive hormone concentrations collected for a complete cycle or monitoring period to categorize the women according to menstrual status. Notably, other studies published on this issue have similarly been limited by small samples. The previously-amenorrheic and previously-eumenorrheic groups of Ducher et al. [28] each consisted of 12 women; whereas, the
study by Ackerman et al. [29] consisted of 16 amenorrheic and 18 eumenorrheic athletes. Due to the lack of data to give us a more comprehensive picture of the reproductive and endocrine environments in the women, we were unable to determine if all women in the eumenorrheic group were having optimal, ovulatory cycles and all women in the amenorrheic group suffered from exercise-associated FHA rather than a non-exercise related endocrine pathology. Additionally, the variety of sport types and loading modalities that were represented may have also served as a potential confounding variable, particularly when considering that more eumenorrheic than amenorrheic women participated in odd impact activities that have been demonstrated to be osteogenic with increases in bone size [34]; whereas, more amenorrheic than eumenorrheic women habitually participated in non-weight bearing activities. A similar study conducted among adult athletes primarily engaging in the same type of weight-bearing activities and loading modality is needed. In addition, the heterogeneity of body composition characteristics of the two groups of exercising women is also a limitation of the current study. Due to the osteogenic influence of lean mass, the significant differences in these variables between groups introduces a confounding influence when determining the effects of menstrual status on bone health; therefore, more studies are needed exploring vBMD, bone geometry, and bone strength in exercising women of varying menstrual status but similar body composition. Further, the lack of a non-exercising control group is also a limitation of the study, hindering the comparison of our results to those of other investigators. Lastly, the lack of metabolic markers known to influence bone metabolism such as leptin and IGF-1 prevents a more complete understanding of the energetic environment of the amenorrheic and eumenorrheic exercising women.
**Conclusion**

Exercising women with a current presentation of amenorrhea demonstrated smaller total bone area and periosteal circumference and, consequently, lower SSI at the proximal tibia compared to their eumenorrheic counterparts. Less lean mass and smaller muscle area compared to eumenorrheic exercising women may have contributed to the reduced bone size and strength in the amenorrheic group. However, metabolic suppression due to an energy deficiency, as is often observed among amenorrheic athletes [16, 17, 48] and was suggested by the lower ratio of measured to predicted REE in the amenorrheic compared to eumenorrheic athletes in the current study, may also have played a role in the decreased bone strength in this population and the apparently compromised response to mechanical loading, particularly when the energy deficit and consequent amenorrhea begins during adolescence and persists into adulthood. Additionally, it appears that proxy indicators of body size and body fat, i.e., BMI and fat mass, are more strongly associated with estimated bone strength at the distal rather than proximal tibia; whereas, muscle forces and habitual exercise demonstrate strong positive correlations with estimated bone strength at the proximal but not distal tibia. However, before conclusions can be drawn regarding bone strength in exercising women with amenorrhea, more research is needed in a larger and more homogeneous sample of exercising women presenting with and without exercise-associated menstrual disturbances.
References


Forwood MR. Mechanical effects on the skeleton: are there clinical implications? Osteoporos Int 2001;12: 77-83.


CHAPTER 4: STUDY 2


**Abstract**

Reproductive function, metabolic hormones, and lean mass have been observed to influence bone metabolism and bone mass. It is unclear, however, if reproductive, metabolic and body composition factors play unique roles in the clinical measures of areal bone mineral density (aBMD) and bone geometry in exercising women. This study compares lumbar spine bone mineral apparent density (BMAD) and estimates of femoral neck cross-sectional moment of inertia (CSMI) and cross-sectional area (CSA) between exercising ovulatory (Ov) and amenorrheic (Amen) women. It also explores the respective roles of reproductive function, metabolic status, and body composition on aBMD, lumbar spine BMAD and femoral neck CSMI and CSA, which are surrogate measures of bone strength. Among exercising women aged 18-30 years, body composition, aBMD, and estimates of femoral neck CSMI and CSA were assessed by dual-energy x-ray absorptiometry. Lumbar spine BMAD was calculated from bone mineral content and area. Estrone-1-glucuronide (E1G) and pregnanediol glucuronide were measured in daily urine samples collected for one cycle or monitoring period. Fasting blood samples were collected for measurement of leptin and total triiodothyronine. Ov (n=37) and Amen (n=45) women aged 22.3±0.5 years did not differ in body mass, body mass index, and lean mass; however, Ov women had significantly higher percent body fat than Amen women. Lumbar spine aBMD and BMAD were
significantly lower in Amen women compared to Ov women (p<0.001); however, femoral neck CSA and CSMI were not different between groups. E1G cycle mean and age of menarche were the strongest predictors of lumbar spine aBMD and BMAD, together explaining 25.5% and 22.7% of the variance, respectively. Lean mass was the strongest predictor of total hip and femoral neck aBMD as well as femoral neck CSMI and CSA, explaining 8.5 – 34.8% of the variance. Upon consideration of several potential osteogenic stimuli, reproductive function appears to play a key role in bone mass at a site composed of primarily trabecular bone. However, lean mass is one of the most influential predictors of bone mass and bone geometry at weight-bearing sites, such as the hip.
Introduction

Amenorrheic exercising women typically present with low bone mineral density (BMD) when compared to their ovulating counterparts [1-3]. The poor bone health observed among amenorrheic exercising women is due to the uncoupling of bone formation and resorption that occurs in an environment of low energy availability and suppressed estrogen activity [4, 5]. Estrogen has known osteogenic benefits, serving as a key inhibitor of osteoclast action [6]. Amenorrheic women present with suppressed concentrations of estrogen which may contribute to elevated resorption and, ultimately, poor bone health as evidenced by low BMD and impaired bone geometry and microarchitecture [4, 5, 7-9]. Likewise, the metabolic environment characteristic of exercising women with functional hypothalamic amenorrhea (FHA), i.e. suppressed insulin-like growth factor-1 (IGF-1), leptin, and total triiodothyronine (TT3), may also contribute to compromised bone health due to its potentially detrimental impact on bone formation [4, 5].

In addition to suppressed reproductive hormone concentrations and altered metabolic profile, another characteristic that may differentiate amenorrheic and ovulatory women is fat mass. Exercising women with FHA typically have a lower fat mass or percent body fat than ovulatory exercising women, serving as another possible contributing factor to poor bone health due to the potentially osteogenic effects of the adipocyte-derived hormone leptin on BMD [10-12]. Furthermore, an energy deficit can cause a decrease in circulating concentrations of leptin prior to changes in fat mass, indicating that a harmful environment for bone health may be present prior to changes in body weight and may be exacerbated by subsequent changes in body composition that
occur as energy deficiency is prolonged [13]. Another large component of body composition, lean mass, has been repeatedly demonstrated to exert strong osteogenic effects on bone mass [14, 15]; however, less is known about the influence of body fat on bone health. Therefore, although reproductive function, metabolic hormones and body composition, particularly lean mass, have been observed to be important for bone health when each component is viewed individually, it is currently not clear whether reproductive function, the metabolic milieu, or body composition, to include both lean body mass and fat mass, is a stronger predictor of bone health among exercising women.

When assessing bone health, BMD is obtained from dual-energy x-ray absorptiometry (DXA), a technique that is only capable of measuring areal BMD (aBMD) rather than true volumetric BMD [16]. Areal BMD tends to underestimate true BMD in small, thin individuals and overestimate true BMD in taller, larger individuals. Therefore, an algorithm that corrects for bone size has been developed to estimate volumetric BMD (bone mineral apparent density or BMAD) at the lumbar spine [16], a site that is prone to low BMD among amenorrheic women and osteoporotic fractures in aged women [2, 3]. Investigators have previously reported low lumbar spine BMAD among amenorrheic adolescent athletes compared to eumenorrheic adolescent athletes and non-athletic girls [3, 8]. Likewise, retired elite gymnasts with a history of amenorrhea were also reported to have lower lumbar spine BMAD compared to retired gymnasts without a history of amenorrhea [9]. Among both adolescent girls and adult women with anorexia nervosa, a severe model of energy deficiency, Karlsson et al. [17] and Misra et al.[18] reported lower lumbar spine BMAD compared to age-matched controls. Therefore, it appears that both an estrogen and energy deficiency contribute to
lower lumbar spine BMAD among adolescent girls and women; however, lumbar spine BMAD has not been assessed, to date, in exercising women with a current presentation of FHA.

In addition, DXA is unable to measure bone geometry, an important component of bone strength. However, three-dimensional techniques such as quantitative computed tomography (QCT) and peripheral QCT (pQCT) which can assess bone geometry involve a higher radiation dose than DXA and may not be as readily available as DXA. Therefore, due to the widespread clinical use of DXA and the importance of bone geometry in determining bone strength and fracture risk, a method of estimating geometric properties of the femoral neck using DXA, termed hip strength analysis (HSA) has been developed [19, 20]. HSA provides an estimate of femoral neck strength via measurements of cross-sectional area (CSA) and cross-sectional moment of inertia (CSMI) [19, 21], and, in essence, has enhanced traditional DXA measurements by allowing for an estimate of not only bone mass but also bone geometry, the two key components of bone strength. Investigations that go beyond DXA-derived aBMD may not only provide a better estimate of bone strength and fracture risk in amenorrheic women but may also help to identify the determinant factors that affect skeletal fragility in this population.

The roles of body composition, metabolic status, and reproductive function in the parameters of bone health derived from DXA such as aBMD, lumbar spine BMAD, and femoral neck CSA and CSMI are currently not well-understood. Therefore, the purpose of this study is twofold. This study seeks 1) to determine if amenorrheic and ovulatory exercising women differ with regard to DXA-derived estimates of volumetric density of
the lumbar spine (lumbar spine BMAD) and femoral neck strength (femoral neck CSMI and CSA) and 2) to explore the respective roles of reproductive function, metabolic status, and body composition, i.e. fat mass and lean mass, in aBMD, lumbar spine BMAD and femoral neck CSMI and CSA. It is hypothesized that 1) amenorrheic exercising women will demonstrate lower lumbar spine BMAD, and lower femoral neck CSMI and CSA compared to ovulatory exercising women, and 2) estrogen and progesterone exposure, age of menarche, leptin and TT3, and lean mass and fat mass will be significant predictors of bone health parameters (aBMD, lumbar spine BMAD, femoral neck CSA and CSMI) among exercising women but will exert differing influences depending on the bone site.

Methods

Study Design

This study is a cross-sectional analysis comparing eumenorrheic exercising women with ovulatory menstrual cycles (Ov, n=37) and exercising women with amenorrhea (Amen, n=45). Women were considered exercising if they participated in at least 2 hours of purposeful physical activity per week. To confirm menstrual status, each woman collected daily urine samples for at least one menstrual cycle if eumenorrheic or one 28-day monitoring period if amenorrheic, and urinary concentrations of reproductive hormones were subsequently assessed. The current study includes data from a cross-sectional study that assessed the impact of menstrual function on cardiovascular and bone health in exercising women and baseline data from a randomized controlled trial designed to determine the effects of a 12-month intervention of increased caloric intake on indices
of bone health and menstrual status in premenopausal women who suffer from severe exercise-associated menstrual disturbances (EAMD).

Participants

Participants were recruited by newspaper advertisements, fliers, and classroom announcements targeting physically active women. Inclusion criteria for this study were: 1) age 18-30 years; 2) weight stable (± 2kg) for at least 3 months; 3) good health and no history of any serious medical conditions; 4) no chronic illness, including hyperprolactinemia and thyroid disease; 5) no current clinical diagnosis of an eating or psychiatric disorder; 6) non-smoking; 7) not taking any form of hormonal therapy for at least 6 months; 8) ≥ 2 hrs/wk aerobic exercise; 9) no history of a clinical diagnosis of polycystic ovarian syndrome or evidence of hyperandrogenism; 10) eumenorrheic, ovulatory cycles of 26-35 days if regularly menstruating; 11) no menses for at least 90 days or ≤3 cycles in the past 6 months if amenorrheic. It has been reported that reproductive function of women who are more gynecologically mature may be less susceptible to perturbation than that of younger women [22]; therefore, to maintain an age-matched sample, the two 30-year old Ov women who were recruited were excluded from analysis.

Study Procedures

During an initial visit, participants were informed of the purpose, procedures, and potential risks of participation in the study before signing an informed consent approved by either the Human Ethics Board at the University of Toronto or Biomedical Institutional Review Board at the Pennsylvania State University. Once consent was obtained, height and weight were measured, and participants completed questionnaires to
assess demographics, medical history, exercise history, menstrual history, eating behaviors [23, 24], and bone health.

Classification of Baseline Menstrual Status

Upon study entry, classification of menstrual status was based on self-reported menstrual histories and was confirmed by urinary estrone-1-glucuronide (E1G), pregnanediol glucuronide (PdG), and luteinizing hormone (LH) profiles and other endocrine measures during a 4-week study period. Participants collected first morning void urine samples throughout the 4-week monitoring period. Ovulatory status was determined by an LH surge and specific hormonal criteria for E1G and PdG as described below. FHA was assessed by confirming no menses in the past 90 days (n=39) or ≤ 3 cycles in the past 6 months (n=6) and documentation of chronically suppressed E1G and PdG profiles observed during the monitoring period. Participants were also asked to record menstrual bleeding patterns and any additional symptoms related to menstrual cycles.

Assessment of Menstrual Function

Menstrual function was monitored daily during the 4-week study period by assessing urinary excretion of E1G, PdG, and LH metabolites and the presence of menses as self-reported on monthly calendars. Cycles were considered eumenorrheic if menses occurred at regular intervals of 26-35 days and amenorrheic if E1G and PdG were chronically suppressed [25]. Ovulatory status was determined by day of the urinary LH surge, identified as an LH peak on the day of or after the midcycle E1G peak [26]. Specific hormonal criteria for detecting ovulation included a LH surge concentration
above 25 mIU/mL, the E1G peak concentration above 35 ng/mL, and the peak PdG concentration above 5 ug/mL during the luteal phase [4, 27, 28].

**Anthropometrics**

Total body weight was measured by a digital scale in the laboratory, and height was measured without shoes. Body mass index (BMI) was calculated as a ratio of weight to height (kg/m$^2$). Baseline values for body weight and BMI were reported as the average of 1-5 measurements collected during study participation.

**Body Composition, Bone Mineral Density, and Hip Strength Analysis**

DXA scans of the total body, lumbar spine, and dual femur (n=74) or left femur (n=8) were performed to assess body composition and BMD. Femoral neck CSMI and CSA were estimated from dual femur scans by HSA as developed by Yoshikawa et al. [19]. HSA is a feature of the GE Lunar software that is used to estimate the structural properties of the hip. From this software option, hip geometry is estimated and a hip strength index is calculated. Femoral neck CSA is a measure of mineralized bone surface at the femoral neck [29] and is calculated with the following equation: \( \text{CSA} = \int_0^w g(x) \, dx = \frac{k \, dx}{p} \Sigma PBM \) where \( g(x) \) is the length of the x-ray through bone, \( dx \) is the distance between scan lines, \( p \) is the assumed average physical density of the bone, \( PBM \) is the pseudo-bone mineral value (based on the x-ray attenuation), and \( k \) is the PBM to bone mineral content conversion factor [19]. Femoral neck CSMI is a measure of distribution of the bone mass around a centroidal axis [29] and is calculated with following equation: \( \text{CSMI} = \frac{k \, dx}{p} \left[ \Sigma PBM \, x^2 - \frac{(\Sigma PBM \, x)^2}{PBM} \right] \) [19]. HSA measurements were obtained from the automatic analysis. Precision for HSA on the Lunar iDXA has been
reported to be 3.78% for CSMI and 3.13% for CSA [30]. To correct for body size, bone mineral apparent density (BMAD), an estimate of volumetric density, was calculated for the lumbar spine L1-L4 site using the following equation: BMAD = (BMC/area^1.5) [16].

The majority of participants were scanned on either a GE Lunar Prodigy DXA scanner (n=51) (GE Lunar Corporation, Madison, WI, enCORE 2002 software, version 6.50.069) or a GE Lunar iDXA scanner (n=23) (GE Lunar Corporation, Madison, WI, enCORE 2008 software version 12.10.113) to obtain body composition and BMD measurements. Remaining participants (n=8) were scanned on a Hologic QDR4500W DXA scanner (Hologic Inc., Bedford, MA); however, for 3 of these participants, an additional dual femur scan was performed on the Lunar iDXA to obtain estimates of femoral neck geometry. Two DXA technicians (one at each site) performed the scans. Consistent with the International Society of Clinical Densitometry guidelines, cross calibration studies were performed to remove systematic bias between the systems. For the cross calibration study between the Lunar Prodigy and Lunar iDXA, fourteen subjects were scanned in triplicate on both machines. The majority (n=8) were scanned on both machines within 5 days; however, there was approximately one month between scans for some subjects (n=6). Body composition, BMD, and femoral neck geometry measurements on the Lunar Prodigy and iDXA systems were found to be highly correlated with r≥0.930 for body composition and r≥0.983 for BMD and femoral neck geometry. For the cross calibration study between the Hologic QDR4500W and the Lunar iDXA, thirty-two subjects were scanned in duplicate on both machines on the same day. High correlations (r≥0.971) between Hologic and iDXA systems were observed for
body composition and BMD measurements. Equations were derived using simple linear regression to remove biases, and BMD and body composition absolute values obtained from both the Lunar Prodigy and the Hologic QDR-4500W were calibrated to the Lunar iDXA. Femoral neck CSA and CSMI were not able to be obtained from the Hologic QDR-4500W; therefore, these measurements were only converted from the GE Lunar Prodigy to the Lunar iDXA. The same software version was used to obtain all HSA measurements.

**Exercise Testing**

Peak aerobic capacity (VO$_{2peak}$) was measured during a progressive treadmill test to volitional exhaustion using an on-line MedGraphics Modular VO$_2$ System (St Paul, MN) or SensorMedics Vmax metabolic cart (Yorba Linda, Calif., USA) using indirect calorimetry as previously published [31].

**Urinary Reproductive Hormone Measurements**

To determine estrogen and progesterone exposure, E1G and PdG urinary metabolites were compared among the participants using a modified trapezoidal integrated area under the curve (AUC) technique. To calculate AUC, the hormone concentrations for two consecutive days of the cycle were averaged; these averages were then summed to provide AUC for the cycle. All urine samples were corrected for specific gravity using a hand refractometer (NSG Precision Cells) to account for hydration status [32] which has been reported to perform as well as creatinine correction for adjusting urinary hormone concentrations [32]. The secretion of E1G and PdG metabolites in the urine parallels serum concentrations of the parent hormones [33]. Microtiter plate competitive enzyme immunoassays were used to measure the urinary
metabolites E1G and PdG. The E1G (R522-2) and PdG (R13904) assays use a polyclonal capture antibody supplied by Coralie Munro University of California (Davis, CA). For samples of 59 women, the inter-assay coefficients of variation for high and low internal controls for the E1G assay are 12.2% and 14.0% respectively. The PdG intra- and inter-assay variability was determined in-house as 13.6% and 18.7%, respectively [7, 26]. Urinary LH was determined by coat-a-count immunoradiometric assay (Siemens Healthcare Diagnostics, Deerfield, IL). The sensitivity of the LH assay is 0.15 mIU/ml. The intra- and inter-assay coefficients of variation were 1.6% and 7.1%, respectively. The intra-assay and inter-assay coefficients of variation for the reproductive hormone assays for the remaining 23 women have been previously published [4, 31].

**Blood Sampling**

Leptin and TT3 were measured from fasting blood samples that were drawn after an overnight 12-hour fast between 0700 and 1000. Samples were collected 1-2 times, and repeated measurements were pooled for hormone analysis. Samples were allowed to clot for at least 30 minutes at room temperature. Samples were then spun in a centrifuge at 4° Celsius for 15 minutes at 3225.6 g-force (3000 rpm) after which serum was transferred into appropriately labeled 1.5 mL microtubules and stored at -80° Celsius until analysis.

**Serum Hormone Analysis**

Serum leptin and TT3 concentrations were measured using previously published methods [10]. All samples from a given participant were analyzed in duplicate.
Statistics and Data Analysis

Prior to analysis, the data were screened for outliers, normality, and homogeneity of variance within each group. Logarithmic transformations were applied to lumbar spine L1-L4 BMD and BMAD data to improve normality and homogeneity of variance for group comparisons. Independent t-tests were performed to determine group differences for variables that were normally distributed and displayed homogeneity of variance. For variables that displayed non-normal distribution and/or non-homogeneity of variance, non-parametric Mann-Whitney U-tests were performed to determine group differences. Analysis of covariance (ANCOVA) using BMI as a covariate was conducted to determine group differences in bone density and geometry after adjusting for the effect of nutritional status and body size on bone density and geometry. Because some variables displayed a non-normal distribution, Spearman correlations were used to determine associations between variables.

Hierarchical linear regression was conducted to determine the strongest predictors of aBMD, BMAD, CSMI, and CSA. Prior to regression analysis, diagnostics were performed to check for influential cases, multicollinearity, heteroscedasticity, linearity, and normality. Variables included in the model were age of menarche, E1G mean, PdG mean, lean mass, fat mass, leptin, and TT3. Predictor variables were chosen because they demonstrated significant bivariate correlations or have previously been shown to be associated with the outcome variables. E1G mean and PdG mean were used as measures of estrogen and progesterone exposure rather than E1G AUC and PdG AUC to eliminate confounding effects of cycle length. To control for indicators of body mass and reproductive function in the model, lean mass and E1G mean were forced into the model.
using blocks 1 and 2; whereas, the remaining 5 predictors were placed in a third block and stepwise regression was conducted for these variables. Only predictors that had a statistically significant contribution to the model were reported.

A p-value <0.05 was considered statistically significant. Analyses were performed using SPSS software (version 19.0; Chicago, IL), and data were reported as mean ± standard error mean (SEM).

Using published results from other investigators [3, 34, 35], a sample size of 36 women per group provides adequate power (1-\(\beta\) = 0.80) to detect differences in aBMD, lumbar spine BMAD, and femoral neck CSA. However, a sample size of 52 women per group would be needed to detect differences in femoral neck CSMI.

**Results**

**Demographics**

Characteristics of the exercising women are presented in Table 4.1. Exercising ovulatory (Ov, n=37) and amenorrheic (Amen, n=45) women aged 22.3±0.5 years did not differ in body weight, height and lean body mass. However, there was a trend toward older age (p=0.056), shorter height (p=0.065), and greater BMI (p=0.050) in the Ov women compared to the Amen women. In addition, percent body fat and fat mass were significantly greater in the Ov women compared to the Amen women. Peak oxygen consumption and self-reported physical activity were not different between groups; however, there was a trend (p=0.073) toward the Amen women engaging in more minutes of exercise each week compared to the Ov women.
This sample of exercising women primarily consisted of recreational athletes (64/82, 78%); the remaining 22% (18/82) of the women were competitive athletes. Based on a classification system modified from Torstveit and Sundgot-Borgen [36], 79% (65/82) of the women participated in a leanness sport which included cycling, running, dance, gym-related cardio, aerobics, rowing, swimming, martial arts, cheerleading, and weight-lifting. Among the Amen and Ov groups, 84% (38/45) and 73% (27/37), respectively, engaged in a leanness sport. The remaining women primarily participated in non-leanness sports to include soccer, rugby, tennis, field hockey, lacrosse, and sailing. The type of weight-bearing exercise that each woman engaged in was also assessed according to a classification system used by Nikander et al. [37] which categorizes...
activities as high impact, odd impact, high magnitude, low impact, and non-weight bearing. As described in Table 4.2, the majority of Ov women participated in low impact (60%) and odd impact (27%) activities with 14% of the women primarily participating in non-weight bearing exercise. Likewise, the common form of exercise among Amen women involved low impact loading (51%), and a similar proportion of Amen women primarily participated in odd-impact and non-impact loading activities (22%).

Table 4.2. Type of weight-bearing exercise of the eumenorrheic ovulatory (Ov) and amenorrheic (Amen) women.

<table>
<thead>
<tr>
<th>Type of Loading</th>
<th>Ov (n=37)</th>
<th>Amen (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High impact</td>
<td>0% (0/37)</td>
<td>0% (0/37)</td>
</tr>
<tr>
<td>Odd impact</td>
<td>27.0% (10/37)</td>
<td>22.2% (10/45)</td>
</tr>
<tr>
<td>High magnitude</td>
<td>0% (0/37)</td>
<td>4.4% (2/45)</td>
</tr>
<tr>
<td>Repetitive, low impact</td>
<td>59.5% (22/37)</td>
<td>51.1% (23/45)</td>
</tr>
<tr>
<td>Repetitive, non-impact</td>
<td>13.5% (5/37)</td>
<td>22.2% (10/45)</td>
</tr>
</tbody>
</table>

High Impact: volleyball, activities involving jumping
Odd impact: dance, soccer, rugby, tennis, field hockey, martial arts, cheerleading, softball, aerobics
High magnitude: body building, strength training
Low impact: Running, cardio exercises at gym, triathlon and pentathlon, walking
Non impact: cycling, swimming, rowing, sailing

Bone mineral density

Areal BMD at the total body and lumbar spine (L1-L4) as well as lumbar spine BMAD were significantly lower in Amen women compared to Ov women (Table 4.3). After adjusting for BMI, the differences for lumbar spine aBMD and BMAD between groups remained significant; however, a trend (p=0.050) toward lower total body aBMD in the Amen women compared to the Ov women was observed after adjusting for BMI.
There was also a trend (p=0.050) toward lower aBMD at the total hip in Amen women compared to Ov women which was maintained albeit the relationship was weaker after controlling for BMI (p=0.097). However, femoral neck aBMD, CSMI and CSA did not differ between groups both before and after adjusting for BMI. Amen women had significantly lower Z-scores at the lumbar spine (p=<0.001) and total hip (p=0.014) compared to Ov women (Figure 4.1).

Table 4.3. Bone density and hip strength analysis characteristics of the eumenorrheic ovulatory (Ov) and amenorrheic (Amen) women.

<table>
<thead>
<tr>
<th></th>
<th>Ov (n=37)</th>
<th>Amen (n=45)</th>
<th>P-value</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone Mineral Density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Body aBMD (g/cm²)</td>
<td>1.157±0.016</td>
<td>1.106±0.013</td>
<td>0.015</td>
<td>0.050</td>
</tr>
<tr>
<td>Lumbar spine (L1-L4) aBMD (g/cm²)</td>
<td>1.199±0.023</td>
<td>1.087±0.014</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lumbar spine (L1-L4) BMAD (g/cm³)</td>
<td>0.166±0.003</td>
<td>0.148±0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Femoral Neck aBMD (g/cm²)</td>
<td>1.068±0.020</td>
<td>1.039±0.019</td>
<td>0.282</td>
<td>0.400</td>
</tr>
<tr>
<td>Total Hip aBMD (g/cm²)</td>
<td>1.095±0.019</td>
<td>1.043±0.018</td>
<td>0.050</td>
<td>0.097</td>
</tr>
<tr>
<td><strong>Femoral Neck Strength</strong> a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSMI (mm⁴)</td>
<td>10,083.0±330.5</td>
<td>9,555.2±333.4</td>
<td>0.265</td>
<td>0.515</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>158.9±3.1</td>
<td>153.0±3.0</td>
<td>0.184</td>
<td>0.342</td>
</tr>
</tbody>
</table>

a n=40 Amen subjects due to missing data
*adjusted for body mass index
aBMD: areal bone mineral density; BMAD: bone mineral apparent density; CSMI: cross sectional moment of inertia; CSA: cross-sectional area
Data are mean±SEM
Metabolic and reproductive characteristics

Circulating leptin and TT3 concentrations were significantly lower in Amen women compared to Ov women (Table 4.4). Likewise, Amen women demonstrated significantly reduced concentrations of reproductive hormones as evidenced by lower E1G AUC and E1G mean as well as PdG AUC and PdG mean compared to Ov women (Table 4.4, Figure 4.2). The average cycle length in the Ov women was 29.4±0.5 days which was significantly longer than the length of the 28-day monitoring period in Amen women (p=0.006). In addition, Ov women had a younger age of menarche than the Amen women (Table 4.4).

Figure 4.1. Bone mineral density Z-scores. This figure demonstrates the Z-score at the lumbar spine, femoral neck, and total hip for ovulatory (Ov) and amenorrheic (Amen) exercising women. *Z-score was significantly lower for Amen women compared to Ov women at the lumbar spine and total hip (p<0.05). Z-scores for femoral neck and total hip represent n=35 Ov and n=39 Amen due to lack of reference data for individuals <20 years of age.
Table 4.4. Reproductive and metabolic characteristics of the eumenorrheic ovulatory (Ov) and amenorrheic (Amen) women.

<table>
<thead>
<tr>
<th></th>
<th>Ov (n=37)</th>
<th>Amen (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reproductive Profile</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of menarche</td>
<td>12.2±0.2</td>
<td>13.2±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E1G AUC (ng*day/ml)</td>
<td>1288.6±96.0</td>
<td>621.9±48.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E1G mean (ng/ml)</td>
<td>45.2±3.4</td>
<td>23.0±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PdG AUC (µg*day/ml)</td>
<td>69.1±5.1</td>
<td>24.4±2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PdG mean (µg/ml)</td>
<td>2.4±0.2</td>
<td>0.9±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>29.4±0.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Metabolic Hormones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>8.6±0.8</td>
<td>5.1±0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TT3 (ng/dl)</td>
<td>93.1±2.4</td>
<td>79.8±3.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

E1G: estrone-1-glucuronide; AUC: area under the curve; PdG: pregnanediol glucuronide; TT3: total triiodothyronine
Data are mean±SEM

Correlation analysis

Spearman correlations to assess the association between metabolic, reproductive, and body composition variables and bone health variables are displayed in Table 4.5. Age of menarche was significantly negatively correlated with lumbar spine aBMD and BMAD. E1G AUC and E1G mean, PdG AUC and PdG mean, body weight, fat mass, lean mass, and TT3 were all significantly positively correlated with lumbar spine aBMD; whereas, E1G AUC and E1G mean, PdG AUC and PdG mean, and percent body fat demonstrated a significant positive correlation with lumbar spine BMAD. Lean body mass and body weight were positively associated with both femoral neck aBMD and total hip aBMD. Lean body mass and body weight were positively correlated with femoral neck CSMI and CSA.
Figure 4.2. 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 E1G and PdG are classic characteristics of the hormonal status in amenorrhea.
Table 4.5. Spearman correlations between reproductive, metabolic, body composition variables and bone health indices in exercising women

<table>
<thead>
<tr>
<th></th>
<th>LS aBMD</th>
<th>LS BMAD</th>
<th>FN aBMD</th>
<th>Total Hip aBMD</th>
<th>FN CSMI</th>
<th>FN CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\rho^*$</td>
<td>$P^{**}$</td>
<td>$\rho$</td>
<td>$P$</td>
<td>$\rho$</td>
<td>$P$</td>
</tr>
<tr>
<td><strong>Reproductive Function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of Menarche</td>
<td>-0.347</td>
<td>0.001</td>
<td>-0.344</td>
<td>0.002</td>
<td>-0.046</td>
<td>0.679</td>
</tr>
<tr>
<td>E1G AUC</td>
<td>0.403</td>
<td>$&lt;0.001$</td>
<td>0.362</td>
<td>0.001</td>
<td>0.008</td>
<td>0.942</td>
</tr>
<tr>
<td>E1G mean</td>
<td>0.399</td>
<td>$&lt;0.001$</td>
<td>0.369</td>
<td>0.001</td>
<td>0.010</td>
<td>0.929</td>
</tr>
<tr>
<td>PdG AUC</td>
<td>0.317</td>
<td>0.004</td>
<td>0.274</td>
<td>0.013</td>
<td>0.006</td>
<td>0.958</td>
</tr>
<tr>
<td>PdG Mean</td>
<td>0.312</td>
<td>0.004</td>
<td>0.277</td>
<td>0.012</td>
<td>0.015</td>
<td>0.897</td>
</tr>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
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</tr>
<tr>
<td>Body weight</td>
<td>0.320</td>
<td>0.003</td>
<td>0.119</td>
<td>0.288</td>
<td>0.311</td>
<td>0.004</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>0.183</td>
<td>0.101</td>
<td>0.225</td>
<td>0.042</td>
<td>-0.051</td>
<td>0.652</td>
</tr>
<tr>
<td>Fat mass</td>
<td>0.251</td>
<td>0.023</td>
<td>0.202</td>
<td>0.069</td>
<td>0.049</td>
<td>0.663</td>
</tr>
<tr>
<td>Lean mass</td>
<td>0.222</td>
<td>0.045</td>
<td>0.013</td>
<td>0.908</td>
<td>0.364</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Metabolic Hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT3</td>
<td>0.235</td>
<td>0.033</td>
<td>0.194</td>
<td>0.081</td>
<td>0.135</td>
<td>0.227</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.108</td>
<td>0.335</td>
<td>0.215</td>
<td>0.052</td>
<td>0.108</td>
<td>0.332</td>
</tr>
</tbody>
</table>

*p indicates Spearman correlation between the variables.
**P indicates the p-value of the correlation.
E1G: estrone-1-glucuronide; AUC: area under the curve; PdG: pregnanediol glucuronide; TT3: total triiodothyronine; LS: lumbar spine; aBMD: areal bone mineral density; BMAD: bone mineral apparent density; FN: femoral neck; CSMI: cross sectional moment of inertia; CSA: cross-sectional area
Regression Analysis

Results of the hierarchical regression analysis are displayed in Table 4.6. To determine the role that reproductive function, metabolic status and body composition play in bone health among exercising women, the following 7 predictors were entered into the model: age of menarche, E1G mean, PdG mean, lean mass, fat mass, leptin, and TT3. Of these 7 predictors, E1G mean and age of menarche were significant predictors of lumbar spine aBMD and BMAD, together explaining 25.5% and 22.7% of the variance, respectively. Of the variance explained by these predictors, E1G mean explained the most, contributing to 18.6% and 13.8% of the variance of lumbar spine aBMD and BMAD, respectively.

The only significant predictor of femoral neck aBMD was lean body mass, accounting for 11.4% of the variance in femoral neck aBMD. For total hip aBMD, lean body mass and age of menarche were significant predictors, together explaining 13.5% of the variance. Similarly, the most significant predictors of femoral neck CSA were lean body mass, age of menarche, and leptin concentration, explaining 27.3%, 5.8%, and 3.6% of the variance, respectively, in femoral neck CSA and thus jointly contributing to 36.7% of the variance. Likewise, lean body mass, age of menarche, and fat mass were significant predictors of femoral neck CSMI, explaining 34.8%, 5.9%, and 4.9% of the variance in femoral neck CSMI, respectively, resulting in a cumulative variability explained by the model of 45.6%.
Table 4.6. Regression analysis of reproductive, metabolic, and body composition variables as potential predictors of indices of bone health in exercising women

<table>
<thead>
<tr>
<th>Variable</th>
<th>β value</th>
<th>P-value</th>
<th>Variability Explained by Variable</th>
<th>Cumulative Variability Explained by Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lumbar spine aBMD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1G cycle mean</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>18.6%</td>
<td></td>
</tr>
<tr>
<td>Age of Menarche</td>
<td>-0.027</td>
<td>0.008</td>
<td>6.9%</td>
<td>25.5%</td>
</tr>
<tr>
<td><strong>Lumbar spine BMAD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1G cycle mean</td>
<td>0.000</td>
<td>0.003</td>
<td>13.8%</td>
<td></td>
</tr>
<tr>
<td>Age of Menarche</td>
<td>-0.005</td>
<td>0.003</td>
<td>8.9%</td>
<td>22.7%</td>
</tr>
<tr>
<td><strong>Femoral Neck aBMD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean Body Mass</td>
<td>0.009</td>
<td>0.002</td>
<td>11.4%</td>
<td>11.4%</td>
</tr>
<tr>
<td><strong>Total Hip aBMD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean Body Mass</td>
<td>0.009</td>
<td>0.002</td>
<td>8.5%</td>
<td></td>
</tr>
<tr>
<td>Age of Menarche</td>
<td>-0.021</td>
<td>0.034</td>
<td>5.0%</td>
<td>13.5%</td>
</tr>
<tr>
<td><strong>Femoral Neck CSA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean Body Mass</td>
<td>2.534</td>
<td>&lt;0.001</td>
<td>27.3%</td>
<td></td>
</tr>
<tr>
<td>Age of Menarche</td>
<td>-3.533</td>
<td>0.014</td>
<td>5.8%</td>
<td></td>
</tr>
<tr>
<td>Leptin Concentration</td>
<td>0.688</td>
<td>0.047</td>
<td>3.6%</td>
<td>36.7%</td>
</tr>
<tr>
<td><strong>Femoral Neck CSMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean Body Mass</td>
<td>277.909</td>
<td>&lt;0.001</td>
<td>34.8%</td>
<td></td>
</tr>
<tr>
<td>Age of Menarche</td>
<td>-362.020</td>
<td>0.013</td>
<td>5.9%</td>
<td></td>
</tr>
<tr>
<td>Fat Mass</td>
<td>130.645</td>
<td>0.013</td>
<td>4.9%</td>
<td>45.6%</td>
</tr>
</tbody>
</table>

E1G: estrone-1-glucuronide; AUC: area under the curve; PdG: pregnanediol glucuronide; aBMD: areal bone mineral density; BMAD: bone mineral apparent density; CSMI: cross sectional moment of inertia; CSA: cross-sectional area
Discussion

Bone strength as assessed by bone mass and geometry appears to be influenced by reproductive function, metabolic hormones, and body composition; however, these osteogenic factors exert their influence at distinct sites and with varying strength. Among exercising women displaying a wide range of reproductive, metabolic, and body composition phenotypes, our results demonstrate that upon consideration of several potential osteogenic stimuli, reproductive function as assessed by the daily measurement of reproductive hormones is a primary determinant of aBMD and estimated volumetric BMD at the lumbar spine; whereas, body composition plays a key role in determining aBMD and estimated geometrical properties such as CSA and CSMI at weight-bearing sites including the femoral neck and total hip. These findings provide further support for the complex interplay between reproductive and energetic environments in promoting optimal systemic bone health and underscore the importance of a multi-faceted approach to treating FHA that includes both weight gain and resumption of menses.

Lumbar spine BMAD was lower among the amenorrheic women compared to the ovulatory women, an observation that is consistent with findings in exercising adolescent girls with amenorrhea and girls or women with anorexia nervosa [3, 8, 12, 17]. Hence, regardless of differences in bone size, exercising women with an estrogen-deficiency and energy-deficiency, as evidenced by lower concentrations of TT3, appear to have compromised skeletal health at the lumbar spine.

On the other hand, femoral neck CSA and CSMI were not significantly different between amenorrheic and ovulatory exercising women, a result that contradicted our hypothesis as well as the findings of other investigators who have reported differences in
femoral neck CSA and CSMI among exercising girls and women [37-40]. Recent investigations in adolescent and adult female athletes revealed that femoral neck CSA was lower in oligoamenorrheic athletes compared to eumenorrheic athletes [39, 40]; however, femoral neck CSMI did not differ between athletic groups [39]. In a sample of female adolescent runners grouped according to bone turnover status, Barrack et al. [38] reported that the runners with elevated bone turnover as assessed by bone-specific alkaline phosphatase (BAP) and cross-linked telopeptides of type I collagen (CTx) of at least the 97th percentile based on a pediatric reference range had significantly lower femoral neck CSA and CSMI than runners with normal bone turnover. Likewise, a cross-sectional analysis of female athletes participating in sports of varying loading modalities revealed that the femoral neck CSA was significantly greater among athletes participating in weight-bearing sports compared to non-athletic women [37]. Those participating in non-weight bearing sports such as swimming and cycling, however, demonstrated no differences in femoral neck CSA compared to non-athletic controls, demonstrating the importance of weight-bearing activities that impose stress on the bones for optimal bone strength and health [37].

It must be noted, however, that the current sample size may not have provided adequate power to detect changes in femoral neck CSMI. Furthermore, differences in DXA systems, software, and HSA algorithms may contribute to the lack of agreement between our results and the findings of others. There are currently two different approaches of HSA. One method, termed General Electric HSA (GE HSA), is a feature of GE Lunar software and calculates geometrical properties at the femoral neck (CSA and CSMI) to obtain a femur strength index that indicates the ability of the femoral neck
to withstand force from a fall [19, 29]. A second approach created by Beck at al. [20] has been termed the John Hopkins HSA (JHU HSA) and involves the calculation hip geometry at three regions of interest: narrowest part of the femoral neck, intertrochanteric region, and the shaft [29]. Both methods have displayed good inter-scan precision of <3% [19, 20] yet are not without their limitations due to the 2-dimensional nature of DXA imaging [19, 21, 29].

In our model, BMD and BMAD at the lumbar spine were most strongly predicted by reproductive function, namely mean estrogen concentration and age of menarche. Over 60% of the lumbar vertebrae consist of trabecular bone which undergoes remodeling more rapidly than cortical bone [6, 41]. Therefore, according to our results, the lumbar vertebrae appear to be more sensitive to the reproductive environment than to metabolic hormones or body composition, likely due to the impact of estrogen on bone turnover.

Estrogen is regarded as an antiresorptive sex steroid, due to its ability to decrease osteoclast proliferation and induce osteoclast apoptosis [6]. Thus, in the presence of suppressed estrogen concentrations as is seen among post-menopausal women and amenorrheic pre-menopausal women, bone resorption typically exceeds bone formation, resulting in a net loss of bone mass [2, 4]. This loss of bone mass occurs more rapidly at sites composed primarily of trabecular bone due to the higher rate of turnover, thereby explaining why estrogen was the strongest predictor of lumbar spine BMD and BMAD in our model. In support of these results, we have previously reported that one of the strongest predictors of lumbar spine BMD in exercising women was mean E1G concentration when creating a model to determine the influence of the metabolic
hormone peptide YY on BMD [42]. Likewise, among women with anorexia nervosa, Lawson et al. [43] also reported that estradiol was a significant predictor of lumbar spine BMD.

Age of menarche has also been shown to be associated with BMD and microarchitecture in pre-, peri-, and post-menopausal women due to its impact on the timing and duration of estrogen exposure during key years of bone mineral accrual [44-48]. Chevalley et al. [46, 48] demonstrated that menarcheal age was inversely associated with aBMD, volumetric BMD, cortical density and cortical thickness at the radius and that a late menarcheal age was associated with greater risk of fracture during childhood and adolescence. Likewise, it was recently reported that age of menarche is inversely related to stiffness and failure load at the distal tibia and radius in female adolescents [49]. The negative impact of late menarche on bone health appears to persist into adulthood, contributing to low lumbar spine aBMD in the peri- and post-menopausal years [44]. An older age of menarche decreases the duration of estrogen exposure during the adolescent years when peak bone mass is achieved, potentially leading to impaired bone mineralization and a poor gain in bone mass at sites such as the lumbar spine.

For aBMD and bone geometry at the weight-bearing femoral neck and total hip sites, lean body mass was the strongest predictor accounting for 8.5 – 34.8% of the variance. These results highlight the dominant role that muscle mass has on bone strength at weight-bearing sites when compared to the roles of fat mass and the reproductive and metabolic environments and, furthermore, provide additional support for the key role of muscle forces in building strong, large bones [14, 15]. In support of our findings, lean mass has been demonstrated to be highly correlated with HSA.
measurements in female athletes [39] and a strong predictor of hip BMD and femoral
neck BMAD among anorexic adolescents [12]. In addition, Petit et al. [50] followed a
sample of premenarcheal girls aged 11.9±0.5 years until the age of 22 years and
measured femoral CSA using Beck’s JHU HSA periodically throughout the adolescent
and young adult years. Lean mass was a significant predictor of change in femoral shaft
CSA from the ages of 17 to 21.5 years and was also found to be a significant predictor of
femoral neck and shaft CSA when the participants reached the age of 21-22 years [50].

Within our model, both fat mass and leptin concentration were observed to be
significant predictors of estimated bone geometry at the weight-bearing femoral neck site.
Fat mass, another primary component of body composition, was observed to be a
significant predictor of femoral neck CSMI; whereas, leptin, a metabolic hormone
secreted from adipocytes, was observed to be a significant predictor of femoral neck
CSA. Leptin has previously been reported to be associated with BMD and BMAD at the
lumbar spine, total hip, and femoral neck among women with anorexia nervosa [12, 43],
and leptin treatment among women with hypothalamic amenorrhea has been reported to
increase bone formation and BMD at the lumbar spine [51-53]. Positive correlations
between leptin and trabecular microarchitecture have also been demonstrated [43]. Based
on our results and those of other investigators, it appears that leptin exerts osteogenic
effects on bone mass in humans; however, the mechanism of action remains unclear. In
the animal model, peripheral administration of leptin stimulates osteoblast proliferation
and inhibits osteoclastogenesis, promoting a gain in bone mass; whereas, central
administration of leptin appears to decrease bone mass, suggesting that leptin has
contrast effects depending on the skeletal region [54]. However, such findings have
not been observed in humans; thus, it is unclear whether the anabolic effect of leptin on bone mass in humans is due to direct actions on bone cells or indirect effects through fat mass, IGF-1, and estrogen [54]. Due to the link between leptin and body fat [55, 56], an increase in leptin typically indicates an increase in fat mass. In addition, increases in leptin coincide with increases in IGF-1 and estrogen, two osteogenic agents known for their strong influence on bone mass [56, 57].

Age of menarche was also a significant predictor of total hip aBMD and femoral neck CSA and CSMI, demonstrating an inverse relationship with these variables. During puberty, the rise in estrogen concentrations that accompanies menarche inhibits periosteal expansion of long bones and stimulates endosteal apposition [46, 47, 58]. Therefore, an older menarcheal age has been associated with an increase in bone diameter, leading to a greater CSA, but a smaller cortical thickness [45-47]. These findings contradict the inverse relationship between age of menarche and femoral neck CSA and CSMI that we observed; however, exercising women may show a different relationship between menarcheal age and bone geometry compared to sedentary women due to the osteogenic effects of exercise. Exercise stimulates periosteal bone formation at the site of stress, leading to increases in bone size, and estrogen enhances the effects of exercise by decreasing the threshold at which mechanical forces via exercise lead to increases in bone formation. As such, the indirect relationship between age of menarche and femoral neck CSA and CSMI may be due to the concomitant effects of exercise and estrogen on bone, working together to stimulate exercise-induced bone formation.

Contrary to our hypothesis, circulating TT3 concentration was not a significant predictor of bone mass or estimated bone geometry at any site. It is believed that the
metabolic hormone TT3 plays a role in the regulation of bone metabolism [59, 60]; however, conflicting results have led to a lack of clarity regarding the effects of TT3 on bone [38, 61, 62]. *In vitro*, TT3 has been observed to stimulate osteoblast activity, thereby enhancing bone formation [59]. These results have been supported *in vivo* by Zanker and Swaine [62] who reported that female runners with the lowest concentrations of TT3 also displayed the lowest concentrations of bone formation markers. Likewise, our dataset showed a strong correlation between TT3 concentration and pro-collagen type I N-terminal propetide (PINP), a marker of bone formation ($\rho=0.467, p<0.001$) as well as a significant positive correlation between TT3 concentration and lumbar spine aBMD (Table 2). However, in a separate report, Zanker and Swaine [61] reported that TT3 was not associated with markers of bone formation among a different sample of female distance runners. Similarly, Barrack et al. [38] grouped female adolescent runners according to bone turnover and found no difference in TT3 among the runners with high bone turnover compared to the runners with normal bone turnover. More research is needed to clarify the influence of this metabolic hormone on bone health.

Strengths of this study include the fairly large sample size and the use of daily urinary reproductive hormone concentrations for an entire cycle or monitoring period 1) to categorize the women according to menstrual status and 2) to provide an indicator of reproductive function, i.e. estrogen and progesterone exposure. Self-reported menstrual history is commonly used for categorization of menstrual status; however, self-report does not allow for the identification of subtle menstrual disturbances that occur in about 50% of exercising women despite apparently regular intermenstrual intervals [7]. This study, on the other hand, is unique in that it confirms self-reported amenorrhea by
assessing daily urinary reproductive hormone concentrations. Furthermore, estrogen and progesterone concentrations obtained from collection of daily urine samples allows for an evaluation of reproductive hormone exposure over an entire cycle, thus capturing the cyclical variations in hormone concentrations.

Limitations of the study include the use of three different DXA systems to obtain body composition and BMD measurements, and the use of two DXA systems to assess femoral neck geometry. Despite our efforts to cross-calibrate the systems, we acknowledge that differences in DXA technology, software, and technicians introduce potential sources of error and perhaps may even contribute to the lack of significant difference that we observed between the ovulatory and amenorrheic women for femoral neck CSA and CSMI as we had hypothesized. HSA algorithms assume that the femur is correctly positioned [19, 21, 29]; therefore differences in femoral positioning among technicians or inadequate femoral anteversion may be a primary source of error in our study. An additional limitation is the absence of a sedentary control group.

Conclusions

In sum, reproductive function, metabolic hormones, and body composition are important contributors to bone strength in exercising women through effects on bone mass or geometry; however, the relative contributions of these osteogenic factors vary in their strength and site of influence. Reproductive function as assessed by estrogen exposure and age of menarche appears to play a key role in bone mass at a site of primarily trabecular bone such as the lumbar spine. On the other hand, body composition, in particular lean mass, is one of the most influential predictors of bone mass and bone geometry at weight-bearing sites. Although demonstrating less of a role
than reproductive function or body composition, leptin concentration, serving as an indicator of metabolic status, is a contributor to bone strength at weight-bearing cortical sites. Interestingly, circulating TT3 concentration was not observed to be a significant predictor at any bone sites in our multivariate regression analysis; however, it did demonstrate a significant positive correlation with lumbar spine aBMD. In light of these findings, an appropriate treatment strategy for restoring bone health in amenorrheic, exercising women involves a multi-faceted approach that targets 1) resumption of menses to restore optimal estrogen concentrations, 2) increases in body weight that will increase lean mass and fat mass, and 3) improvements in energy status to increase metabolic hormone concentrations.
References


CHAPTER 5: STUDY 3

Mallinson RJ, Williams NI, Hill BR, Allaway HC, and De Souza MJ. The response of bone mineral density, estimated bone geometry, and bone health-related factors to an intervention of increased caloric intake among young women with exercise-associated menstrual disturbances.

Abstract

Among exercising women with menstrual dysfunction and low bone mass as a consequence of an energy deficiency, the non-pharmacological treatment approach of increased caloric intake to achieve weight gain and resumption of menses has improved bone mineral density (BMD) in reports of small follow-up investigations and case studies. However, the impact of a controlled intervention of increased caloric intake among women with exercise-associated menstrual disturbances (EAMD) on bone health and related factors such as body composition, metabolic status, and reproductive function has not been explored. Purpose: The purpose of this study was 1) to assess the impact of a 12-month intervention of increased caloric intake on areal BMD (aBMD), bone mineral apparent density (BMAD) and estimated bone geometry (femoral neck cross-sectional area (CSA) and cross-sectional moment of inertia (CSMI)) among women with EAMD, 2) to determine the impact of the intervention on factors known to influence bone health to include estrogen exposure, body composition, and the metabolic environment, and 3) to explore associations between changes in factors known to influence bone health and changes in aBMD and estimated bone geometry during the intervention. Methods: Exercising women between the ages of 18-35 years were recruited. Women who presented with EAMD defined as no menses in the past 3 months or \( \leq 6 \) cycles in the past 12 months were randomized to the intervention group which was instructed to increase
caloric intake 20-40% above baseline total energy expenditure needs (EAMD+Cal) or a control group which was instructed to maintain dietary and exercise behaviors (EAMD Control). Women who reported regular menstrual cycles for the past 6 months and presented with an ovulatory cycle at baseline were assigned to the Ovulatory Control (Ov Control) group. Repeated measures of dietary intake; body composition, aBMD, and estimated femoral neck geometry via dual-energy x-ray absorptiometry; circulating concentrations of the metabolic hormones leptin and insulin-like growth factor-1; and estrogen exposure via collection of daily urinary metabolites were obtained at baseline, month 3, month 6, and post-intervention (months 7-16). Results: Exercising women aged 23.0±1.0 years did not differ (p>0.05) in height, weight, body mass index, exercise volume, or body composition. Baseline lumbar spine aBMD and BMAD were lower (p=0.002) in the EAMD+Cal group compared to the Ov Control group. A 22% increase in energy intake in the EAMD+Cal group resulted in an increase in body mass and fat mass of 2.4 kg (4.5%) and 1.3 kg (11.7%), respectively, from baseline (p≤0.013). Lumbar spine aBMD and BMAD increased non-significantly by 2.2% and 2.1%, respectively, in the EAMD+Cal group yet remained lower than the Ov Control group (p≤0.003). Femoral neck CSA increased by 1.8% in the EAMD+Cal group (group*time interaction, p=0.058). Leptin concentrations demonstrated a greater percent change in the EAMD+Cal group compared to the Ov Control group (p=0.015). Percent change in fat mass and lean mass were associated with percent change in lumbar spine aBMD and estimated geometry at the femoral neck, respectively (p<0.05). Percent change in estrogen exposure was correlated with percent change in femoral neck and total hip aBMD (p<0.05) and lumbar spine aBMD and BMAD (p<0.10). Conclusion. An
intervention of increased caloric intake among women with EAMD holds potential for an effective treatment strategy to improve factors known to influence BMD, thereby also improving bone health.
Introduction

Non-pharmacological treatment of low bone mineral density (BMD) among women and girls with functional hypothalamic amenorrhea (FHA) as a result of an energy deficiency has focused on weight gain and resumption of menses. Such an approach has been extensively explored among girls or young women with anorexia nervosa [1-5], a population that represents a severe model of energy deficiency. Investigators have reported annual increases in lumbar spine and hip BMD of 3.1% and 1.8%, respectively, among anorexic women who experience weight gain concomitant with resumption of menses [1]. Furthermore, unlike that observed in non-recovered anorexic patients, no further decreases in lumbar spine BMD measures have been reported among anorexic girls who gained weight and resumed menses [2]. Interestingly, weight gain and resumption of menses do not always have to occur concomitantly for beneficial bone health outcomes. Investigators have demonstrated that weight gain, independent of resumption of menses, is associated with increases in spine [3, 4] and hip BMD [1, 3] among anorexic women. Conversely, resumption of menses without concomitant weight gain among anorexic women has been associated with a significant increase in lumbar spine BMD [1]. As such, the non-pharmacological approach of increased energy intake to achieve weight gain and resumption of menses appears to be an appropriate treatment strategy for low BMD caused by the synergistic effects of an energy- and estrogen-deficiency.

Exploration of the reversal of bone loss among physically-active women with exercise-associated menstrual disturbances (EAMD), which are also a consequence of an energy deficiency albeit less severe than that observed among the anorexic population,
has been limited to case studies [6, 7] and follow-up investigations [8-11]. The case studies demonstrated 17-26% increases in hip and spine BMD after weight gain in endurance athletes with amenorrhea and low BMD [6, 7]. Follow-up investigations ranging from 15-24 months in duration revealed increases in lumbar spine BMD among amenorrheic athletes who gained weight and, for the majority, also resumed menses; however, there was a lack of normalization of BMD values to the BMD observed in regularly-menstruating athletes [9, 10]. Similarly, an 8-year follow-up investigation of former amenorrheic and oligomenorrheic athletes demonstrated that lumbar spine BMD remained persistently low despite recovery of regular menstrual cycles and/or oral contraceptive use for several years [8]. It must be noted, however, that these women gained minimal weight during the period between study visits [8]. Therefore, results from these studies provide the impetus for further exploration of the effectiveness of a non-pharmacological strategy such as increased energy intake to address low BMD in exercising women with EAMD. Reports from interventions that focus on increased caloric intake and weight gain in a larger population of exercising women with EAMD are currently lacking and are, therefore, necessary.

Improvements in BMD with weight gain and resumption of menses, either combined or separately, may be attributed to changes in the components of body mass, i.e., lean mass and fat mass, and changes in the underlying hormonal environment, i.e., reproductive and metabolic hormones. Among girls and women undergoing nutritional treatment for anorexia nervosa, change in lean mass demonstrated a strong positive association with change in BMD [1, 12]. The influence of changes in fat mass on changes in BMD is not as clear. Among healthy girls and young women, investigators
observed that change in lean mass was a stronger predictor than change in fat mass of BMD change at all sites during the period of longitudinal growth; however, fat mass was a stronger predictor of change in femoral neck BMD during the period of post-linear growth [13]. Furthermore, lean mass and fat mass have been reported to be significant predictors of BMD at the lumbar spine [14] and hip [14, 15] among healthy premenopausal women, albeit lean mass is the stronger predictor [14]. As such, it appears that fat mass influences BMD; however, the mechanism underlying the effect of fat mass on bone health may be mediated through circulating concentrations of leptin and estrogen. Adipose tissue serves as a producer of the metabolic hormone leptin as well as small amounts of estrogen due to the aromatization of testosterone to estrogen [16]; however, after adjusting for circulating concentrations of leptin and estradiol, fat mass remains a significant predictor of BMD although the relationship is weaker after adjustment for leptin [15].

The metabolic hormones, leptin and insulin-like growth factor-1 (IGF-1), and the reproductive hormone, estrogen, are typically suppressed among energy-deficient women and girls with menstrual dysfunction [12, 17-19], serving as a link between EAMD and low BMD due to their influence on bone turnover. Leptin and IGF-1 stimulate bone formation [20-22] whereas estrogen inhibits bone resorption [23], thus highlighting the importance of optimal metabolic and reproductive environments on bone health. In fact, pharmacological treatment with recombinant human leptin [24], IGF-1 [25] and oral estrogen [26] has resulted in increases in BMD among young amenorrheic women, suggesting that physiological changes in these hormones may be associated with change in BMD among young, exercising women with EAMD.
The purpose of this paper was 1) to assess the impact of a 12-month intervention of increased caloric intake on DXA-derived areal BMD (aBMD), bone mineral apparent density (BMAD) and estimated bone geometry (femoral neck cross-sectional area (CSA) and cross-sectional moment of inertia (CSMI)) among women with EAMD, 2) to determine the impact of the intervention on factors known to influence bone health to include estrogen exposure, body composition, and the metabolic environment (leptin and IGF-1 concentrations), and 3) to explore associations between changes in factors known to influence bone health and changes in aBMD and estimated bone geometry during the intervention. It was hypothesized that women with EAMD undergoing an intervention of increased caloric intake would demonstrate an increase in lumbar spine, femoral neck and total hip aBMD and lumbar spine BMAD, but would not demonstrate an increase in femoral neck CSA and CSMI. In addition, it was hypothesized that estrogen exposure, fat mass, lean mass, and circulating concentrations of leptin and IGF-1 would increase in conjunction with an increase in body weight among exercising women with EAMD undergoing an intervention of increased caloric intake. Furthermore, it was anticipated that change in body weight and its components (i.e. fat mass and lean mass) as well as change in circulating concentrations of reproductive and metabolic hormones (i.e., estrogen, leptin, and IGF-1) would be positively associated with change in aBMD during the intervention.

Methods

Study Design: A prospective repeated measures design was used to determine the effect of an intervention of increased energy intake on DXA-derived parameters of bone health in exercising women who suffer from severe exercise-associated menstrual
disturbances (EAMD), including oligomenorrhea (long and inconsistent menstrual cycles of 36-90 days) and functional hypothalamic amenorrhea (FHA, the absence of menses for >90 days). Women with EAMD who increased energy intake across the intervention (EAMD+Cal) that ranged from 7-12 months in duration were compared to control groups with EAMD (EAMD Control) and ovulatory cycles (OV Control). This study included data from a randomized controlled trial (RCT) that was designed to assess the effects of 12 months of increased energy intake (20-40% above baseline energy requirements) on indices of bone health and menstrual status in women with EAMD, including FHA and oligomenorrhea, vs. exercising control participants with (EAMD Control) and without menstrual disturbances (OV Control). The study was conducted at two sites, University of Toronto (UT) and the Pennsylvania State University (PSU) over 7 years. Participants (OV Control and EAMD women) were recruited on a rolling basis and observed for 12 months. All participants were “exercising”, which was defined as participation in purposeful physical activity for at least two hours/week. Our analysis included only those women who completed the RCT. The majority of women completed 12 months of the study (n=40); however, 5 women in the Ov Control group and 3 women in the EAMD+Cal completed 7-10 months of the RCT. Additionally, post-intervention data for one Ov Control was collected 16 months after the start of the RCT.

Participants: Participants were young adult women (18-35 yrs) at UT and PSU. Recruitment was accomplished through the university campus and community newspapers, television, radio, and bulletin fliers. Eligibility criteria for this study were: (1) aged 18-35yr; (2) good health as determined by a medical exam; (3) body mass index (BMI) 16-25 kg/m²; (4) no chronic illness, including hyperprolactinemia and thyroid
disease; (5) currently participating in at least two hours/week of purposeful exercise; (6) non-smoker; (7) not currently dieting; (8) not taking any hormonal therapy for at least six months; (9) no current clinical diagnosis of eating or psychiatric disorders; (10) not pregnant or lactating or planning a pregnancy; (11) no medication use that would alter metabolic or reproductive hormone concentrations; and (12) no other contraindications that would preclude participation in the study. Women reporting regular menstrual cycles of 26-35 days for the past 6 months were eligible for the OV Control group; whereas, women who reported no menses in the past three months or ≤6 cycles in the past 12 months were eligible for the EAMD groups.

The progression of participants through the study is displayed in Figure 5.1. One hundred ninety-two women signed an informed consent and were assessed for eligibility during the screening phase of the study. Fifty-six women were excluded or withdrew during screening; therefore, 136 women entered the baseline period. Twenty-eight women did not complete baseline due to the time commitment (n=7), a menstrual status inconsistent with the protocol (n=5), non-compliance (n=2), starting to take oral contraceptives (n=2), and other reasons (n=12). One hundred and eight women entered the intervention. Thirty-nine women were assigned to the Ov Control group, and 35 and 34 women were assigned to the EAMD Control and EAMD+Cal groups, respectively. Fifty-five women did not complete more than 6 months of the study. Twenty-two women in the Ov Control group, 15 women in the EAMD Control group, and 16 women in the EAMD+Cal group completed >6 months of the study. Our analysis included 20 Ov Controls, 15 EAMD Controls, and 13 women in the EAMD+Cal group. Five women
**Table 5.1. Progression of study participants through the randomized controlled trial.**

- **Screening**
  - n=192

- **Baseline**
  - n=136
  - Screening Failure (n=56):
    - High BMI (n=6)
    - Moving/not in area (n=6)
    - No longer interested (n=10)
    - Time commitment (n=6)
    - Hormone abnormalities (n=7)
    - Medical concerns (n=3)
    - Inconsistent menstrual status (n=2)
    - Did not meet study criteria (n=11)
    - Other reasons (n=5)

- **Intervention**
  - n=15
  - Baseline Failure (n=28):
    - Time commitment (n=7)
    - Inconsistent menstrual status (n=5)
    - Non-compliance (n=2)
    - Started taking oral contraceptives (n=2)
    - Moving (n=1)
    - No longer interested (n=1)
    - Hormone abnormalities (n=1)
    - Other reasons (n=9)

  - Completing > 6 months
  - n=22

  - Ov Control
    - n=39
  - EAMD Control
    - n=35
  - EAMD+Cal
    - n=34

  - Excluded from analysis (n=5):
    - Inconsistent menstrual status (n=2)
    - Missing data for primary outcome (n=3)

  - Intervention Failure (n=55):
    - Non-compliant (n=9)
    - Inconsistent menstrual status (n=10)
    - Time commitment (n=9)
    - Moving (n=6)
    - Started taking oral contraceptives/hormonal medication (n=6)
    - No longer interested (n=2)
    - Medical concerns (n=3)
    - Other reasons (n=10)

- **Post-Intervention Analysis**
  - n=20
  - Ov Control
    - n=20
  - EAMD Control
    - n=15
  - EAMD+Cal
    - n=13
were excluded from our analysis due to missing post-intervention data for the primary outcome variables (n=3) and inconsistent menstrual status (n=2).

**Sport Categorization based on Loading Type.** To describe the type of mechanical loading that the participants were habitually exposed to, the primary sport of each participant was grouped based on loading type according to a classification system by Nikander et al. [27, 28]. The primary exercise mode of each participant was assigned to one of five loading modalities as follows: high impact; odd impact; high magnitude; repetitive, low impact; and repetitive, non-impact. The high-impact loading group consisted of sports such as volleyball and hurdles that included maximal jumping and leaping. The odd-impact loading group included activities with rapidly accelerating and decelerating movements that often occur in directions that are not customary to activities of daily living. The high-magnitude loading group consisted of activities such as weightlifting that involved high muscle force production. Weight-bearing endurance sports such as distance running were classified as repetitive, low-impact activities; whereas, non-weight bearing endurance sports such as swimming and cycling were grouped as repetitive, non-impact activities.

**Screening Procedures:** During the screening period, participants were informed of the purpose, procedures, and potential benefits/risks of study participation prior to signing an Informed Consent approved by the Biomedical Institutional Review Board at PSU or the Human Ethics Boards at UT. Once consent was obtained, height (cm) and weight (kg) were measured, and questionnaires were completed to assess demographic information, medical and menstrual history, eating attitudes and behaviors [29, 30], exercise participation, bone health, and psychological health [31-34]. A physical exam
was performed to determine health status and to rule out any physical signs or symptoms of polycystic ovarian syndrome (i.e., acne, hirsutism) or eating disorders. To rule out endocrine and metabolic disease, a fasting blood sample was obtained and analyzed for complete blood count, basic chemistry panel, and an endocrine panel, which included follicular stimulating hormone, luteinizing hormone (LH), estradiol, prolactin, thyroid stimulating hormone, thyroxine, total and free testosterone, and dehydroepiandrosterone sulfate. Participants completed a 3-day diet log for assessment of dietary energy intake and a 7-day exercise log for assessment of purposeful exercise energy expenditure. A research psychologist with trained expertise in clinical eating disorders completed a semi-structured interview with each participant to rule out current clinical eating disorders and other psychiatric disorders. Participants met with a registered dietitian for a structured interview designed to assess the participants’ eating patterns, relationship with food, and food preferences in an effort to determine participants’ willingness to comply with the study protocol. A dual-energy x-ray absorptiometry (DXA) scan was performed to assess body composition (percent body fat, fat mass (kg), and lean body mass (LBM) (kg)) and bone mineral density (BMD) at the total body, lumbar spine, and dual femur. For 3 women (2 Ov Control and 1 EAMD+Cal), body composition and aBMD measurements at the beginning of month 2 were used as baseline measurements to maintain within-subject consistency of scanner used.

**Baseline Procedures:** During baseline, participants collected daily urine samples for a 28-day monitoring period if in the EAMD group or for an entire menstrual cycle if in the OV Control group, and recorded menses on menstrual calendars. Participants began a calcium and vitamin D run-in period on day 1 of week 1 of baseline which
coincided with the first day of menses for the Ov Control group and a random day for the EAMD groups. All groups received oral calcium and vitamin D₃ supplements to ensure that they consumed the adequate intake (AI) of 1200 mg/per day of calcium and 400 IU of vitamin D (usual dietary intake was considered in achieving this goal and supplemented when necessary). Calcium and vitamin D₃ were used as control measures similar to other studies of bone health [35-38]. The dosage targeted was the current recommendation for AI for both calcium and vitamin D₃ [39, 40].

During baseline week 3, participants arrived at the Women’s Health and Exercise Laboratory between 600-830hr (fasted and having refrained from exercise and caffeine for the prior 24hr and alcohol for the prior 12hr) and completed the following: (1) body weight and body composition measurement (via DXA), (2) resting energy expenditure (REE) assessment, (3) blood sampling for the determination of metabolic hormones (specifically leptin and IGF-1), iv) peak oxygen uptake (VO₂peak) test to evaluate aerobic fitness (often completed on a separate occasion) and v) a 3-day diet log and a 7-day exercise and activity log (see below).

1) Anthropometric Assessment: Total body weight was measured to the nearest 0.1 kg each week during baseline. Height was measured to the nearest 1.0 cm. BMI was calculated as the body mass divided by height squared (kg/m²). Baseline values for body weight and BMI were reported as the average of all baseline and screening measurements.

2) Resting Energy Expenditure Test: REE was determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA, USA) by methods previously published in detail [41, 42]. REE was adjusted for lean body mass
(REE/LBM) using lean mass measurements from the screening or baseline total body DXA scan. We also compared lab-assessed REE to a prediction equation [43] for REE to estimate how much each individual’s measured REE differed from the predicted REE (REE/pREE) [42, 44-47]. In women with anorexia nervosa [48-50], the majority of data published utilized the Harris-Benedict equation [43] to predict REE, and as such, we determined that this equation was most useful for our purposes. REE was used to calculate baseline energy expenditure needs as explained below.

3) Dietary Energy Intake: Energy intake (kcal·day⁻¹) was assessed using three-day diet logs recorded for two week days and one weekend day, as previously described [42, 51]. Baseline values for energy intake were reported as the average of baseline and screening measurements. Three-day diet logs recording energy intake have been shown to provide comparable data to 7-day logs in women who may under-report their energy intake, including lean women [52]. Additionally, 3-day diet logs have been shown to reduce participant burden and improve compliance [53]. On-site registered dietitians met with the participants to instruct them on how to accurately record energy intake. Participants were specifically instructed to measure (using standard measuring cups/tools) and record all food and beverages consumed in detail. The nutrient data from the 3-day logs were coded and analyzed using the Nutrition Data System for Research (NDSR 2008 Version; University of Minnesota; Minneapolis, MN, USA).

4) Purposeful Exercise Energy Expenditure: Participants kept logs of their purposeful exercise each week of the study as per a previous publication [42, 51]. These logs provided a measurement of exercise volume over a 7-day period (min/wk). Purposeful exercise energy expenditure (EEE) was estimated at baseline using a polar
heart rate monitor. Energy expended during these purposeful exercise sessions was measured using the OwnCal feature of the Polar S610 or RS400 heart rate monitors (Polar Electro Oy, Kempele, Finland) [54]. The OwnCal feature has been validated for the use in calculating exercise energy expenditure from heart rate. The Polar S601 and RS400 heart rate monitors include rest in their estimation of energy expenditure. To estimate only exercise energy expenditure, we subtracted the measured REE (kilocalories/min) from the Polar heart rate monitors’ estimation of energy expenditure. For purposeful exercise sessions in which participants did not wear the Polar S610 or RS400 heart rate monitors, the Ainsworth et al. [55, 56] compendiums of physical activities were used to determine the appropriate metabolic equivalent (MET) level for the exercise performed [57]. To calculate the energy expended during the exercise session, the MET level was multiplied by the duration (min) of the exercise session and the measured REE (kcal/min). The MET value includes a resting component. To estimate only exercise energy expenditure, we subtracted the measured REE (kcal/min) from this value.

5) VO\textsubscript{2peak} Test: VO\textsubscript{2peak} (mL/kg\textsuperscript{*}min) was measured during a progressive treadmill test to volitional exhaustion using indirect calorimetry on a single occasion during the study as per a previous publication [42].

6) Blood Sampling and Serum Hormone Measurement: Fasting blood samples were collected between 0700 and 1000h once during week 3 of baseline and once at the end of baseline. The latter two samples were pooled for all baseline hormone analyses. After collection, samples were stored and processed as previously described [42].
Serum leptin concentration was measured using a solid-phase sandwich enzyme-linked immunoassay (ELISA) for total leptin (Millipore, St. Charles, MI). The content of leptin in samples was calculated from a standard curve generated in each assay with recombinant human leptin. The inter-assay and intra-assay coefficients of variation for the low control were 6.2% and 4.6%, respectively. This assay is sensitive to leptin concentrations of 0.78 ng/ml; however, for samples below this sensitivity, a sensitive assay procedure was performed, reducing the sensitivity of the assay to 0.195 ng/ml. For the samples that remained below this low sensitivity, the sensitivity of the assay (0.195 ng/ml) was used to estimate the concentration of leptin in the sample. IGF-1 was analyzed using an enzyme-linked immunosorbent assay (ELISA) (Enzo Life Sciences, Farmingdale, NY). Sensitivity for the assay was 34.2 pg/mL. For samples below the sensitivity of the assay, the value for sensitivity (34.2 pg/ml) was used to estimate IGF-1 concentration. The intra-assay and inter-assay coefficients of variation were 5.8% and 7.1%, respectively. All samples from a given participant were analyzed in duplicate.

Classification of Baseline Menstrual Status: Initial classification of menstrual status prior to the intervention was based on self-reported menstrual history, results of physical exam, and urinary estrone-1-glucuronide (E1G), pregnanediol glucuronide (PdG), and LH profiles. Participants recorded menses and/or other menstrual symptoms (i.e., cramps, spotting, discharge, etc.) daily on menstrual calendars. Eumenorrheic women collected daily urine samples for one menstrual cycle, oligomenorrheic women for no more than 90 days, and women with FHA for one 28-day monitoring period. Menstrual cycle length was defined as the number of days from the first day of menses up to the day preceding the next menses [18, 58]. Daily first morning void urine samples
were assayed for LH, E1G, and PdG to assess ovulatory status. Ovulatory status was determined by the day of the urinary LH surge, identified as an LH peak on the day of or within a few days after the mid-cycle E1G peak [18, 58]. Specific hormonal criteria for detecting ovulation included a LH surge concentration above 25 mIU/mL, an E1G peak concentration above 35 ng/mL and a peak PdG concentration above 5 µg/mL during the luteal phase [18, 41, 59, 60]. An anovulatory cycle was defined as a cycle in which minimal increases in E1G was observed concomitantly with a failure of LH to rise at midcycle, or when a luteal phase exhibited no increase in PdG concentration from a 5-day follicular phase baseline or when the peak PdG value was below 2.49 µg/mL [41, 60, 61].

Menstrual status was defined as EAMD if a participant was FHA (reported no menses for the past 3 months) or oligomenorrheic (reported irregular menses at intervals of 36-90 days). Menstrual status was defined as ovulatory if a participant was eumenorrheic (reported regular menses at intervals of 26-35 days) and ovulatory. Self-reported menstrual status was then confirmed prospectively by classifying menstrual cycles by length of the intermenstrual interval, length of follicular and luteal phases, the presence of menses, urinary concentrations of E1G and PdG, and by ovulatory status (ovulatory or anovulatory) as described in previous publications from our lab [18, 58]. As such, women within the EAMD group were categorized as amenorrheic if they reported no menses in the 3 months prior to the intervention and presented with suppressed concentrations of E1G and PdG at baseline and oligomenorrheic if they reported menses within the 90 days prior to the intervention or demonstrated hormonal activity indicative of ovulation during the baseline period.
Urinary Reproductive Hormone Measurements. To determine estrogen and progesterone exposure, concentrations of E1G and PdG urinary metabolites were assessed using a modified trapezoidal integrated area under the curve (AUC) technique. To calculate AUC, the hormone concentrations for two consecutive days of the cycle were averaged; these averages were then summed to provide AUC for the cycle (if eumenorrheic or oligomenorrheic) or monitoring period (if amenorrheic). All urine samples were corrected for specific gravity using a hand refractometer (NSG Precision Cells) to account for hydration status [62] which has been reported to perform as well as creatinine correction for adjusting urinary hormone concentrations [62]. The secretion of E1G and PdG metabolites in the urine parallels serum concentrations of the parent hormones [63]. Microtiter plate competitive enzyme immunoassays were used to measure the urinary metabolites E1G and PdG. The E1G (R522-2) and PdG (R13904) assays use a polyclonal capture antibody supplied by Coralie Munro University of California (Davis, CA). The inter-assay coefficients of variation for high and low internal controls for the E1G assay are 12.2% and 14.0% respectively. The PdG intra- and inter-assay variability was determined in-house as 13.6% and 18.7%, respectively [18, 61]. For samples below or above the sensitivity of the assay, the high and low sensitivity values were used to estimate E1G (3.9-250 ng/ml) and PdG (97.5-25,000 ng/ml) concentrations. Urinary LH was determined by coat-a-count immunoradiometric assay (Siemens Healthcare Diagnostics, Deerfield, IL). The sensitivity of the LH assay is 0.15 mIU/ml. The intra- and inter-assay coefficients of variation were 1.6% and 7.1%, respectively.
**Intervention Procedures**

Prior to the start of the intervention, participants with EAMD were randomly assigned to either a treatment group (EAMD+Cal) or a control group (EAMD Control). An ovulatory control group was also included (OV Control).

1) **Energy Prescription:** The EAMD participants randomly assigned to the treatment group (EAMD+Cal) were provided an energy prescription of increased energy intake 20-40% above baseline energy requirements and asked to maintain their usual exercise training regimen for the intervention phase of the study. Baseline energy requirements for this study were operationally defined as the sum of laboratory-measured REE and purposeful EEE. Participants in the EAMD+Cal group were requested to increase their energy intake through the use of nutritional and sports energy supplements or with foods they typically eat. Energy bars (primarily PowerBars, Clif Bars) that contained approximately 220-300 calories were provided by the research staff and used as a strategy to increase energy intake throughout the day. Participants in the EAMD Control and Ov Control groups were asked to maintain baseline physical activity level and energy intake, and were monitored in a similar manner to the EAMD+Cal group.

2) **Nutritional Intake Monitoring:** The EAMD+Cal participants met with a registered dietitian at screening and baseline then bi-weekly for the first 3 months and monthly for the remainder of the study. The dietitian evaluated whether the participant was a good candidate for a study in which they may have to increase energy intake. The dietitian also provided the participant with specific instruction on how to record a 3-day diet log to accurately assess their food and beverage intake during the study. Calcium intake was also assessed [64]. Those participants with dietary habits that did not comply
with study protocol were excluded. Participants were monitored by the dietitian for compliance to energy prescription (i.e., review participants’ diet logs and provide strategies to achieve prescribed energy intake) and changes in nutritional and eating behavior characteristics. The participants in the EAMD and OV Control groups met with the registered dietitian at monthly and 3-month intervals throughout the study, respectively.

3) Psychological Status/Behavior Monitoring: The EAMD+Cal participants met with a clinical psychologist bi-weekly for the first three months and then monthly for the remainder of the study to monitor general psychological and eating behavior status and provide assistance in implementing the energy prescription and other lifestyle changes to ensure compliance to the intervention. The participants in the EAMD and OV Control groups met with the clinical psychologist at monthly and 3-month intervals throughout the study, respectively.

4) Study Protocol: Assessment of body mass, energy intake (3-day diet logs), body composition (lean mass and fat mass via DXA), and metabolic hormones (fasting leptin and IGF-1) were repeated at months 3 and 6 of the intervention and at post-intervention (months 7-16). For one woman in the EAMD Control group, metabolic hormones at month 5 of the intervention are reported because the woman was not able to visit the lab at month 6. Areal BMD at the total body, lumbar spine, and dual femur was assessed at month 6 and post-intervention (months 7-16). OV Control women collected daily urine samples for an entire menstrual cycle on 2-3 occasions during the intervention; whereas EAMD+Cal and EAMD Control groups collected daily urine samples for the entire intervention. Menstrual bleeding/symptoms and exercise training
were monitored throughout the study using menstrual calendars and seven-day purposeful exercise logs.

5) Recovery of menstrual function. Successful recovery of menses was defined as the first occurrence of menstrual bleeding during the intervention, if amenorrheic, or a shortened intermenstrual interval, i.e. more frequent menses, during the intervention compared to the 12-month period immediately preceding the study, if oligomenorrheic.

Primary Outcome Measures:

1) Areal BMD and Femoral Neck Geometry. DXA scans of the total body, lumbar spine, and dual femur were performed to assess areal BMD. To correct for body size, BMAD, an estimate of volumetric density, was calculated for the lumbar spine L1-L4 site using the following equation: $BMAD = (BMC/\text{area}^{1.5})$ [65].

Femoral neck CSMI and CSA were estimated from dual femur scans by HSA as developed by Yoshikawa et al. [66]. HSA is a feature of the GE Lunar software that is used to estimate the structural properties of the hip. From this software option, hip geometry is estimated and a hip strength index is calculated. Femoral neck CSA is a measure of mineralized bone surface at the femoral neck [67] and is calculated with the following equation: 

$$CSA = \int_0^w g(x) \, dx = \frac{k \, dx}{p} \sum PBM$$

where $g(x)$ is the length of the x-ray through bone, $dx$ is the distance between scan lines, $p$ is the assumed average physical density of the bone, $PBM$ is the pseudo-bone mineral value (based on the x-ray attenuation), and $k$ is the PBM to bone mineral content conversion factor [66]. Femoral neck CSMI is a measure of distribution of the bone mass around a centroidal axis [67] and is calculated with following equation:

$$CSMI = \frac{k \, dx}{p} \left[ \sum PBM x^2 - \left( \frac{\sum PBMx}{PBM} \right)^2 \right]$$ [66].

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HSA measurements were obtained from the automatic analysis. Precision for HSA on the Lunar iDXA has been reported to be 3.78% for CSMI and 3.13% for CSA [68].

Participants were scanned on either a GE Lunar Prodigy DXA scanner (n=22) (GE Lunar Corporation, Madison, WI, enCORE 2002 software, version 6.50.069) or a GE Lunar iDXA scanner (n=26) (GE Lunar Corporation, Madison, WI, enCORE 2008 software version 12.10.113). Two DXA technicians (one at each site) performed the scans for BMD. Consistent with the International Society of Clinical Densitometry guidelines, cross calibration studies were performed to remove systematic bias between the systems. For the cross calibration study between the Lunar Prodigy and Lunar iDXA, fourteen subjects were scanned in triplicate on both machines. The majority (n=8) were scanned on both machines within 5 days; however, there was approximately one month between scans for some subjects (n=6). Body composition, BMD, and femoral neck geometry measurements on the Lunar Prodigy and iDXA systems were found to be highly correlated with $r \geq 0.930$ for body composition and $r \geq 0.983$ for BMD and femoral neck geometry. Equations were derived using simple linear regression to remove biases, and BMD and body composition absolute values obtained from the Lunar Prodigy were calibrated to the Lunar iDXA. The same software version was used to obtain all HSA measurements.

2) $E1G \ AUC$. To assess change in estrogen exposure from baseline to post-intervention, 3-month projected baseline E1G AUC was compared to 3-month E1G AUC during the latter half of the intervention. For each woman, baseline E1G AUC was multiplied by 3 (for eumenorrheic baseline cycles and 28-day monitoring period) or by 2 (for oligomenorrheic cycles) to estimate 3-month baseline estrogen exposure. Beginning
with the last complete cycle, E1G AUC for the latter half of the intervention was calculated for the comparable number of days as the 3-month projected baseline E1G AUC. For 6 Ov Control subjects, 2-month E1G exposure was assessed to maintain consistency of number of days between baseline and intervention E1G AUC.

**Statistical Analyses:**

Data was screened for normality using the Shapiro-Wilk test, homogeneity of variance using Levene's test, sphericity, and outliers prior to analysis. For variables that violated the assumptions, log and square root transformations were applied to establish normality and homogeneity of variance. If the distribution was not able to be fixed with a transformation, non-parametric analyses were used when possible.

Baseline characteristics were assessed using a one-way analysis of variance (ANOVA). If a main effect of group was observed, post hoc analyses using Gabriel's test were performed. Gabriel's procedure is recommended for use when sample sizes between groups are unequal [69]. The log transformation was applied to the following variables for baseline comparisons: self-reported volume of habitual exercise, total body BMD, femoral neck CSMI, and E1G AUC and mean. The Kruskal Wallis test was performed for variables that violated the ANOVA assumptions and were not transformed. When a significant difference among groups was observed, follow-up analyses using Mann Whitney U tests with the Bonferroni correction to adjust for multiple comparisons were completed.

Repeated measures ANOVA using a one between, one within design with two factors (group and time) were conducted for longitudinal analyses of BMD, estimated femoral neck geometry, body composition, and metabolic and reproductive hormones.
Repeated measures ANOVA was conducted using both absolute measures and percent change scores. Three groups were included in the analysis, i.e. EAMD+Cal, EAMD Control, and Ov Control. When assessing absolute measurements and percent change of body composition and metabolic hormones over time, four time points were used (baseline, month 3, month 6, post-intervention); whereas, 3 time points (baseline, month 6, and post-intervention) were used to assess absolute measurements and percent change of BMD and estimated bone geometry variables. Two time points (baseline and end of study) were used to assess change in E1G AUC during the intervention. When significant main effects were observed, pairwise comparisons were performed using the Bonferroni correction to adjust for multiple comparisons. When a significant interaction was observed, simple effects tests were completed using the Bonferroni correction to adjust for multiple comparisons. For longitudinal analysis of percent change in energy intake from baseline to the latter part of the study, a one-way ANOVA was used to determine differences in percent change of energy intake among groups. One woman in the EAMD Control group was missing month 6 data from the DXA scan and, therefore, was excluded from longitudinal analyses of BMD, estimated bone geometry, and body composition. In addition, one woman in the EAMD Control did not have reliable longitudinal data for E1G AUC and, therefore, was excluded from longitudinal analyses of reproductive hormones.

Pearson correlations were performed to determine associations between change in reproductive, metabolic, and body composition characteristics and change in bone health characteristics during the intervention. For variables that did not display a normal distribution according to the Shapiro Wilk test (change in leptin concentrations and E1G
AUC), Spearman correlations were performed. Correlation analyses were performed in the entire sample of exercising women.

A p-value <0.05 was considered statistically significant, and two-tailed p-values were reported. Analyses were performed using SPSS software (version 19.0; Chicago, IL), and data were reported as mean ± standard error mean (SEM).

**Results**

**Baseline Characteristics**

*Demographics and Body Composition.* Baseline demographics and body composition of the exercising women are provided in Table 5.1. Ov Control (n=20), EAMD Control (n=15), and EAMD+Cal (n=13) women did not differ (p>0.05) in age, height, weight, BMI, or body composition characteristics to include percent body fat, fat mass, and lean mass. Self-reported participation in habitual physical activity and laboratory-assessed VO2peak were also similar among groups. Women in the EAMD Control and EAMD+Cal groups reported a younger age of menarche compared to the Ov Control group (p=0.001). The primary sport type of the majority of the women (58%) involved repetitive, low impact loading. Specifically, in the EAMD+Cal group, 69% (9/13) engaged in repetitive, low impact activities; whereas, 8% (1/13) participated in odd impact activities, 8% (1/13) participated in high magnitude activities, and 15% (2/13) regularly engaged in non-impact activities. In the EAMD Control group, 53% (8/15) of the women participated in low-impact activities, 13% (2/15) engaged in high impact activities, 20% (3/15) engaged in odd impact activities, and another 13% (2/15) engaged in non-weight bearing activities. In the Ov Control group, 55% (11/20) of the women
participated in low-impact activities; whereas, 40% (8/20) and 5% (1/20) engaged in odd impact and non-weight bearing activities, respectively.

Table 5.1. Baseline demographic and body composition characteristics of the three groups of exercising women.

<table>
<thead>
<tr>
<th></th>
<th>Ov Control (n=20)</th>
<th>EAMD Control (n=15)</th>
<th>EAMD+Cal (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>24.2±1.2</td>
<td>21.2±0.8</td>
<td>23.7±1.0</td>
<td>0.127</td>
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<td>Age of Menarche (yrs)</td>
<td>12.2±0.2</td>
<td>14.0±0.6a</td>
<td>13.9±0.4a</td>
<td><strong>0.001</strong></td>
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<tr>
<td>Height (cm)</td>
<td>163.6±1.1</td>
<td>166.3±1.5</td>
<td>165.6±1.8</td>
<td>0.344</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.9±1.0</td>
<td>57.9±2.1</td>
<td>57.3±1.9</td>
<td>0.760</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0±0.3</td>
<td>20.9±2.3</td>
<td>20.9±0.6</td>
<td>0.135</td>
</tr>
<tr>
<td>Physical activity (min/wk)</td>
<td>520.0±88.7</td>
<td>451.8±56.4</td>
<td>600.4±129.4</td>
<td>0.856</td>
</tr>
<tr>
<td>VO₂ peak (ml/kg*min) *</td>
<td>48.5±1.9</td>
<td>45.5±2.7</td>
<td>48.6±1.7</td>
<td>0.578</td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>% Body Fat</td>
<td>25.4±0.9</td>
<td>24.4±1.5</td>
<td>24.2±1.1</td>
<td>0.721</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>14.9±0.6</td>
<td>14.4±1.2</td>
<td>13.8±0.8</td>
<td>0.669</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>41.8±0.8</td>
<td>40.8±1.2</td>
<td>41.6±1.5</td>
<td>0.812</td>
</tr>
</tbody>
</table>

*p<0.05 Ov Control vs. EAMD+Cal and EAMD Control

BMI: body mass index; VO₂: oxygen consumption; Ov: ovulatory; EAMD: exercise-associated menstrual disturbances

Data are mean±SEM
Bone Mineral Density and Estimated Bone Geometry. Table 5.2 describes baseline aBMD and estimated femoral neck geometry. Baseline lumbar spine (L1-L4) aBMD (p<0.001) and BMAD (p=0.001) were lower in the EAMD+Cal group compared to the Ov Control group. Consequently, lumbar spine (L1-L4) Z-score was also lower in the EAMD+Cal group compared to the Ov Control group (p<0.001). aBMD and Z-scores did not differ (p>0.05) at any other site among groups. Baseline femoral neck CSA and CSMI were also not significantly different among groups.

Table 5.2. Baseline bone density and hip strength analysis characteristics of the three groups of exercising women.

<table>
<thead>
<tr>
<th></th>
<th>Ov Control (n=20)</th>
<th>EAMD Control (n=15)</th>
<th>EAMD+Cal (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone Mineral Density</strong></td>
<td></td>
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</tr>
<tr>
<td>Total Body aBMD (g/cm²)</td>
<td>1.160±0.022</td>
<td>1.125±0.030</td>
<td>1.079±0.024</td>
<td>0.090</td>
</tr>
<tr>
<td>L1-L4 aBMD (g/cm²)</td>
<td>1.170±0.022</td>
<td>1.122±0.041</td>
<td>1.027±0.024 a</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>L1-L4 Z-score</td>
<td>0.1±0.2</td>
<td>-0.4±0.3</td>
<td>-1.1±0.2 a</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>L1-L4 BMAD (g/cm³)</td>
<td>0.164±0.003</td>
<td>0.154±0.005</td>
<td>0.140±0.004 a</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Femoral Neck aBMD (g/cm²)</td>
<td>1.061±0.029</td>
<td>1.089±0.040</td>
<td>0.996±0.039</td>
<td>0.222</td>
</tr>
<tr>
<td>Femoral Neck Z-score*</td>
<td>0.7±0.3</td>
<td>1.0±0.3</td>
<td>0.1±0.3</td>
<td>0.162</td>
</tr>
<tr>
<td>Total Hip aBMD (g/cm²)</td>
<td>1.087±0.027</td>
<td>1.068±0.038</td>
<td>0.987±0.037</td>
<td>0.105</td>
</tr>
<tr>
<td>Total Hip Z-score*</td>
<td>0.8±0.3</td>
<td>0.7±0.3</td>
<td>-0.1±0.3</td>
<td>0.102</td>
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<tr>
<td><strong>Femoral Neck Strength</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSMI (mm⁴)</td>
<td>1,0112.5±406.1</td>
<td>8,843.4±491.8</td>
<td>9963.0±711.6</td>
<td>0.135</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>157.9±4.1</td>
<td>154.8±6.0</td>
<td>150.3±6.4</td>
<td>0.610</td>
</tr>
</tbody>
</table>

a p<0.05 Ov Control vs. EAMD+Cal
* n=18 Ov Control, 11 EAMD Control, 11 EAMD+Cal due to lack of reference data for women <20 years of age
aBMD: areal bone mineral density; BMAD: bone mineral apparent density; L1-L4: lumbar spine L1-L4; CSMI: cross sectional moment of inertia; CSA: cross-sectional area; Ov: ovulatory; EAMD: exercise-associated menstrual disturbances
Data are mean±SEM
Reproductive and Metabolic Characteristics. Seventy-seven and 23% of women in the EAMD+Cal group were amenorrheic and oligomenorrheic, respectively; whereas, 40% of the women in the EAMD Control group were amenorrheic and 60% were oligomenorrheic at the start of the intervention. E1G AUC and E1G mean concentration of the baseline cycle (if menstruating) or 28-day monitoring period (if amenorrheic) were lower in the EAMD+Cal group compared to the Ov Control (E1G AUC: p=0.004; E1G mean: p=0.001) and EAMD Control (E1G AUC: p<0.001; E1G mean: p<0.001) groups (Table 5.3). PdG AUC and PdG mean concentration of the baseline cycle or 28-day monitoring period were lower in the EAMD+Cal (PdG AUC: p<0.001; PdG mean: p<0.001) and EAMD Control (PdG AUC: p=0.003; PdG mean: p=0.001) groups compared to the Ov Control group. Furthermore, the EAMD+Cal group also demonstrated lower baseline PdG AUC (p=0.002) and PdG cycle mean (p=0.005) compared to the EAMD Control group. There were no significant differences in circulating leptin and IGF-1 concentrations at baseline among groups. However, baseline REE (p=0.008) and REE/LBM (p=0.006) were suppressed in the EAMD Control group compared to the Ov Control group, resulting in a baseline REE/pREE ratio that was also lower than the ratio observed in the Ov Control group (p=0.002).
Table 5.3. Baseline reproductive and metabolic characteristics of the three groups of exercising women.

<table>
<thead>
<tr>
<th></th>
<th>Ov Control (n=20)</th>
<th>EAMD Control (n=15)</th>
<th>EAMD+Cal (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reproductive Profile</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E1G AUC (ng*day/ml)</td>
<td>1046.3±86.1</td>
<td>1522.8±251.3</td>
<td>624.8±124.7ac</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E1G mean (ng/ml)</td>
<td>36.4±1.0</td>
<td>41.4±3.9</td>
<td>22.5±4.6ac</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PdG AUC (ug*day/ml)</td>
<td>79.4±5.1</td>
<td>68.9±16.0b</td>
<td>27.7±4.6ac</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PdG mean (ug/ml)</td>
<td>2.8±0.2</td>
<td>1.8±0.2b</td>
<td>1.0±0.2ac</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>29.5±0.4</td>
<td>35.9±3.9</td>
<td>29.8±1.8</td>
<td>0.159</td>
</tr>
<tr>
<td><strong>Metabolic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>9.3±1.2</td>
<td>6.8±1.8</td>
<td>6.4±1.4</td>
<td>0.126</td>
</tr>
<tr>
<td>IGF-1 (pg/ml)</td>
<td>58.6±3.7</td>
<td>56.0±4.8</td>
<td>55.0±3.6</td>
<td>0.803</td>
</tr>
<tr>
<td>REE (kcal/day)</td>
<td>1310.2±32.0</td>
<td>1152.8±40.7b</td>
<td>1219.8±57.2</td>
<td>0.016</td>
</tr>
<tr>
<td>REE/LBM (kcal/day/kg LBM)</td>
<td>31.3±0.6</td>
<td>28.2±0.8b</td>
<td>29.7±0.9</td>
<td>0.014</td>
</tr>
<tr>
<td>REE/pREE</td>
<td>0.94±0.02</td>
<td>0.82±0.02b</td>
<td>0.87±0.03</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\(^a\) p<0.05 Ov Control vs. EAMD+Cal
\(^b\) p<0.05 Ov Control vs. EAMD Control
\(^c\) p<0.05 EAMD Control vs. EAMD+Cal

E1G: estrone-1-glucuronide; AUC: area under the curve; PdG: pregnanediol glucuronide; IGF-1: insulin-like growth factor-1; REE: resting energy expenditure; pREE: predicted REE using Harris Benedict equation; LBM: lean body mass; Ov: ovulatory; EAMD: exercise-associated menstrual disturbances

Data are mean±SEM

Longitudinal Analysis

*Intervention Length & Recovery of Menstrual Function:* Mean duration of the RCT was 10.9 months for the EAMD+Cal group, 12.0 months for the EAMD Control group, and 11.6 months for the Ov Control group. Sixty-nine percent of the EAMD+Cal group recovered menstrual function during the intervention compared to 47% of the EAMD Control group.

*Changes in Energy Intake:* One woman in the EAMD+Cal group was non-compliant to the intervention and therefore did not follow a prescribed caloric intake. As
such, she was excluded from the following analyses. The EAMD+Cal group (n=12) increased energy intake by 12% (+211 kcal/day) at month 1, 26% (+484 kcal/day) at month 3, 31% (+583 kcal/day) at month 6, and 25% (+454 kcal/day) by the end of the intervention. This intake was >90% of the prescribed caloric intake (mean: 98% for month 1, 3, 6, and post-intervention), demonstrating good compliance to the intervention. Figure 5.2 depicts the actual and prescribed caloric intake during the intervention (Figure 5.2A) and the ratio of actual:prescribed energy intake (Figure 5.2B). Percent change from baseline to post-intervention in energy intake among the groups is shown in Figure 5.3. Upon inclusion of all completers of the RCT, the EAMD+Cal group (n=13) increased energy intake by 21.6% compared to 3.1% and -6.5% in the EAMD Control and Ov Control groups, respectively. The percent change from baseline in energy intake for the EAMD+Cal group was greater (p=0.038) than the percent change for the Ov Control group.
Figure 5.2. A) Actual energy intake and prescribed energy intake during the intervention in the EAMD+Cal group (n=12). B) Ratio of actual energy intake to prescribed energy intake at different time points of the intervention. The EAMD+Cal group (n=12) consumed >90% of the prescribed energy intake. One woman in the EAMD+Cal was non-compliant to the intervention and was therefore excluded from this analysis.
Changes in Body Composition: Absolute Measurements. Results of the repeated measures ANOVA assessing change in body composition across the intervention are displayed in Table 5.4. The EAMD+Cal group increased body mass by 0.8 kg at month 3, 1.7 kg at month 6, and 2.4 kg by post-intervention. This increase in body mass translated to changes in fat mass and lean mass, respectively, of 0.6 and -0.1 kg at month 3, 1.2 and 0.1 kg at month 6, and 1.3 and 0.8 kg at post-intervention in the EAMD+Cal group. A group*time interaction was observed for body mass (p=0.007) and fat mass (p=0.008). In the EAMD+Cal group only, body mass at month 6 (p<0.001) and post-intervention (p=0.002) was greater than body mass at baseline. Body mass was also

![Figure 5.3](image_url)  
**Figure 5.3.** Percent change in energy intake during the intervention among the Ov Control (n=20), EAMD Control (n=15), and EAMD+Cal (n=13) women. Change is from baseline to the last available diet record (range: months 6-13). The EAMD+Cal group demonstrated a significantly greater percent change in energy intake compared to the Ov Control group. One woman in the EAMD+Cal was not compliant to the intervention. Exclusion of this woman results in a 24.9% increase in caloric intake in the EAMD+Cal group, which remains greater than the percent change in energy intake for the Ov Control group (p=0.020).
greater at month 6 than month 3 (p=0.035) in the EAMD+Cal group. Similarly, fat mass in the EAMD+Cal group was significantly greater at month 6 (p=0.004) and post-intervention (p=0.013) compared to baseline. No interaction or main effects for group and time were observed for lean mass during the intervention.

Changes in Body Composition: Percent Change. Percent changes in body mass and body composition from baseline are depicted in Figure 5.4. In the EAMD+Cal group, body mass increased by 1.5% at month 3, 3.2% at month 6, and 4.5% at post-intervention. Fat mass and lean mass, respectively, changed by 5.1% and -0.03% at month 3, 9.5% and 0.3% at month 6, and 11.7% and 2.1% at post-intervention. There was a group*time interaction for percent change in body mass (p=0.004) and fat mass (p=0.004). In the EAMD+Cal group, the percent change in body mass from baseline was greater than the percent change observed in the Ov Control group at month 3 (p=0.040), month 6 (p<0.001), and post-intervention (p=0.003), and also greater than the percent change observed in the EAMD Control group at month 6 (p=0.009). Likewise, the percent change from baseline in fat mass of the EAMD+Cal group was greater at all time points (months 3, 6, and post intervention) than the percent change in the Ov Control group (p≤0.025). Body mass and fat mass increased from baseline in the EAMD+Cal group at month 6 (p≤0.001) and post-intervention (p≤0.003) compared to baseline; however, the percent changes from baseline in body mass and fat mass for the EAMD Control and Ov Control groups were not significant at any intervention time point. No significant interaction or main effects were observed for percent change from baseline in lean mass during the intervention.
Table 5.4. Body composition and hormonal characteristics during the intervention.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Month 3</th>
<th>Month 6</th>
<th>Post-Intervention</th>
<th>Group Effect P-value</th>
<th>Time Effect P-value</th>
<th>Group*Time Interaction P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Composition</strong></td>
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<tr>
<td><strong>Body Mass (kg)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ov Control</td>
<td>58.9±1.0</td>
<td>58.7±1.0</td>
<td>58.5±1.0</td>
<td>58.7±1.1</td>
<td>0.961</td>
<td>0.012</td>
<td>0.007</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>57.9±2.1</td>
<td>58.0±2.2</td>
<td>58.1±2.1</td>
<td>58.4±2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAMD+Cal</td>
<td>57.3±1.9</td>
<td>58.1±1.8</td>
<td>59.0±1.8</td>
<td>59.7±1.5</td>
<td>0.996</td>
<td>0.019</td>
<td>0.008</td>
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<tr>
<td><strong>Fat Mass (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ov Control</td>
<td>14.9±0.6</td>
<td>14.6±0.6</td>
<td>14.5±0.6</td>
<td>14.7±0.7</td>
<td>0.996</td>
<td>0.019</td>
<td>0.008</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>14.6±1.2</td>
<td>15.0±1.2</td>
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<td>15.1±1.2</td>
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<tr>
<td>EAMD+Cal</td>
<td>13.8±0.8</td>
<td>14.4±0.7</td>
<td>15.0±0.7</td>
<td>15.1±0.5</td>
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<tr>
<td><strong>Lean Mass (kg)</strong></td>
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<td></td>
<td></td>
<td>0.750</td>
<td>0.086</td>
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<tr>
<td>Ov Control</td>
<td>41.8±0.8</td>
<td>42.2±0.9</td>
<td>42.1±0.9</td>
<td>42.0±0.8</td>
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<tr>
<td>EAMD Control</td>
<td>40.9±1.3</td>
<td>40.8±1.4</td>
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<td>41.2±1.5</td>
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<tr>
<td>EAMD+Cal</td>
<td>41.6±1.5</td>
<td>41.5±1.5</td>
<td>41.7±1.6</td>
<td>42.4±1.5</td>
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<tr>
<td><strong>Metabolic Hormones</strong></td>
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</tr>
<tr>
<td><strong>Leptin (ng/ml)</strong></td>
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<td></td>
<td></td>
<td></td>
<td>0.659</td>
<td>0.986</td>
<td>0.352</td>
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<tr>
<td>Ov Control</td>
<td>9.3±1.2</td>
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<td>7.6±1.0</td>
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<tr>
<td>EAMD Control</td>
<td>6.8±1.8</td>
<td>6.7±1.8</td>
<td>6.5±1.8</td>
<td>6.4±2.5</td>
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<tr>
<td>EAMD+Cal</td>
<td>6.4±1.4</td>
<td>7.4±1.3</td>
<td>7.8±1.4</td>
<td>8.3±2.0</td>
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<td><strong>IGF-1 (pg/ml)</strong></td>
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<td>0.823</td>
<td>0.314</td>
<td>0.175</td>
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<tr>
<td>Ov Control</td>
<td>58.6±3.7</td>
<td>52.1±3.9</td>
<td>55.6±3.0</td>
<td>55.4±3.6</td>
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</tr>
<tr>
<td>EAMD Control</td>
<td>56.0±4.8</td>
<td>55.7±4.1</td>
<td>55.8±4.3</td>
<td>55.9±3.9</td>
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<tr>
<td>EAMD+Cal</td>
<td>55.0±3.6</td>
<td>56.9±4.1</td>
<td>56.8±3.8</td>
<td>62.9±4.3</td>
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<tr>
<td><strong>Reproductive Hormone</strong></td>
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<td></td>
<td><strong>0.002</strong></td>
<td>0.134</td>
<td>0.261</td>
</tr>
<tr>
<td><strong>E1G AUC (ng*day/ml)</strong></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ov Control</td>
<td>2733.2±232.8</td>
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<td></td>
<td>2802.7±268.3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EAMD Control</td>
<td>3496.2±361.4</td>
<td></td>
<td></td>
<td>3757.7±513.8</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>EAMD+Cal</td>
<td>1845.5±379.4</td>
<td></td>
<td></td>
<td>2222.2±319.8</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

n=14 EAMD Control for fat mass, lean mass, and E1G AUC

E1G: estrone-1-glucuronide; AUC: area under the curve; IGF-1: insulin-like growth factor-1; Ov: ovulatory; EAMD: exercise-associated menstrual disturbances
Data are mean±SEM
Figure 5.4. Percent change of body composition during the intervention. A) Percent change in body mass (kg). A significant group*time interaction was observed, indicating that the EAMD+Cal group had a significant increase in body mass compared to baseline and month 3. The EAMD+Cal group demonstrated a greater increase in body mass compared to the Ov control at Month 3, Month 6, and Post-intervention and compared to the EAMD Control group at Month 6. B) Percent change in fat mass (kg). There was a significant group*time interaction, indicating that the EAMD+Cal had a significant percent increase in fat mass at Month 6 and Post-intervention compared to baseline and month 3. The EAMD+Cal group demonstrated a greater increase in fat mass compared to Ov Control group at Month 3, Month 6, and post-intervention. The EAMD Control group demonstrated a significant increase in fat mass compared to the Ov Control group at Month 6. C) Percent change in lean mass (kg).

☐ indicates the EAMD+Cal group (n=13); ○ is the EAMD Control group (n=15 for body mass, n=14 for fat mass and lean mass); △ indicates the Ov Control group (n=20). a indicates p<0.05 EAMD+Cal vs. Ov Control. b indicates p<0.05 EAMD+Cal vs. EAMD Control. *indicates p<0.05 from baseline. **indicates p<0.05 from baseline and month 3.
B

Group Effect: p=0.001
Time Effect: p=0.005
Group*Time Interaction: p=0.004

Fat Mass % change

Baseline Month 3 Month 6 Post-Intervention

Ov Control
EAMD Control
EAMD+Cal

Group Effect: p=0.439
Time Effect: p=0.188
Group*Time Interaction: p=0.314

Lean Mass % change

Baseline Month 3 Month 6 Post-Intervention

Ov Control
EAMD Control
EAMD+Cal
Bone Mineral Density and Estimated Bone Geometry: Absolute Measurements.

Absolute aBMD measurements and estimated bone geometry across the intervention are displayed in Table 5.5. There was no group*time interaction for lumbar spine aBMD and BMAD; however, main effects for group and time were observed for lumbar spine aBMD (group: p=0.005; time: p=0.002) and BMAD (group: p=0.002; time: p=0.015). The EAMD+Cal group presented with lower lumbar spine aBMD (p=0.003) and BMAD (p=0.001) compared to the Ov Control group. Lumbar spine aBMD steadily increased during the intervention such that aBMD at month 6 (p=0.011) and post-intervention (p=0.013) were greater than that observed at baseline. Lumbar spine BMAD also increased from baseline to month 6 (p=0.043). There were no significant interactions or main effects for total hip aBMD and femoral neck aBMD, CSMI, and CSA.
Table 5.5. Bone mineral density and hip strength analysis characteristics during the intervention.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Month 6</th>
<th>Post-Intervention</th>
<th>Group Effect P-value</th>
<th>Time Effect P-value</th>
<th>Group*Time Interaction P-value</th>
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<tr>
<td><strong>Bone Mineral Density</strong></td>
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<tr>
<td><em>L1-L4 aBMD (g/cm²)</em></td>
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</tr>
<tr>
<td>Ov Control</td>
<td>1.170±0.022</td>
<td>1.183±0.021</td>
<td>1.176±0.019</td>
<td>0.005</td>
<td>0.002</td>
<td>0.169</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>1.129±0.044</td>
<td>1.130±0.041</td>
<td>1.136±0.041</td>
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<td>EAMD+Cal</td>
<td>1.027±0.024</td>
<td>1.042±0.027</td>
<td>1.050±0.028</td>
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<tr>
<td><em>L1-L4 BMAD (g/cm³)</em></td>
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<tr>
<td>Ov Control</td>
<td>0.164±0.003</td>
<td>0.166±0.003</td>
<td>0.164±0.003</td>
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<td>0.002</td>
<td>0.015</td>
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<tr>
<td>EAMD Control</td>
<td>0.155±0.006</td>
<td>0.155±0.006</td>
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<td>EAMD+Cal</td>
<td>0.140±0.004</td>
<td>0.142±0.004</td>
<td>0.143±0.004</td>
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<td><strong>Femoral Neck aBMD (g/cm²)</strong></td>
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<tr>
<td>Ov Control</td>
<td>1.061±0.029</td>
<td>1.062±0.030</td>
<td>1.065±0.029</td>
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<td>0.199</td>
<td>0.218</td>
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<td>EAMD Control</td>
<td>1.098±0.041</td>
<td>1.092±0.042</td>
<td>1.091±0.043</td>
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<td>EAMD+Cal</td>
<td>0.996±0.039</td>
<td>0.995±0.037</td>
<td>0.997±0.036</td>
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<tr>
<td><strong>Total Hip aBMD (g/cm²)</strong></td>
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<tr>
<td>Ov Control</td>
<td>1.087±0.027</td>
<td>1.093±0.027</td>
<td>1.090±0.026</td>
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<td>0.072</td>
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<td>EAMD Control</td>
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<td>1.079±0.039</td>
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<tr>
<td>EAMD+Cal</td>
<td>0.987±0.037</td>
<td>0.984±0.036</td>
<td>0.989±0.035</td>
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<tr>
<td><strong>Femoral Neck Strength</strong></td>
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<tr>
<td><em>CSMI (mm⁴)</em></td>
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<td></td>
<td>0.265</td>
<td>0.726</td>
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<tr>
<td>Ov Control</td>
<td>10112.5±406.1</td>
<td>9984.8±414.1</td>
<td>10051.2±397.0</td>
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<tr>
<td>EAMD Control</td>
<td>8955.3±514.5</td>
<td>9071.7±527.6</td>
<td>9046.9±561.0</td>
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<tr>
<td>EAMD+Cal</td>
<td>9963.0±711.6</td>
<td>9946.6±692.1</td>
<td>10084.7±734.4</td>
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<td><em>CSA (mm²)</em></td>
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<tr>
<td>Ov Control</td>
<td>157.9±4.1</td>
<td>157.2±4.1</td>
<td>157.2±3.9</td>
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<tr>
<td>EAMD Control</td>
<td>156.3±6.2</td>
<td>156.3±6.3</td>
<td>156.1±6.4</td>
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<tr>
<td>EAMD+Cal</td>
<td>150.3±6.4</td>
<td>148.8±6.3</td>
<td>152.6±5.9</td>
<td></td>
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<td></td>
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</table>

n=14 EAMD Control
aBMD: areal bone mineral density; BMAD: bone mineral apparent density; L1-L4: lumbar vertebrae 1-4; CSMI: cross sectional moment of inertia; CSA: cross-sectional area; Ov: ovulatory; EAMD: exercise-associated menstrual disturbances
Bone Mineral Density and Estimated Bone Geometry: Percent Change. Percent changes from baseline in aBMD and BMAD are displayed in Figure 5.5. Post-intervention, lumbar spine aBMD increased by 2.2% in the EAMD+Cal group compared to 0.8% and 0.6% increases in the EAMD Control and Ov Control groups, respectively. Similarly, lumbar spine BMAD demonstrated a 2.1% increase in the EAMD+Cal group compared to the 0.6% and 0.3% increases in lumbar spine BMAD in the EAMD Control and Ov Control groups, respectively. There was neither a significant group*time interaction nor main effect of group for percent change of lumbar spine aBMD and BMAD; however, there was a time effect (lumbar spine aBMD: \( p=0.002 \); lumbar spine BMAD: \( p=0.012 \)). Lumbar spine aBMD and BMAD demonstrated an increase from baseline to month 6 (\( p\leq0.040 \)) and post-intervention (\( p\leq0.038 \)). No interactions or main effects were observed for percent change in total hip and femoral neck aBMD across the intervention. Total hip aBMD changed by 0.3%, -0.2, and 0.3% in the EAMD+Cal, EAMD Control, and Ov Control groups, respectively, from baseline to post-intervention. Similarly, femoral neck aBMD changed by 0.2%, -0.8%, and -0.6% from baseline to post-intervention in the EAMD+Cal, EAMD Control, and Ov Control groups, respectively. Percent changes from baseline in estimated bone geometry at the femoral neck are displayed in Figure 5.6. From baseline to post-intervention, the EAMD+Cal group demonstrated a 1.8% increase in femoral neck CSA compared to 0.2% and 0.3% decreases in the EAMD Control and Ov Control groups, respectively. Femoral neck CSMI changed by 1.2%, 0.7%, and -0.5% in the EAMD+Cal, EAMD Control, and Ov Control groups, respectively. Neither statistically significant interactions nor main
effects were observed for percent change in femoral neck CSA and CSMI across the intervention.

**Figure 5.5.** Percent change of areal bone mineral density (aBMD) during the intervention. A) Percent change in lumbar spine (L1-L4) aBMD. B) Percent change in lumbar spine (L1-L4) bone mineral apparent density (BMAD). A significant time effect was observed for lumbar spine aBMD and BMAD, indicating an increase in lumbar spine aBMD and BMAD during the study. C) Percent change in femoral neck aBMD. D) Percent change in total hip aBMD. □ indicates the EAMD+Cal group (n=13); ○ is the EAMD Control group (n=14); Δ indicates the Ov Control group (n=20).
Baseline Month 6 Post-Intervention

Femoral Neck aBMD % change

Group Effect: p=0.633
Time Effect: p=0.091
Group*Time Interaction: p=0.696

Total Hip aBMD % change

Group Effect: p=0.422
Time Effect: p=0.748
Group*Time Interaction: p=0.372
Figure 5.6. Percent change in estimated femoral neck geometry. A) Percent change in femoral neck (FN) cross-sectional moment of inertia (CSMI). B) Percent change in femoral neck (FN) cross-sectional area (CSA).

□ indicates the EAMD+Cal group (n=13); ○ is the EAMD Control group (n=14); Δ indicates the Ov Control group (n=20).
Metabolic and Reproductive Hormones: Absolute Measurements. Measurements of metabolic and reproductive hormones across the intervention are displayed in Table 5.4. No interactions or main effects were observed for circulating concentrations of leptin and IGF-1 during the intervention. Although there was neither a significant group*time interaction nor main effect of time for E1G exposure, a group effect (p=0.002) was observed. The EAMD+Cal group demonstrated lower E1G AUC compared to the Ov Control (p=0.044) and EAMD Control (p=0.002) groups.

Metabolic and Reproductive Hormones: Percent Change. Percent changes in metabolic hormones across the intervention are displayed in Figure 5.7. Circulating leptin concentrations increased by 83.0% in the EAMD+Cal group from baseline to post-intervention compared to a 4.9% increase in the EAMD Control group and a 3.1% decrease in the Ov Control group. There was neither a significant group*time interaction nor main effect of time for percent change in leptin concentrations; however, a group effect (p=0.013) was observed. The EAMD+Cal group demonstrated greater increase in circulating leptin concentration compared to the Ov Control (p=0.015) group.

Circulating concentrations of IGF-1 increased by 16.5% in the EAMD+Cal group from baseline to post-intervention compared to a 4.4% increase and 3.0% decrease in the EAMD Control and Ov Control groups, respectively. No significant interaction or main effects were observed for percent change in IGF-1 concentrations.
Figure 5.7. Percent change of metabolic hormones during the intervention. A) Percent change in circulating leptin concentrations. A significant group effect was observed, indicating that the EAMD+Cal group demonstrated a greater percent change in leptin concentrations than the Ov Control group. The difference in percent change between the EAMD+Cal and EAMD Control group did not achieve statistical significance (p=0.059). B) Percent change in circulating concentrations of insulin-like growth factor-1 (IGF-1).

□ indicates the EAMD+Cal group (n=13); ○ is the EAMD Control group (n=15); Δ indicates the Ov Control group (n=20).
Figure 5.8 displays the percent change in 2- to 3-month E1G AUC from baseline to the end of the intervention. The EAMD+Cal group demonstrated an increase of 47.2% in E1G AUC compared to increases of 14.7% and 6.1% in the EAMD Control and Ov Control groups, respectively. Neither a significant interaction nor main effects were observed for percent change in E1G AUC.

Figure 5.8. Percent change of estrone-1-glucuronide (E1G) area under the curve (AUC) from baseline to the end of the study. A 3-month baseline E1G AUC was estimated based on E1G AUC of the baseline cycle or 28-day monitoring period. Three-month E1G AUC during the latter half of the study was compared to this projected baseline AUC to determine percent change in E1G AUC during the study. There was no significant difference among groups. N=14 for EAMD Control group due to missing data.
Correlation Analysis

Significant correlations between change in bone health variables (baseline to post-intervention) and change in body composition and reproductive function during the intervention are displayed in Table 5.6. Percent change in body composition demonstrated the strongest correlations with percent change in bone health variables. Percent change in body mass from baseline to post-intervention was positively correlated with percent change in femoral neck CSA ($r=0.300$, $p=0.038$). Likewise, post-intervention percent change in lean mass was associated with change in femoral neck CSMI ($r=0.331$, $p=0.022$) and CSA ($r=0.322$, $p=0.026$). Percent change in fat mass at month 3 ($r=0.328$, $p=0.023$) and month 6 ($r=0.366$, $p=0.011$) of the intervention was positively correlated with percent change in lumbar spine BMAD. Similarly, percent change in fat mass at month 3 was also associated with change in lumbar spine aBMD ($r=0.328$, $p=0.023$). Percent change in 2- to 3-month E1G AUC from baseline to the end of the study was positively correlated with percent change in femoral neck ($\rho=0.308$, $p=0.035$) and total hip ($\rho=0.320$, $p=0.028$) aBMD. Percent change in circulating leptin and IGF-1 concentrations were not significantly correlated with baseline to post-intervention percent change in any bone health variables.
Table 5.6. Pearson correlations between changes in reproductive and body composition variables and changes in bone health indices across the intervention in exercising women

<table>
<thead>
<tr>
<th></th>
<th>%Δ LS aBMD</th>
<th>%Δ LS BMAD</th>
<th>%Δ FN aBMD</th>
<th>%Δ Total Hip aBMD</th>
<th>%Δ FN CSMI</th>
<th>%Δ FN CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r*</td>
<td>P**</td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
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<tr>
<td><strong>Body Composition</strong></td>
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<tr>
<td>Body Mass</td>
<td></td>
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<tr>
<td>% Δ post-intervention</td>
<td>0.094</td>
<td>0.525</td>
<td>0.147</td>
<td>0.318</td>
<td>-0.049</td>
<td>0.739</td>
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<td>Fat Mass</td>
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<tr>
<td>%Δ month 3</td>
<td>0.328</td>
<td>0.023</td>
<td>0.366</td>
<td>0.011</td>
<td>0.011</td>
<td>0.940</td>
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<td>%Δ month 6</td>
<td>0.216</td>
<td>0.144</td>
<td><strong>0.291</strong></td>
<td><strong>0.047</strong></td>
<td>-0.100</td>
<td>0.502</td>
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<td>Lean Mass</td>
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<tr>
<td>% Δ post-intervention</td>
<td>0.040</td>
<td>0.789</td>
<td>0.101</td>
<td>0.496</td>
<td>-0.114</td>
<td>0.442</td>
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<td><strong>Reproductive Hormone</strong></td>
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<tr>
<td>%Δ E1G AUC</td>
<td>0.286</td>
<td>0.051</td>
<td>0.282</td>
<td>0.055</td>
<td><strong>0.308</strong></td>
<td><strong>0.035</strong></td>
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</tbody>
</table>

*r indicates Pearson correlation between the variables.
**P indicates the p-value of the correlation
aSpearman correlation due to non-normal distribution
E1G: estrone-1-glucuronide; AUC: area under the curve; LS: lumbar spine; aBMD: areal bone mineral density; BMAD: bone mineral apparent density; FN: femoral neck; CSMI: cross sectional moment of inertia; CSA: cross-sectional area
Discussion

Among exercising women with menstrual dysfunction, an intervention of increased energy intake appears to be well-received and holds potential for an effective treatment strategy to improve factors known to influence BMD. In the EAMD+Cal group, weight gain of 2.4 kg was observed secondary to the increased energy intake that was, on average, 33% above baseline total energy expenditure at the time points assessed. Metabolic status and reproductive function improved as evidenced by increases in circulating leptin concentrations and E1G exposure during the latter months of the intervention. Although improvements in BMD and estimated bone geometry as a result of the intervention failed to reach statistical significance, the increases observed in lumbar spine aBMD, BMAD, and femoral neck CSA suggest that the intervention may be having a desired influence, but more time and women are likely required to achieve statistical significance. Among the factors assessed in this study that are known to influence bone health, changes in body composition and estrogen exposure appear to be more strongly associated with changes in aBMD and estimated bone geometry than changes in metabolic status.

We hypothesized that the intervention of increased energy intake would result in increases in aBMD. Although a significant increase in aBMD was not observed in the EAMD+Cal group, the 2.2% percent increase in lumbar spine aBMD compared to the average increase of 0.7% in the two control groups suggests that BMD, particularly at the lumbar spine, may be improving as a result of the intervention. A time effect was observed for lumbar spine aBMD and BMAD from baseline to post-intervention when assessing both the absolute measurements and percent change, findings suggestive of an
improvement in lumbar spine BMD over time. The relatively short duration of the intervention and the small sample size may have hindered the ability to observe a statistical difference among groups. Interestingly, a group effect for lumbar spine aBMD and BMAD was also observed when assessing the absolute aBMD measurements, indicating that lumbar spine aBMD and BMAD in the EAMD+Cal group remained lower than that of the Ov Control group throughout the duration of the intervention. This finding is consistent with reports of other investigators who have observed persistently low BMD in formerly amenorrheic athletes or women/adolescents with anorexia nervosa, despite weight gain and/or resumption of menses [5, 8-10, 12, 70, 71]. In fact, in a small sample of formerly amenorrheic athletes who gained, on average, 1.9 kg of body mass and resumed menses, Drinkwater et al. [9] observed a significant increase in lumbar spine aBMD after 15 months; however, lumbar spine aBMD remained below that of regularly-menstruating athletes. Furthermore, a 24-month follow-up investigation of amenorrheic dancers revealed robust increases in lumbar spine aBMD (14.4%) that coincided with an average weight gain of 5 kg, yet lumbar spine aBMD continued to be significantly lower than that of eumenorrheic dancers [10]. As such, although weight gain subsequent to increased energy intake may improve lumbar aBMD in exercising women with EAMD, it is possible that BMD may never fully recover in a manner that normalizes BMD to that observed in ovulatory exercising women, particularly when the menstrual dysfunction persists into adulthood. Due to the large increases that occur in bone mass, geometry, and bone strength during the adolescent years, the optimal time to reverse low bone mass as a result of an energy deficiency and menstrual dysfunction is during adolescence rather
Contrary to our hypothesis, minimal changes were observed for femoral neck and total hip aBMD as a result of the intervention. Although investigators have reported an increase in hip aBMD among anorexic women who gained weight and resumed menses compared to those who did not gain weight or recover menstrual function [1], our results are not surprising when taking into consideration the proportion of cortical and trabecular bone in the femoral neck and total hip sites as well as the nature of the population of interest. Compared to the lumbar vertebrae, the femoral neck and total hip are composed of a greater percentage of cortical bone [73]. Cortical bone has a slower rate of bone turnover than trabecular bone, the primary type of bone found in the vertebrae, and therefore may be less sensitive to changes in the metabolic and hormonal environment that accompanies weight gain. Furthermore, in our sample of exercising women, femoral neck and total hip aBMD did not differ among groups at baseline which is consistent with the results of other investigators who have compared BMD between amenorrheic and eumenorrheic athletes [74, 75]. A lifestyle of habitual physical activity, particularly weight-bearing activity of the lower extremities which characterized the majority of our women’s exercise patterns, may provide some protection against losses in bone mass at these weight-bearing sites [10]. Because gains in BMD tend to be the most robust with the initiation of a treatment aimed to improve BMD when the initial values are low, the relatively normal aBMD values of the EAMD groups at the femoral neck and total hip may preclude robust increases during an intervention of relatively short duration. Further, within an adolescent anorexic population who gained weight and recovered
menstrual function, investigators have also reported a smaller annual change in femoral neck BMD (0.5%) compared to lumbar spine BMD (3.0%) [72]. Considering that these results were observed in the adolescent population whose bones may respond more readily to improvements in weight and menstrual function compared to adults [72], the small and insignificant increases that we observed in total hip and femoral neck aBMD are understandable.

On the other hand, case reports of amenorrheic athletes who gained weight have demonstrated robust improvements in BMD. Frederickson et al. [6] reported a 25.5% and 19.5% increase in lumbar spine and hip BMD, respectively, after a gradual weight gain of 17 kg over the course of 8 years in an athlete who presented with amenorrhea and very low BMD. Similarly, Zanker et al. [7] observed a 16.9% increase in hip BMD after weight gain of 8 kg over 36 months in an endurance athlete with primary amenorrhea and low BMD. It must be noted, however, that the weight gain in these athletes was 3-7 times what we observed in our women, and the duration of time that these women were followed greatly exceeded the length of our intervention.

Interestingly, an unexpected finding was the relatively large, albeit non-significant (group*time interaction p=0.058), 1.8% increase in femoral neck CSA at post-intervention for the EAMD+Cal group compared to an average change of -0.3% in the EAMD Control and Ov Control groups. The reason for this change is difficult to determine; however, it is proposed that the improved metabolic and nutritional state of the EAMD+Cal women may have allowed the women to acquire greater osteogenic benefits from exercise-induced mechanical loading. Additionally, it is speculated that the small increase in lean mass during the latter part of the study may have aided an increase
in bone size, particularly at weight-bearing sites that experience muscles forces with weight-bearing exercise such as that engaged in by the majority of the women.

The observed responses of aBMD and estimated bone geometry to the intervention may be explained by the changes in body composition and reproductive function that were also occurring as a result of the intervention. In adolescent athletes, fat mass has been observed to be strongly associated with lumbar spine BMAD; whereas, lean mass demonstrates a strong association with hip BMD [76]. The weight gain observed in the EAMD+Cal group was primarily attributed to an increase in fat mass, which is consistent with the composition of weight gain among anorexic women and adolescents undergoing treatment [1, 3, 5]. Interestingly, however, the gain in fat mass was the primary contributor to the increase in body mass during the first 6 months of the intervention; whereas, lean mass was the dominant contributor to weight gain during the second half of the intervention. The differing change in fat mass and lean mass during the intervention was evidenced in the correlations of change in fat mass and lean mass with change in parameters of bone health during the intervention. Percent change in fat mass at month 3 and month 6 when the most robust increases in fat mass were occurring was associated with post-intervention percent change in the lumbar spine aBMD and BMAD. On the other hand, minimal change in fat mass occurred from month 6 to post-intervention, and no associations were observed between post-intervention percent change in fat mass and percent change in aBMD. Conversely, percent change in lean mass at post-intervention, when the most robust change in lean mass was observed, was associated with post-intervention percent change in estimated geometry of the femoral neck, i.e., femoral neck CSMI and CSA.
The increase in fat mass and, to a lesser extent, lean mass that we observed with an increase in body mass may begin to explain the underlying mechanism for the improvement in BMD subsequent to weight gain that has been observed in numerous studies of amenorrheic athletes and women and adolescents with anorexia nervosa [1-4, 9]. The benefits of lean mass on both bone mass [76] and bone geometry [77-79] as a result of muscle forces on bone [80-82] have been well-established, and change in fat free mass has previously been reported to be a more important predictor of improvement in BMD among recovering anorexic women than either change in body weight or fat mass [1]. Therefore, perhaps a more interesting finding in our study is the influence of change in fat mass on change in aBMD. Other investigators have reported a relationship between fat mass and BMD [14, 15, 76], particularly among those beyond the years of longitudinal growth which is a period of time when the influence of lean mass on bone mass appears to dominate [13]. However, the mechanism by which fat mass influences bone health is currently unclear. Adipose tissue may play a favorable role in bone remodeling due to its production of the anorexigenic hormone leptin [20] and its aromatization of androgens to estrogens [16]. In vitro, leptin has been observed to increase osteoblast proliferation [20, 83], and in vivo, treatment with recombinant human leptin has resulted in increases in markers of bone formation [84] and lumbar spine BMD [24]. Estrogen, on the other hand, inhibits osteoclast action and bone resorption [23]. In this way, fat mass may indirectly have a favorable influence on bone turnover, thus leading to increases in bone mass. However, investigators have demonstrated that even after adjusting for concentrations of leptin and estradiol, fat mass remains significantly correlated with BMD [15]. Likewise, our results demonstrate that change in fat mass
appears to have an influence on the change in bone mass independent of, or at least stronger than, the influence of change in leptin concentration and estrogen exposure.

Paralleling the increase in fat mass during the intervention in the EAMD+Cal group, circulating leptin concentrations also steadily increased, resulting in a baseline to post-intervention percent change of 83%. Although the increase in leptin concentrations may have played a role in the small increases observed in lumbar spine aBMD due to its proposed stimulatory action on bone formation [24, 84], we did not observe a significant correlation between change in leptin concentration and post-intervention change in aBMD. Although the reason for our findings is unclear, it is possible that the effects of leptin on bone mass observed in other studies are mediated through fat mass or other hormones known to affect bone metabolism and BMD [20, 85-88]. In a sample of premenopausal women, fat mass remained a significant predictor of BMD after adjusting for leptin concentration, although the relation between fat mass and BMD was weaker [15]. The exact mechanism for the influence of fat mass on BMD independent of leptin is not clear; however, it may be mediated through other hormones that are regulated by the energetic environment and may be altered with changes in body fat [88]. In addition, the increases in BMD that were observed with administration of recombinant human leptin in hypoleptinemic and amenorrheic women were not detected after 9 months of treatment [84] but were noted after 2 years of treatment [24], indicating that the length of our intervention may not be long enough to see the effects of increased leptin on BMD, particularly since the most robust increase in leptin concentrations was noted at post-intervention.
Estrogen exposure increased during the intervention in the EAMD+Cal group; however, this change failed to be significantly different over time and compared to the change observed in the control groups. Oligomenorrheic women do not demonstrate the same profile of suppressed estrogen and progesterone concentrations that is characteristic of amenorrheic women. It is notable that oligomenorrheic women were included in the EAMD groups, thus the increase in estrogen exposure that is observed in amenorrheic women who resume menses may be masked. However, even after excluding the oligomenorrheic women from the analysis, no significant difference in percent change of estrogen exposure among the groups was observed. Therefore, another possible reason underlying the lack of statistical significance observed for change in estrogen exposure is that some women in both EAMD groups recovered menstrual function. Sixty-nine percent of the women in the EAMD+Cal group recovered menstrual function most likely as a result of the intervention; however, 49% of women in the EAMD Control group spontaneously recovered menstrual function. As such, an increase in estrogen exposure was noted in both groups. Nevertheless, the change in estrogen exposure was positively associated with change in aBMD at the weight-bearing total hip and femoral neck sites. As such, these results provide further support for the positive influence of estrogen on bone mass and highlight the importance of optimal menstrual function for bone health.

A strength of this study lies in the nature of the intervention. To date, the impact of non-pharmacological treatment approaches to menstrual dysfunction and low bone mass in exercising women have not been explored in a controlled intervention. Furthermore, we demonstrated relatively good compliance to the intervention with a caloric intake in the EAMD+Cal group that was, on average, 98% of the prescribed
caloric intake. This good compliance suggests that the non-pharmacological approach of increased caloric intake and subsequent weight gain is a favorable treatment strategy of EAMD among exercising women. Limitations of this study include the small sample size, varied length of the intervention for the participants, and the relatively short duration of the intervention. In addition, longitudinal assessments of BMD and estimated bone geometry using DXA should be interpreted with caution when weight change occurs between scans due to the potential for spurious measurements of bone mineral content and bone area as tissue thickness changes [89, 90]; however, the weight change in this study may not be substantial enough for these measurement anomalies to occur [89]. To observe the long-term impact of the increases in body mass and its components, i.e., fat mass and lean mass, leptin and IGF-1 concentrations, and estrogen exposure, an intervention of at least 2 years is recommended. Additionally, the fact that not all women in the EAMD+Cal group resumed menses and some women in the EAMD Control group spontaneously resumed menses may contribute to the lack of a statistically significant increase in aBMD and bone geometry in the EAMD+Cal group compared to the other groups. Therefore, a subanalysis of changes in BMD and estimated bone geometry among those women who did and did not resume menses may provide further insight into the mechanisms underlying changes in bone mass in exercising women who are characterized by the unique profile of reproductive suppression and habitual mechanical loading.
Conclusion

In conclusion, an intervention of increased energy intake among exercising women with EAMD favorably increased lumbar spine aBMD and femoral neck CSA, although statistical significance was not achieved. An intervention of longer duration in a larger sample is needed to determine if these favorable increases will continue. Although the changes observed are encouraging with respect to determining appropriate and effective non-pharmacological treatments for EAMD and low bone mass in exercising women, it is notable that the lumbar aBMD and BMAD of the EAMD+Cal group remained below that of the Ov Control group, emphasizing the persistent nature of low bone mass caused by an energy deficiency and the importance of prevention and intervention during adolescence when the bones appear to more readily respond to improvements in the metabolic and hormonal profile [10, 72]. Interestingly, change in body composition appeared to be more strongly associated with change in bone health parameters when compared to the association of changes in the metabolic hormones leptin and IGF-1 and, to some degree, changes in estrogen exposure with changes in aBMD and estimated bone geometry. However, change in estrogen exposure but not leptin and IGF-1 appeared to also be important for improvements in bone mass. As such, weight gain concomitant with improved reproductive function is an attractive strategy for addressing menstrual dysfunction and low bone mass in athletes with menstrual dysfunction; however, an intervention of longer duration with a larger sample is needed before strong conclusions can be drawn.
References


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[63] Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Relationship of serum estradiol and progesterone concentrations to the excretion profiles
CHAPTER 6: STUDY 4

Mallinson RJ, Williams NI, Olmsted MP, Scheid JL, Riddle ES, and De Souza MJ. A case report of recovery of menstrual function following a nutritional intervention in two exercising women with amenorrhea of varying duration.

Abstract

Increasing caloric intake is a promising treatment for exercise-associated amenorrhea, but strategies have not been fully explored. The purpose of this case report was to compare and contrast the responses of two exercising women with amenorrhea of varying duration to an intervention of increased energy intake. Two exercising women with amenorrhea of short (3 months) and long (11 months) duration were chosen to demonstrate the impact of increased caloric intake on recovery of menstrual function and bone health. Repeated measures of dietary intake, eating behavior, body weight, body composition, bone mineral density, resting energy expenditure, exercise volume, serum metabolic hormones and markers of bone turnover, and daily urinary metabolites were obtained. Participant 1 was 19 years old and had a body mass index (BMI) of 20.4 kg/m² at baseline. She increased caloric intake by 276 kcal/day (13%), on average, during the intervention, and her body mass increased by 4.2 kg (8%). Participant 2 was 24 years old and had a BMI of 19.7 kg/m². She increased caloric intake by 1881 kcal/day (27%) and increased body mass by 2.8 kg (5%). Resting energy expenditure, triiodothyronine, and leptin increased; whereas, ghrelin decreased in both women. Resumption of menses occurred 23 and 74 days into the intervention for the women with short-term and long-term amenorrhea, respectively. The onset of ovulation and regular cycles corresponded with changes in body weight. Recovery of menses coincided closely with increases in caloric intake, weight gain, and improvements in the metabolic environment; however, the nature of
restoration of menstrual function differed between the women with short-term versus long-term amenorrhea.
Introduction

Exercising women frequently present with a chronic energy deficiency resulting from inadequate caloric intake to compensate for energy expenditure [1, 2]. In this population, energy expenditure may be high due to the added energy cost of exercise. Therefore, when daily energy intake does not match energy expenditure, there may be inadequate fuel to support all physiological processes [3]. As a result, the physiological consequences of an energy deficiency involve a cascade of metabolic and hormonal alterations that can suppress the reproductive axis and cause menstrual disturbances such as functional hypothalamic amenorrhea (FHA) and low bone mass [4, 5]. The optimal treatment strategy for women with exercise-associated amenorrhea and low bone mass is to target the source of the problem, i.e. the energy deficiency, by initiating a lifestyle intervention that includes an increase in energy intake, and, if necessary, a decrease in exercise energy expenditure (EEE) [6]. Weight gain often occurs secondary to such treatment and has been observed to be a clinically positive outcome associated with resumption of menses and enhanced bone health in exercising women [7-9].

A few investigators have reported case studies of amenorrheic, exercising women who have increased caloric intake and gained weight [7-10]. Dueck et al. [10] and Kopp-Woodroffe et al. [8] described a case study of five amenorrheic athletes who increased caloric intake for 12 to 20 weeks, resulting in weight gain of 1-3 kg and the resumption of menses in 3 of 5 participants during the intervention. Fredericson and Kent [7] reported a case study of an amenorrheic athlete who gained weight over the course of 5 years, resulting in the maintenance of normal menstrual cycles and improved bone health. Similarly, Zanker et al. [9] followed an amenorrheic athlete for 12 years and reported
increases in bone mineral density (BMD) of the proximal femur with increases in body mass index (BMI). There are, however, no case studies published to date that document the simultaneous changes in energetic and metabolic status and the associated effects on hormonal attributes of reproductive recovery and bone health in amenorrheic exercising women. Indeed, the case studies reported to date have limited their findings solely to the outcome of recovery of menses rather than the documentation of the hormonal aspects of menstrual recovery that include estrogen exposure, progesterone exposure, and ovulation over the course of 12 months of increasing energy intake. The absence of detailed reports describing the metabolic and hormonal environment surrounding resumption of menses in exercising women with FHA has resulted in a lack of evidence on which to base effective dietary treatment strategies. As such, the value of this case report lies in the opportunity to study the manifestation and resolution of this complex problem using detailed hormonal analyses in an effort to gain a better understanding about the interplay of factors that may contribute to the induction and reversal of FHA in exercising women.

Therefore, the purpose of this case report was to compare and contrast the recovery of two exercising women with current FHA of varying duration (short-term vs. long-term) to a 12-month nutritional intervention. Thus, this case report will describe, in detail, the changes in energetic status, and the hormonal aspects of recovery of menstrual function and bone health in two amenorrheic exercising women.

**Nutritional Intervention Methods**

*Study Design*

For the purposes of this case report, two exercising amenorrheic women (age 19-24 years) with current amenorrhea of short (3 months) and long (11 months) duration
were chosen to demonstrate the impact of increased caloric intake on the hormonal aspects of recovery of menstrual function and bone health. The two individuals were chosen because they both demonstrated good compliance to an intervention of 12 months of increased caloric intake targeted to exceed baseline total energy expenditure (TEE) needs by 20-30%, and the ongoing nature of the intervention precludes inclusion of the entire sample of women that participated in the intervention. Both women successfully resumed menses. The presence of amenorrhea at the beginning of the intervention was confirmed by the analysis of daily urinary excretion of estrone-1-glucuronide (E1G) and pregnanediol glucuronide (PdG) metabolites for one 28-day monitoring period. Both women were recreationally active, engaging in >7 hours of exercise per week at baseline. The primary outcome variables in the 12-month intervention were indices of energy status, bone health and menstrual status.

Inclusion Criteria

The two women in this case report were exercising women who met the following inclusion criteria: 1) age 18-35 years, 2) BMI 16-25 kg/m², 3) weight stable (± 2kg) for the past 6 months, 4) no history of any serious medical conditions, 5) no current clinical diagnosis of an eating or psychiatric disorder, 6) non-smoking, 7) no medication use that would alter metabolic or reproductive hormone concentrations, 8) ≥ 3 hrs/wk aerobic exercise, 9) no menses for the past 3 months, and 10) no history of a clinical diagnosis of polycystic ovarian syndrome (PCOS), or a free androgen index (FAI), calculated as (total testosterone (nmol/L)/sex hormone binding globulin (SHBG) (nmol/L)) * 100) > 6 [11]. In addition, the women in this case report presented with current amenorrhea of varying
duration, i.e., short-term amenorrhea defined as the cessation of menses for <100 days and long-term amenorrhea defined as the absence of menses for >100 days [12].

Screening Procedures

Participants signed an informed consent approved by the Institutional Review Board at the University of Toronto or Pennsylvania State University. Height and weight were measured, and participants completed questionnaires to assess medical history, exercise and menstrual history, eating behaviors, and psychological health. A physical exam was performed and blood sample was collected to determine overall health. A semi-structured psychological interview was conducted to ensure that the women were not experiencing major psychiatric disorders, and a registered dietitian assessed eating patterns and food preferences. Dual-energy x-ray absorptiometry (DXA) scans were performed to assess BMD and body composition.

Baseline Procedures

During a 4-week baseline period, menstrual calendars and daily urine samples for the assessment of menstrual function were collected. Body weight was measured weekly. At week 3 of baseline, energetic markers (leptin, ghrelin, total triiodothyronine (TT3)), markers of bone formation and resorption, body composition, resting energy expenditure (REE), and dietary intake were assessed. Participants also completed a test of aerobic fitness.

Classification of Baseline Menstrual Status

Upon study entry, classification of menstrual status was based on self-reported menstrual histories, which was confirmed by a 28-day urinary profile of E1G, PdG, and
luteinizing hormone (LH) profiles during a 4-week baseline period. FHA was assessed by confirming a negative pregnancy test, normal endocrine panel, no menses in the past 90 days, and documentation of chronically suppressed E1G and PdG profiles observed during the baseline period.

**Intervention Procedures for Energy Calculations**

Both participants were asked to increase their caloric intake 20-30% above baseline TEE while maintaining their usual exercise training regimen. For the purpose of this report, baseline TEE was operationally defined as the sum of REE and purposeful EEE. Energy bars that contained approximately 250-300 kilocalories were provided by the research staff to increase caloric intake. The target increase in caloric intake was gradually achieved by a slow increase in calories during the first several weeks of the intervention to encourage compliance. A registered dietitian met with the participants regularly to provide strategies to meet the target caloric intake. Participants also regularly met with a clinical psychologist or licensed clinical social worker to monitor general psychological health.

**Assessment of Menstrual Function During the Intervention**

Menstrual function was monitored daily during the intervention by assessing urinary excretion of E1G, PdG, and LH metabolites and the presence of menses as self-reported on monthly calendars. The methods used for the assessment and categorization of menstrual cycles are detailed and have previously been published [2].
Recovery of Menstrual Function Categories

To describe the recovery of menstrual function, we classified recovery using several definitions of recovery that ranged in hormonal and clinical relevance. Recovery Category 1 was described simply as “recovery of menses.” The successful recovery of menses after the baseline period was defined as the first occurrence of menstrual bleeding during the intervention. For further analysis of the recovery of menstrual function, Recovery Category 2 was described as resumption of menses preceded by ovulation based on increases in urinary E1G (above 35 ng/ml), PdG (above 2.5 μg/ml), and mid-cycle LH (above 25 mIU/ml) concentrations [2, 13]. Recovery Category 3 was described as resumption of menses followed by at least 2 menstrual cycles of less than 36 days each.

Anthropometrics

Total body weight was measured by a digital scale during each week of the baseline period and every two weeks during the intervention. Height was measured during the screening period, and BMI was calculated as a ratio of weight to height (kg/m²). Baseline values for body weight and BMI were reported as the average of all baseline and screening measurements.

Eating Behavior Assessment

Participants completed the Three Factor Eating Questionnaire (TFEQ) and Eating Disorder Inventory 2 (EDI-2) at screening and at months 2, 3, 6, 9, and 13 (post-study) to assess eating behavior. The TFEQ is a 51-item questionnaire with three subscales – cognitive dietary restraint (CDR), disinhibition, and hunger. Cognitive dietary restraint was evaluated according to the following ranges established by Stunkard and Messick.
0-10 indicated low CDR, 11-13 indicated high CDR, and 14-21 indicated the clinical range. The EDI-2 is a 91-item questionnaire with 8 subscales and 3 provisional subscales, as previously reported [15]. Scores on the first 8 subscales were compared to published means and 95% confidence intervals of eating disorder patients and non-patient college females to assess for symptoms of disordered eating and associated psychological features [16].

**Body Composition and Bone Mineral Density**

DXA scans of the total body, lumbar spine, and dual femur were performed to assess body composition and BMD. Body composition was measured at screening and baseline and during months 1, 2, 3, 6, 9, and 13 (post-study). BMD was assessed at all three sites at screening, month 6, and month 13 (post-study). The participants were scanned on either a GE Lunar Prodigy or Lunar iDXA (GE Lunar Corporation, Madison, WI). Consistent with the International Society of Clinical Densitometry guidelines, a cross calibration study was performed to remove systematic bias between the systems as previously published [17].

**Dietary Energy Intake**

Dietary energy intake was assessed from 3-day diet logs (2 weekdays and 1 weekend-day) completed during week 3 of baseline and each month during the intervention as previously published [17]. Participants met with a registered dietician regularly who trained them how to record dietary intake accurately and reviewed the completed energy intake logs. Participants received written guidelines regarding proper measurement and reporting of food portions and preparation.
Resting Energy Expenditure

REE was determined by indirect calorimetry during week 3 of baseline and months 2, 3, 6, 9, and 13 (post-study) (Sensormedics Vmax metabolic cart, Yorba Linda, CA). Methods explaining the measurement of REE have been published in detail elsewhere [17]. Predicted REE (pREE) was also calculated using the Harris Benedict equation [18]. We compared the lab-assessed REE to the predicted REE (REE/pREE) to estimate how much the measured REE deviated from the predicted REE. A reduced ratio of measured REE to Harris-Benedict predicted REE of 0.60-0.80 has been reported during periods of low body weight and prior to refeeding in anorexic women [19-21]. We have previously published data using a ratio of REE/pREE <0.90 as the operational definition of an energy deficiency [1, 4, 15, 22]. As such, in this study, a ratio <0.90 was used to discriminate between being energy deficient and energy replete.

Purposeful exercise energy expenditure

Purposeful EEE was estimated at baseline and monthly during the intervention using a polar heart rate monitor. Participants completed exercise logs where all purposeful exercise sessions greater than 10 minutes in duration were recorded for a 7-day period. Energy expended during these purposeful exercise sessions was measured using the OwnCal feature of the Polar S610 or RS400 heart rate monitors (Polar Electro Oy, Kempele, Finland) [23]. The OwnCal feature has been validated for the use in calculating EEE from heart rate. The Polar S601 and RS400 hear rate monitors include rest in their estimation of energy expenditure. To estimate only EEE, we subtracted the most recently measured REE (kcal/min) from the Polar heart rate monitors’ estimation of energy expenditure. For purposeful exercise sessions in which participants did not wear
the Polar S610 or RS400 heart rate monitors, the Ainsworth et al. [24, 25] compendiums of physical activities were used to determine the appropriate metabolic equivalent (MET) level for the exercise performed [26]. To calculate the energy expended during the exercise session, the MET level was multiplied by the duration (min) of the exercise session and the measured REE (kcal/min). The MET value includes a resting component. To estimate only EEE, we subtracted the most recently measured REE (kcal/min) from this value.

Participants also recorded the type and duration of purposeful physical activity using daily exercise logs to provide a measure of exercise volume during the study.

Exercise Testing

Maximal aerobic capacity (VO_{2\text{max}}) was measured during a progressive treadmill test to volitional exhaustion using an on-line MedGraphics Modular VO_{2} System (St Paul, MN) or SensorMedics Vmax metabolic cart (Yorba Linda, Calif., USA) during week 3 of baseline using methods previously published [27].

Urinary Reproductive Hormone Measurements

To determine estrogen and progesterone exposure, E1G and PdG urinary metabolites were assessed using a modified trapezoidal integrated area under the curve (AUC) technique. To calculate AUC, the hormone concentrations for two consecutive days of the cycle were averaged; these averages were then summed to provide AUC for the cycle. The methods for measuring urinary reproductive hormones have been previously published [2]. The inter-assay coefficients of variation for high and low internal controls for the E1G assay are 12.2% and 14.0%, respectively. The PdG intra- and inter-assay variability was determined in-house as 13.6% and 18.7%, respectively [2],
Urinary LH was determined by coat-a-count immunoradiometric assay (Siemens Healthcare Diagnostics, Deerfield, IL). The sensitivity of the LH assay is 0.15 mIU/ml. The intra- and inter-assay coefficients of variation were 1.6% and 7.1%, respectively.

### Blood Sampling

Blood was collected, processed, and stored after an overnight fast between 0700 and 1000 once during week 3 of baseline and once at the end of baseline using methods previously published in detail [17]. The latter two samples were pooled for all baseline hormone analyses. In addition, blood samples were collected during months 2, 3, 4, 5, 6, 9, 13 (post-study).

### Serum Hormone Analysis

The metabolic hormones TT3, leptin, and ghrelin were measured using previously published methods [17, 28]. Bone markers including pro-collagen type 1 amino-terminal propeptide (P1NP) and collagen type 1 cross-linked C-telopeptide (CTx) were also measured. P1NP was analyzed by radioimmunoassay (RIA) (Immunodiagnostic Systems, Inc., Scottsdale, AZ). The sensitivity of the assay was 2 ug/L. Intra-assay and inter-assay coefficients of variation were between 6.5-10.2% and 6.0-9.8%, respectively. CTx was analyzed by enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostic Systems, Inc., Scottsdale, AZ). The sensitivity of the assay was 0.02 ng/mL. Intra-assay and inter-assay coefficients of variation for the low control were 3.0 and 10.9%, respectively. All samples from a given participant were analyzed in duplicate.
Case Presentation

Participant 1: Long-term Amenorrhea

Characteristics at Baseline (Table 6.1): This participant was a 19-year old recreationally active college student who participated in a wide variety of activities such as running, weightlifting, rock climbing, hiking, and downhill skiing. At baseline, she reported 12 hours of physical activity each week and averaged about 9 hr/wk of purposeful EEE during the study. Despite presenting with a normal BMI of 20.4 kg/m² and body fat of 20.6%, she had been amenorrheic for 11 months when the intervention commenced and urinary analysis of E1G and PdG confirmed suppressed ovarian activity (Figure 6.1). She presented with a dietary CDR score of 12 which is elevated but not above the clinical threshold of 14 [14]. Scores on the subscales of the EDI-2 were within or below the normal range for college-aged women and did not indicate disordered eating (Table 6.2). The baseline semi-structured psychological interview revealed that the participant felt good about herself and her healthy eating pattern. There was no evidence of current or past eating disorders. Over the course of the study, Participant 1 reported having no difficulty following the energy intake prescriptions.

Changes in Energetic Status: The participant was instructed to gradually increase her daily dietary intake by 500 kcal/day which represented an increase of 31% above her baseline energy requirement (TEE) and a target caloric intake for the intervention of 2,600 kcal/day. The participant’s caloric intake was 2,143 kcal/day at baseline and increased to an average intake of 2,419 kcal/day during the intervention. Exercise volume remained relatively constant throughout the intervention, ranging from 7-12 hr/wk. Weekly EEE averaged 685 kcal/day with a range of 319 to 1,013 kcal/day.
During the intervention, the participant demonstrated a progressive weight gain of 1.8 kg at month 3, 2.1 kg at month 6, and 4.2 kg after month 12 of the intervention when compared to her baseline weight. The increase in weight coincided with an increase in BMI from 20.5 kg/m² at baseline to 22.0 kg/m² after month 12. Fat and lean mass (LBM) increased by 11.7% and 8.3%, respectively, which translated to an increase of 1.3 kg of fat mass and 3.4 kg of lean mass. Percent body fat increased from 20.6% to 21.1%. The greatest increase in fat mass was observed at month 9 with an increase of 2.0 kg from baseline, and a concomitant increase in circulating leptin concentration of 105.7% from baseline to month 9. An increase in REE from 27.20 to 32.61 kcal/day/kg LBM was observed from baseline to month 12. The REE/pREE ratio also increased from 0.81 at baseline to 1.01 at the end of the study, demonstrating an improvement in energy status. Further evidence of an improved energy state is corroborated by a 39.4% increase in TT3 and a 59.2% decrease in ghrelin concentrations (Table 6.3).

Changes in Menstrual Status: After 2.5 months (74 days) in the intervention, menses resumed (Figure 6.1). However, due to the anovulatory nature of the cycle preceding resumption, estrogen exposure, as assessed by E1G AUC, was not improved from the baseline period to the time period preceding resumption. For the first two months after resumption, two consistently eumenorrheic but anovulatory cycles of 28-33 days in length were observed (Figure 6.1). About 6 months into the intervention, however, she experienced another brief episode of amenorrhea with 92 days elapsing between menses. Approximately 8 months in the intervention and 3 months after her last menses (92 days), she resumed menses for a second time. A long intermenstrual interval of 68 days characterized the first cycle after resumption. During this time, a decrease in
caloric intake of approximately 400 kcal/day in the face of a consistent volume of EEE was observed. The participant was informed of the decrease in caloric intake and was instructed again to increase her daily intake to 2,600 kcal/day. She was moderately successful, increasing her intake to approximately 2,350 kcal/day. Consequently, the cycle following the second resumption was ovulatory but characteristic of an inadequate luteal phase, representing the first ovulatory cycle that this participant experienced during the intervention. Estrogen exposure during the 28 days preceding the ovulation-associated menses increased 64.3% compared to the baseline cycle. Furthermore, despite its anovulatory nature, the length of the subsequent and final cycle during the study declined sharply with an intermenstrual interval of 21 days.

*Changes in Bone Health:* As Table 6.4 demonstrates, the participant had a low BMD at the lumbar spine at baseline. After the 12-month intervention, no increases in BMD were observed at any skeletal site; however, P1NP, a marker of bone formation, increased by 49.6%.
Table 6.1. Baseline descriptives of the cases.

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Participant 1</th>
<th>Participant 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.0</td>
<td>165.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<td>54.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.4</td>
<td>19.7</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>20.6</td>
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<table>
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<tr>
<th>Reproductive Characteristics</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of Menarche (yr)</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Gynecological Age (yr)</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Duration of Amenorrhea (days)</td>
<td>330</td>
<td>90</td>
</tr>
<tr>
<td>Duration until Resumption</td>
<td>74</td>
<td>23</td>
</tr>
<tr>
<td>(days in intervention)</td>
<td></td>
<td></td>
</tr>
<tr>
<td># Cycles during Intervention</td>
<td>6</td>
<td>9</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Training Characteristics</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Physical Activity (min/wk)*</td>
<td>761</td>
<td>438</td>
</tr>
<tr>
<td>VO₂max (ml/kg/min)</td>
<td>50.1</td>
<td>43.5</td>
</tr>
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</table>

* Self-reported exercise during baseline
BMI: body mass index; VO₂max: maximal oxygen consumption
Table 6.2. Baseline, month 6, and post-intervention scores on subscales of the Three Factor Eating Questionnaire and Eating Disorder Inventory-2.

<table>
<thead>
<tr>
<th>Eating Behavior Scores</th>
<th>Participant 1</th>
<th>Participant 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary Cognitive Restraint</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Baseline</em></td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><em>Month 6</em></td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><em>Post-Intervention</em></td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td><strong>Drive for Thinness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Baseline</em></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>Month 6</em></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Post-Intervention</em></td>
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<td>4</td>
</tr>
<tr>
<td><strong>Body Dissatisfaction</strong></td>
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<td></td>
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<tr>
<td><em>Baseline</em></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>Month 6</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Post-Intervention</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Perfectionism</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Baseline</em></td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td><em>Month 6</em></td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td><em>Post-Intervention</em></td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 6.3. Baseline measurements and the 6-month and 12-month percent change for metabolic hormone concentrations

<table>
<thead>
<tr>
<th>Metabolic Hormones</th>
<th>Participant 1</th>
<th>Participant 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leptin (µg/ml)</strong></td>
<td>5.1</td>
<td>2.4</td>
</tr>
<tr>
<td><em>6 month % change</em></td>
<td>-19.8</td>
<td>230.9</td>
</tr>
<tr>
<td><em>12 month % change</em></td>
<td>-17.3</td>
<td>279.8</td>
</tr>
<tr>
<td><strong>Total Ghrelin (pg/ml)</strong></td>
<td>1,806.9</td>
<td>1,656.4</td>
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<tr>
<td><em>6 month % change</em></td>
<td>-35.9</td>
<td>-15.2</td>
</tr>
<tr>
<td><em>12 month % change</em></td>
<td>-59.2</td>
<td>-12.1</td>
</tr>
<tr>
<td><strong>Total Triiodothyronine (ng/dl)</strong></td>
<td>53.1</td>
<td>68.9</td>
</tr>
<tr>
<td><em>6 month % change</em></td>
<td>8.0</td>
<td>6.3</td>
</tr>
<tr>
<td><em>12 month % change</em></td>
<td>39.4</td>
<td>31.5</td>
</tr>
</tbody>
</table>
Table 6.4. Baseline measurements and the 6-month and 12-month percent change for bone marker concentrations and bone mineral density.

<table>
<thead>
<tr>
<th></th>
<th>Participant 1</th>
<th>Participant 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone Markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1NP (μg/L)</td>
<td>52.90</td>
<td>36.95</td>
</tr>
<tr>
<td>6 month % change</td>
<td>5.6</td>
<td>22.6</td>
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<tr>
<td>12 month % change</td>
<td>49.6</td>
<td>51.6</td>
</tr>
<tr>
<td>CTx (ng/ml)</td>
<td>0.65</td>
<td>0.64</td>
</tr>
<tr>
<td>6 month % change</td>
<td>-23.1</td>
<td>-29.0</td>
</tr>
<tr>
<td>12 month % change</td>
<td>17.7</td>
<td>-36.1</td>
</tr>
<tr>
<td><strong>Bone Mineral Density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar Spine Z-score</td>
<td>-1.6</td>
<td>-1.4</td>
</tr>
<tr>
<td>Lumbar Spine BMD (g/cm²)</td>
<td>0.983</td>
<td>1.056</td>
</tr>
<tr>
<td>6 month % change</td>
<td>1.7</td>
<td>2.6</td>
</tr>
<tr>
<td>12 month % change</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Femoral Neck Z-score</td>
<td>0.5*</td>
<td>-0.6</td>
</tr>
<tr>
<td>Femoral Neck BMD (g/cm²)</td>
<td>1.062</td>
<td>0.994</td>
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<tr>
<td>6 month % change</td>
<td>-2.8</td>
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<td>12 month % change</td>
<td>-4.3</td>
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<tr>
<td>Hip Z-score</td>
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<tr>
<td>Hip BMD (g/cm²)</td>
<td>0.996</td>
<td>0.955</td>
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<td>6 month % change</td>
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<td>-0.4</td>
</tr>
<tr>
<td>12 month % change</td>
<td>-2.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Z-score at month 6

BMD: bone mineral density; CTx: collagen type 1 cross-linked C-telopeptide; P1NP: pro-collagen type 1 amino-terminal propeptide
Figure 6.1. Reproductive hormone profile of Participant 1. This figure displays the reproductive hormone profile during the study for Participant 1 and the changes in caloric intake, body weight, and energy status that coincided with each category of menstrual recovery. Arrows indicate menses. Body weight was measured within 1 week of menses. ‡ indicates data were collected 2 weeks before menses. † indicates data were collected 6 weeks after menses. ○ indicates E1G; Δ indicates PdG. %BF: percent body fat; BMI: body mass index; BW: body weight; E1G: estrone-3-glucuronide; PdG: pregnanediol glucuronide; REE/pREE: measured resting energy expenditure/predicted resting energy expenditure; TT3: total triiodothyronine

Baseline
- BW: 54.7 kg
- Fat mass: 11.1 kg
- %BF: 20.6
- BMI: 20.4 kg/m²
- REE/pREE: 0.81

Recovery of Menses & Resumption of Regular Cycles
- Caloric Intake ↑ 338 kcal ‡
- BW ↑ 2.5 kg
- Fat Mass ↑ 0.4 kg ‡
- %BF: 20.4 ‡
- TT3 ↑ 2.2% ‡
- REE/pREE: 0.85 ‡

Menses preceded by Ovulation
- Caloric Intake ↑ 307 kcal ‡
- BW ↑ 3.3 kg
- Fat Mass ↑ 1.3 kg †
- %BF: 21.1 †
- TT3 ↑ 39.4% †
- REE/pREE: 1.01 †

Day of Intervention
- 74
- 107
- 135
- 226
- 294
- 315

Intermenstrual Interval
- 33 d
- 28 d
- 92 d
- 68 d
- 21 d

# of days of menses
- 5
- 6
- 4
- 5
- 5
- 6
Participant 2: Short-term Amenorrhea

*Characteristics at Baseline (Table 6.1):* This participant was a 24-year old graduate student who participated in approximately 7 hours of exercise each week, consisting of dancing, running, and weight training. She presented with a normal BMI of 19.7 kg/m² and percent body fat of 22.7%; however, at the start of the intervention, she had not had menses for three months, and her menstrual history revealed multiple extended episodes of amenorrhea. Menarche occurred at 13 years of age. At age 16, she experienced an 8-month episode of amenorrhea. After she resumed menses, she had regular cycles until the age of 21 years when she experienced a prolonged episode of amenorrhea for 2.5 years that she associated with low food intake, stress, and excessive exercise. During this time of amenorrhea, she weighed 43 kg but gained about 10 kg to bring her to the weight of 53.8 kg which was measured at the baseline period of this report. Ten months prior to starting this study, she resumed sporadic menses, reporting 5 cycles during those months. Menstrual disturbances were still present, however, as confirmed by self-reported long cycles and suppressed concentrations of E1G and PdG measured at baseline.

The participant presented with an elevated but not clinical dietary cognitive restraint score of 12 and scores that were above normal for college-aged women and within the range for eating disorder patients for the following four subscales of the EDI-2: ineffectiveness, perfectionism, interpersonal distrust, and interoceptive awareness [16] (Table 6.2). The baseline semi-structured psychological interview revealed that Participant 2 had a history of clinical diagnosis of anorexia nervosa and although she no longer met criteria for a clinical eating disorder, she continued to have associated
characteristics such as perfectionism, social anxiety and reservations about trusting others.

**Changes in Energy Status:** The participant was instructed to gradually increase daily dietary intake by 400 kcal/day, representing an increase of 27% above her baseline energy requirements (TEE) and a target caloric intake of 1,900 kcal/day. Her caloric intake increased from 1,482 kcal/day at baseline to an average intake of 1,917 kcal/day for the first six months of the study. During the latter 6 months, an average intake of 1,838 kcal/day was observed. Exercise volume ranged from 3 to 7 hr/wk during the intervention with the exception of one month during which 10 hours of purposeful EEE were reported. Weekly EEE averaged 237 kcal/day with a range of 30 to 508 kcal/day.

The participant gradually gained weight for the first 6 months of the intervention such that by month 6, her weight had increased by 2.4 kg. After 12 months, the total weight gain was 2.8 kg, indicating that her weight remained relatively stable during the last 6 months of the study. Coinciding with this increase in weight, BMI increased from 19.7 kg/m² to 20.7 kg/m², and fat mass steadily increased with a total gain of 2.2 kg (17.5% increase). Interestingly, lean mass decreased 1.4 kg (-3.3%) after 12 months which primarily occurred during the last 6 months of the study. Leptin concentrations increased during the study (279.8% increase) (**Table 6.3**). Improvement in energy status was demonstrated by an increase in REE from 28.1 kcal/day/kg LBM to 32.8 kcal/day/kg LBM at the completion of the study which coincided with an increase in the REE/pREE ratio from 0.87 to 0.94. Further evidence for this improved energy state was an increase in TT3 (31.2%) and a decrease in ghrelin (-12.1%) (**Table 6.3**).
Changes in Menstrual Status: The participant resumed menses 23 days after the start of the intervention, an event that was preceded by ovulation (Figure 6.2). Estrogen exposure increased 139.4% from baseline to the cycle preceding the resumption of menses. However, menses was not reported for the following 4 months and chronically suppressed concentrations of E1G and PdG were observed, confirming the presence of another episode of amenorrhea. During this period of amenorrhea, body weight and caloric intake decreased slightly toward baseline values then increased again, leading to a second resumption of menses 144 days (~5 months) into the intervention. For the remaining 7 months of the study, 8 more cycles were reported, with consistent cycle lengths of 24-29 days (Figure 6.2). Despite consistent intermenstrual intervals, the cycles were characterized by subtle menstrual disturbances. Of the 10 cycles reported during the study, 6 were ovulatory and 4 were anovulatory. Of the ovulatory cycles, all of them displayed a luteal phase defect. Four cycles were characterized by both a short and inadequate luteal phase, one cycle had just a short luteal phase, and one cycle had an inadequate luteal phase.

Changes in Bone Health: As depicted in Table 6.4, low BMD at the lumbar spine and hip were observed at baseline. No significant increases in BMD were observed; however, P1NP increased by 51.6% and CTx decreased 36.1%, demonstrating a favorable change in bone turnover.
Figure 6.2. Reproductive hormone profile for Participant 2. This figure displays the reproductive hormone profile during the study for Participant 2 and the changes in caloric intake, body weight, and energy status that coincided with each category of menstrual recovery. Arrows indicate menses. ‡ indicates data were collected 5 weeks after menses. † indicates data were collected 3 days after menses. ○ indicates E1G; Δ indicates PdG. %BF: percent body fat; BMI: body mass index; BW: body weight; E1G: estrone-3-glucuronide; nr: not reported PdG: pregnanediol glucuronide; REE/pREE: measured resting energy expenditure/predicted resting energy expenditure; TT3: total triiodothyronine

<table>
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<tr>
<th>Day of Study</th>
<th>E1G (ng/ml)</th>
<th>PdG (ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>30</td>
<td>0.00</td>
<td>0.00</td>
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<thead>
<tr>
<th>Baseline</th>
<th>Recovery of Menses Preceded by Ovulation</th>
<th>Resumption of Regular Cycles</th>
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</thead>
<tbody>
<tr>
<td>• BW: 54.0 kg</td>
<td>• Caloric Intake ↑ 231 kcal ‡</td>
<td>• Caloric Intake ↑ 913 kcal †</td>
</tr>
<tr>
<td>• Fat mass: 12.6 kg</td>
<td>• BW ↑ 1.0 kg ‡</td>
<td>• BW ↑ 2.4 kg †</td>
</tr>
<tr>
<td>• % BF: 22.7</td>
<td>• Fat Mass ↑ 0.9 kg ‡</td>
<td>• Fat Mass ↑ 1.0 kg †</td>
</tr>
<tr>
<td>• BMI: 19.7 kg/m²</td>
<td>• % BF: 24.0 ‡</td>
<td>% BF: 23.8 †</td>
</tr>
<tr>
<td>• REE/pREE: 0.87</td>
<td>• TT3 ↑ 2.0% ‡</td>
<td>• TT3 ↑ 6.3% †</td>
</tr>
<tr>
<td></td>
<td>• REE/pREE: 0.90 ‡</td>
<td>• REE/pREE: 0.89 †</td>
</tr>
</tbody>
</table>

Day of Study

Intermenstrual Interval

Pre-Intervention Baseline Period

Day of Intervention

# of days of menses

Intermenstrual Interval

% of days

Table:

<table>
<thead>
<tr>
<th>Day of Study</th>
<th>E1G (ng/ml)</th>
<th>PdG (ug/ml)</th>
</tr>
</thead>
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</tr>
<tr>
<td>28 d</td>
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Discussion

This case report examined the effects of a 12-month controlled intervention of increased caloric intake in two exercising women with current amenorrhea of varying duration and documents for the first time the simultaneous response of markers of energetic status, daily changes in reproductive hormones, and markers of bone health. The two women in this case report successfully gained weight and resumed menses in response to the non-pharmacological intervention of increased caloric intake. We also document the onset of ovulatory function and regular inter-menstrual intervals in these women and highlight the improved energetic milieu that preceded the reproductive milestones.

Resumption of menses successfully occurred in both women with an intervention that increased caloric intake rather than decreased EEE, a strategy that may be attractive to both athletes and coaches because it does not interfere with training volume or intensity. The increase in caloric intake was tailored to the individualized energy requirements of each participant and was associated with an increase in body weight and an improved energy status. On average, an increase in body weight of 3.5 kg was observed commensurate with an increase in REE from baseline to the completion of the study. In our lab, we have used the ratio of REE/pREE as an indicator of energy status and have operationally defined an energy deficiency as a ratio <0.90 [4, 15, 22]. Both women presented with a ratio <0.90 at baseline, indicative of an energy deficient state. Previous reports of the REE/pREE ratio in amenorrheic exercising women have ranged from 0.80 to 0.95 [4, 27, 29] and in anorexic women from 0.60 to 0.80 [19-21]. The two women in this case report resumed menses and experienced an increase in REE such that
the REE/pREE ratio improved to above 0.90 at the completion of the intervention, indicative of an improvement in energy status and reversal of the energy deficiency.

Likewise, changes in TT3 and ghrelin concentrations paralleled the changes in body weight and REE and provide support for the critical importance of an energy replete state for the successful resumption of menses. Interestingly, fasting concentrations of TT3 increased and ghrelin decreased during the intervention in both women. TT3 is a well-known marker of energy status and is often suppressed among amenorrheic athletes when compared to their ovulating counterparts and sedentary women [1, 27]. In fact, it has been shown in the non-human primate model that induction of amenorrhea via an increase in exercise volume and energy expenditure results in a significant decrease in circulating concentrations of TT3 that is reversed with an increase in caloric intake and resumption of menses [30]. Ghrelin, on the other hand, is an orexigenic hormone that regulates appetite and is commonly elevated among amenorrheic exercising women [27, 31]. Therefore, an increase in fasting concentrations of TT3 and a decrease in ghrelin provide evidence for improvements in energy status.

In response to the intervention, each woman successfully resumed menses as defined by the occurrence of menstrual bleeding and experienced at least one cycle that was preceded by ovulation. However, in association with varying duration of amenorrhea, the changes observed for each woman in dietary intake, body weight, and the energetic environment that were associated with the reproductive milestones varied. For Participant 1 with long-term amenorrhea, it appeared that weight gain greater than 2 kg coincided with recovery of menses and a gain of about 3 kg coincided with ovulation. However, for Participant 2 with short-term amenorrhea, minimal change in weight prior
to the first menses during the study was observed, but approximately 2 kg of weight gain was necessary before the onset of regular cycles. It should be noted, however, that upon entrance into the study Participant 2 reported experiencing long intermenstrual intervals in the previous year, indicative of an oligomenorrheic profile. Thus, it appears that upon commencement of the intervention, this woman was presumably in the early stages of recovery, and the first menses observed during the study may have been another sporadic menses similar to those that she had been experiencing for the past 10 months. Robust increases in caloric intake and subsequent weight gain may have aided resumption of regular intermenstrual intervals as evidenced by consistent cycles of 24-29 days in length for the last 7 months of the study.

Body composition and the metabolic milieu at baseline may have played a role in both the time to and quality of recovery of menses. At baseline, both women presented with a BMI and percent body fat within the normal range for exercising women; however, Participant 2 (short-term amenorrhea) presented with a greater percent body fat at baseline than Participant 1. Body fat has been recognized as playing an important permissive role in reproductive function through the effects of leptin, an adipocyte-derived metabolic hormone [32, 33]. Leptin binds to receptors in the hypothalamus, stimulating the release of gonadotropin-releasing hormone [34, 35] and thereby playing a regulatory role in reproductive function via its influence on gonadotropin pulsatility and reproductive steroid production [36]. Alterations in leptin secretion parallel changes in fat mass; however, leptin secretion is also sensitive to acute alterations in circulating concentrations of glucose [37] and insulin [38]. Consequently, a change in leptin concentrations may occur prior to a change in fat mass [36]. In this way, leptin may be
mediating recovery of menstrual function prior to notable changes in fat mass. In this case report, Participant 2 with short-term amenorrhea demonstrated robust increases in fat mass and leptin concentrations within the first 6 months of the intervention and, coinciding with this increase in leptin, displayed both an ovulatory cycle and resumption of regular cycles early in the intervention. On the other hand, Participant 1 with long-term amenorrhea gained minimal fat mass and showed no increase in leptin concentrations during the first 6 months of the intervention despite an increase in circulating T3. Interestingly, she did not experience an ovulatory cycle until month 11 after demonstrating a gain in fat mass of 2.0 kg and increase in leptin concentration of 106% at month 9 of the intervention. Of further interest is that body fat and leptin concentration decreased again by month 12; whereas, REE and T3 concentration continued to increase during the last few months of the intervention. Therefore, the woman with short-term amenorrhea seemed to recover faster secondary to robust increases in fat mass and leptin early in the intervention; whereas, the woman with long-term amenorrhea required more time to achieve an ovulatory cycle and demonstrated cycles of greater inconsistency, coinciding with inconsistent changes in fat mass and circulating leptin concentration. As such, in agreement with other investigators [33], leptin concentration, which is strongly correlated with percent body fat [17], likely plays a role in recovery of menses. Furthermore, the woman with long-term amenorrhea (Participant 1) maintained a lower percent body fat as well as greater exercise volume throughout the intervention compared to the woman with short-term amenorrhea (Participant 2), providing further potential reasons for the differences observed during recovery of menstrual function.
Of interest, however, is that neither woman experienced complete recovery of menstrual function as defined by the occurrence of consistent ovulation and regular cycles of 26-35 days during the course of the intervention. Despite the onset of menses, subtle menstrual cycle disturbances or long intermenstrual intervals were observed throughout the study. The presence of subtle menstrual cycle disturbances in exercising women who are regularly cycling is not uncommon [2, 13]. In fact, it has been reported that about 52% of exercising women experience subtle menstrual cycle disturbances in the face of apparently regular cycles [2]. Thus, it is plausible that women who are recovering from amenorrhea may also experience these subtle menstrual cycle disturbances prior to complete recovery of optimal menstrual function which may require more time than 12 months.

Further, it is notable that both women experienced a decrease in energy intake during the intervention that corresponded with long intermenstrual intervals consistent with the definition of amenorrhea and oligomenorrhea. This non-compliance with the prescribed energy intake, whether inadvertent or intentional, for a period of time during the intervention may have also contributed to the time course of recovery of menstrual function and the lack of complete recovery of optimal menstrual function. However, both women increased caloric intake again after this period of non-compliance, coinciding with ovulation and the onset of regular cycles for Participant 1 and 2, respectively. These events further demonstrate the importance of adequate energy intake on menstrual function among exercising women.

No improvements in bone health for either woman were observed, likely secondary to the relatively short intervention of 12 months. For bone health outcomes, a
longer intervention of 18-24 months may be required to realize significant changes to bone density and strength. Neither woman demonstrated a clinically significant increase in BMD as defined by a change that exceeded the least significant change; however, P1NP, a marker of bone formation, increased by approximately 50% in both women. This favorable change in bone turnover may indicate that more significant BMD changes may have been observed if the participants were followed for a longer duration of time.

Other case studies of amenorrheic athletes who gained weight demonstrated significant improvements in bone health [7, 9]. Frederickson et al. [7] reported a 25.5% and 19.5% increase in lumbar spine and hip BMD, respectively, over 8 years after a gradual weight gain of 17 kg in an athlete who presented with amenorrhea and very low BMD. Similarly, Zanker et al. [9] observed a 16.9% increase in hip BMD after weight gain of 8 kg over 36 months in an endurance athlete with primary amenorrhea and low BMD. These case studies demonstrate that weight gain can lead to significant increases in BMD if an adequate energy state is achieved and adequate time has passed to allow for measurable changes in BMD. It must be noted, however, that in larger samples which have primarily been composed of anorexic women and adolescents, investigators have reported both minimal changes and increases in BMD with weight gain [39, 40], highlighting the need for more research in this area.

Strengths of this case report include the detailed assessments of energy status, the metabolic environment, menstrual function, and bone health for a 12-month period. Furthermore, characterizing changes and improvements in menstrual function using urinary metabolites of reproductive hormones collected daily for 12 months provides the opportunity to examine subtle changes in menstrual function that coincide with
improvements in the energetic and metabolic environments. A limitation of this case report is the omission of non-exercise activity thermogenesis from the calculation of TEE as a result of problems encountered with the accelerometers used for the study, therefore resulting in a lack of reliable data for this variable.

Conclusion

This case report provides further support for the role of energy deficiency in menstrual dysfunction among exercising women and the benefits of an adequate energy intake on reproductive health. Resumption of menses coincided closely with weight gain and improvements in energy status that were achieved by increases in caloric intake. This case report also demonstrates that the nature of recovery of menstrual function among exercising women with FHA may differ according to individual differences in duration of amenorrhea, body composition, exercise volume, and the metabolic milieu. Therefore, the response to an increase in caloric intake as well as the time course of menstrual recovery is unique to each woman; however, it appears that improvements in energy status are closely linked to improvements in menstrual function. Further research is needed in larger samples to determine the primary contributors to resumption of menses in amenorrheic, exercising women.
References


CHAPTER 7

Conclusions

The loss of bone mass or failure to achieve optimal peak bone mass among female athletes as a result of an energy deficiency and subsequent menstrual dysfunction is a lifelong health concern as it can predispose women to osteoporosis and fractures later in life [1]. As such, it is imperative to understand both the skeletal characteristics of amenorrheic athletes that may contribute to increased fracture risk as well as physiological factors that may influence bone mass and bone geometry in this population in an effort to develop effective strategies for prevention and treatment of low bone mineral density (BMD) among athletes with exercise-associated menstrual disturbances (EAMD). Therefore, the overall purpose of this dissertation was to explore the determinants of bone health in exercising women with severe menstrual cycle disturbances in an effort to gain further insight into effective treatment strategies for the prevention and reversal of low bone mass in this population. To that end, four studies were conducted to achieve the following aims: 1) to compare volumetric BMD (vBMD), bone geometry, and estimated bone strength between amenorrheic and eumenorrheic exercising women (Study 1), 2) to explore the respective roles of reproductive function, metabolic status, and body composition on areal BMD (aBMD) and estimated bone geometry (Study 2), 3) to assess the impact of a 12-month intervention of increased caloric intake on bone health and related factors in exercising women with EAMD (Study 3), and 4) to examine the responses of two amenorrheic exercising women to a 12-month intervention involving an increase in energy intake (Study 4).

Using two-dimensional imaging techniques such as dual-energy x-ray absorptiometry (DXA) and dual-photon absorptiometry, it has been well-established that
amenorrheic athletes have low aBMD compared to eumenorrheic athletes [2-5]. However, studies that have assessed bone health using three-dimensional techniques, which have the ability to assess estimated bone strength due to their capability to measure bone geometry, are limited and have focused collectively on athletes with amenorrhea during the adolescent and young adult years [6-9]. In Study 1, a three-dimensional imaging technique, peripheral quantitative computed tomography (pQCT), was used to assess true volumetric BMD (vBMD), bone geometry, and estimated bone strength at the tibia among amenorrheic and eumenorrheic exercising women. Our purpose was to understand the characteristics of bone beyond DXA-derived aBMD that may contribute to poor bone health among young women with the most severe form of EAMD. The primary finding of this study was that amenorrheic exercising women presented with smaller bone area and lower estimated bone strength at the proximal tibia compared to their regularly-menstruating counterparts, suggesting that the metabolic suppression typically characteristic of amenorrheic athletes [10, 11] may impair the typical osteogenic response of bone to mechanical loading, particularly when the energy deficit and reproductive suppression begin during adolescence and persist into adulthood. However, less lean mass and smaller muscle area in the amenorrheic women may have also contributed to the smaller bone size and lower bone strength in this group, particularly when considering that lean mass and muscle area were positively associated with bone area and estimated strength at the proximal tibia.

In light of these findings, the results from Study 2 brought further clarity to the role of body composition and reproductive function in bone health of exercising women. In Study 2, we determined whether reproductive function, metabolic status, or body
composition, all of which impact BMD [5, 12-15], was the strongest predictor of aBMD at varying sites, lumbar spine bone mineral apparent density (BMAD), and femoral neck cross-sectional area (CSA) and cross-sectional moment of inertia (CSMI), which served as estimates of femoral neck geometry. Upon consideration of the aforementioned potential osteogenic stimuli, reproductive function (as assessed by mean estrogen concentrations during a menstrual cycle or monitoring period and age of menarche) was a primary determinant of lumbar spine aBMD and BMAD, a site of primarily trabecular bone; whereas, body composition, in particular lean mass, played a key role in determining aBMD and estimated geometrical properties, i.e., CSA and CSMI, at weight-bearing sites including the femoral neck and total hip.

If reproductive function and body composition are strong determinants of bone health at differing sites in exercising women, would changing these factors translate to improvements in bone health in exercising women with EAMD who often present with poor bone health? In Study 3, we addressed this question by assessing the impact of an intervention of increased caloric intake that resulted in weight gain and improvements in the metabolic and reproductive environment on aBMD, lumbar spine BMAD, and femoral neck CSA and CSMI among women with severe EAMD. The influence of such an intervention on bone health has not been previously reported, and current reports of improvements in bone health with weight gain among amenorrheic athletes are limited to case studies [16, 17] and follow-up investigations [18-21] that did not include a controlled intervention component and only assessed changes in aBMD. A primary finding of this study was that lumbar spine aBMD and BMAD increased non-significantly by 2.2% and 2.1%, respectively, and femoral neck CSA increased by 1.8%
in the intervention group, suggesting that the intervention of increased caloric intake may be having its desired influence, but more time and women are likely necessary to achieve statistical significance. Consistent with reports of other investigators [19-21], low aBMD at the lumbar spine among women with EAMD when compared to their ovulating and menorrheic counterparts persisted, suggesting that the complete reversal of low BMD, particularly among young adults, may not be attainable. In agreement with the findings of Study 1 and Study 2, change in body composition and reproductive function demonstrated strong associations with change in bone health parameters during the intervention.

To closely examine the impact of the intervention of increased caloric intake on factors known to influence bone health in exercising women, the final study was a case report that described, in detail, changes in energetic status and the hormonal aspects of recovery of menstrual function and bone health in two exercising women with amenorrhea of varying duration. Other case reports describing the energetic and reproductive response to a short intervention (12-20 weeks) of increased caloric intake among amenorrheic exercising women have, for the most part, demonstrated that increased caloric intake is an effective treatment strategy for the recovery of menstrual function among amenorrheic exercising women [22, 23]. To date, however, there are no reports that document the simultaneous changes in markers of energetic and metabolic status, and the associated effects on reproductive recovery and bone health in amenorrheic exercising women over the course of 12 months of increased caloric intake. In response to the intervention, both women successfully gained weight and resumed menses, eventually presenting with regular inter-menstrual intervals and ovulation as the
randomized controlled trial (RCT) progressed. Recovery of menstrual function was closely linked to weight gain and improvements in energetic and metabolic status as evidenced by an increase in body mass, resting energy expenditure, and the metabolic hormones leptin and triiodothyronine, and a decrease in ghrelin. Although no clinically significant increases in aBMD were observed as a result of weight gain, improved energy status, and resumption of menses, an increase in a marker of bone formation (procollagen type 1 amino-terminal propeptide (P1NP)) was observed, indicating that skeletal tissue may be responding favorably to the physiological changes occurring as a result of the intervention.

In sum, results from the four studies demonstrate that among exercising women with impaired bone health as a consequence of severe EAMD, weight gain and resumption of menses which coincide with favorable changes in lean mass, fat mass, and estrogen concentrations may be beneficial for improvements in bone health. Body composition to include both lean mass and fat mass appears to be an important determinant of bone health, i.e., bone mass and bone geometry, among exercising women. Further, optimal reproductive function and estrogen exposure are essential for maintenance of a healthy skeletal framework. On the other hand, the metabolic hormones leptin, insulin-like growth factor-1, and triiodothyronine appear to play less of a role in bone health among exercising women. However, we acknowledge the limitations of our studies. Further, we acknowledge that within the integrative nature of physiology, these hormones likely play a role in bone health, especially when considering the inextricable link of metabolic status and metabolic hormones to reproductive function [10, 24, 25] and body composition [10, 26].
Future Directions

In general, each of the studies presented in this dissertation should be performed in a larger sample of exercising women to confirm our primary findings. Due to the current gap in the literature with respect to skeletal characteristics of exercising women with EAMD that go beyond DXA-derived estimates of BMD, i.e., vBMD, bone geometry, estimated bone strength, and the trabecular and cortical components of bone, assessing these bone health parameters in a large cohort of exercising women with a range of menstrual disturbances will provide further insight into the impact of an energy deficit and suppressed reproductive function on bone health and risk for future clinical outcomes such as fractures and osteoporosis. Furthermore, conducting an intervention of increased caloric intake to restore optimal menstrual function and improve bone health in a larger sample of exercising women with EAMD will provide greater clarity and certainty with respect to the changes occurring as a result of the intervention and may provide valuable insight into appropriate and effective non-pharmacological treatment approaches for women presenting with the female athlete triad, i.e., a syndrome consisting of an energy deficit, menstrual dysfunction, and low bone mass.

The outcomes assessed in each study should also be assessed with an additional grouping variable of sport type, or perhaps more specifically, loading modality of the primary sport of each woman. In the studies presented herein, exercising women were grouped according to menstrual status regardless of the type of sport in which they primarily engaged. Because bone responds differently to the various types of loading forces, the influence of reproductive function, metabolic status, and body composition on bone mass and bone geometry may differ depending on the type of mechanical loading to
which the bone is regularly exposed. Nikander et al. [27, 28] examined the influence of loading modality on bone structure among female athletes using DXA-derived estimates of bone geometry [27] and pQCT-derived measures of vBMD, bone geometry, and estimated bone strength [28]. However, no studies to date have grouped exercising women according to menstrual status and primary loading modality, thereby determining if bones respond differently to an energy deficit and reproductive dysfunction if exposed to different loading impacts.

Follow-up studies similar to Study 1 and Study 2 presented herein should include not only amenorrheic and eumenorrheic exercising women but also sedentary women as has been done in other studies assessing bone health in amenorrheic athletes [4, 6, 8]. We demonstrated that bone health and estimated bone strength are compromised in amenorrheic exercising women compared to their eumenorrheic counterparts. However, the energy- and estrogen-deficient environment of exercising women with amenorrhea coupled with the mechanical loading from habitual physical activity represents a unique situation for bone health outcomes. With the absence of a sedentary control group, we are unable to determine if the mechanical loading from habitual exercise provides a protective effect on bone health among exercising amenorrheic women despite the unfavorable influence of metabolic and reproductive suppression on bone mass and, perhaps, bone geometry.

A further follow-up to Study 2 in which we determined the respective roles of reproductive function, metabolic status, and body composition in DXA-derived bone health measures would be to complete a similar study assessing the roles of the aforementioned potential osteogenic factors (reproductive function, metabolic status, and
body composition) in bone health measures obtained from pQCT, i.e., total, trabecular, and cortical vBMD and bone area, and estimated bone strength. Understanding which potentially osteogenic factors are strong contributors to the health of not only the whole bone but also the trabecular and cortical compartments may provide insight into appropriate prevention and treatment strategies for poor bone health among amenorrheic athletes.

Similarly, a follow-up study to Study 3 which investigated the impact of an intervention of increased caloric intake on DXA-derived measures of bone health in exercising women with EAMD would be to explore the impact of the intervention on bone health measures obtained from pQCT, i.e., total, trabecular, and cortical vBMD and bone area, and estimated bone strength. To date, one longitudinal pQCT study has been conducted among women recovering from anorexia nervosa [29]; however, the impact of weight gain on bone health measures from pQCT has not been described among athletes with EAMD. Understanding the impact of the non-pharmacological treatment approach of weight gain secondary to increased caloric intake on trabecular and cortical vBMD, bone geometry, and estimated bone strength may provide valuable information regarding the characteristics and components of bone that are influenced by the intervention, thereby allowing for the development of focused and effective treatment strategies.

Skeletal tissue is known for its slow rate of change, with the various bone sites changing at different rates depending on the proportions of trabecular and cortical bone at the sites [30]. As such, the 12-month intervention of increased caloric intake explained in Study 3 and Study 4 may not have been of adequate duration to see significant changes in aBMD. Rather, an intervention of 24 months may be more appropriate to determine if
weight gain and recovery of menstrual function secondary to an increase in caloric intake is effective for improving aBMD and estimated bone geometry among exercising women with EAMD. Other investigators have reported no change in aBMD after 9 months of recombinant human leptin treatment in amenorrheic women [31]; however, continuation of the treatment for a total treatment duration of 24 months resulted in a significant improvement in lumbar spine aBMD [32]. Furthermore, among anorexic adolescents with improved nutritional status and gains in body fat, significant improvements in aBMD were not observed until 21 months after the initial assessment [33]. A follow-up investigation of 24 months in amenorrheic athletes who gained weight demonstrated robust and significant improvements in aBMD from baseline that exceeded the changes observed at a 12-month follow-up visit [19]. Results from these studies suggest that significant changes in aBMD may be observed following weight gain and improvements in the metabolic environment if an appropriate time period has elapsed between assessments. In Study 3, we observed improvements in lumbar spine aBMD and BMAD and femoral neck CSA that were not statistically significant, and in Study 4, we observed improvements in a marker of bone formation. These results suggest that the intervention may be having the desired effect on bone health, but more time is needed to observe clinically and statistically significant results.
References


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