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STRESS FRACTURES AND ENERGY DEFICIENCY IN PREMENOPAUSAL EXERCISING WOMEN

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ABSTRACT

Stress fractures are a common injury among exercising women and often interrupt training and hinder athletic performance. Furthermore, the presence of a stress fracture may indicate other underlying health problems in female athletes such as an energy deficiency, menstrual disturbances, or low bone mass. An energy deficiency has been shown to impact hormone concentration and, in turn, bone health through suppressed bone formation or low bone mass. As such, the purpose of this paper is to determine whether exercising women with stress fractures have suppressed resting energy expenditure (REE) and metabolic hormones, such as total triiodothyronine (TT3) and insulin-like growth factor-1 (IGF-1). Exercising women with a lower limb stress injury (SFx) and healthy exercising controls (NSFx) were recruited. As indicators of energy status, resting energy expenditure was assessed using indirect calorimetry, and a blood sample was collected for measurement of circulating TT3 and IGF-1 concentrations. SFx (n=15) and NSFx (n=13) women aged 21.1 and 20.6 respectively, differed significantly in height and lean mass. Serum concentrations of TT3 and IGF-1 did not differ between groups. Similarly, REE and the ratio of REE/pREE were not different between the SFx and NSFx women. However, the SFx group demonstrated a trend toward a lower REE/kg LBM ($p=0.083$) and higher exercise volume ($p=0.093$) compared to the NSFx group. Assessment of exercise volume as high (cutoff) or normal (cutoff) revealed that a greater proportion of SFx women (73%) participated in a high volume of exercise compared to the NSFx women (23%). When categorizing the women based on exercise volume, the high exercise volume group (add in here the cutoff) demonstrated a higher concentration of IGF-1 ($p<0.001$) compared to the normal exercise volume group (add in cutoff). Energy status, as assessed by REE and circulating concentrations of IGF-1 and TT3, was unable to discriminate between exercising women

with and without a lower limb bone injury. However, the high volume of exercise seen in the stress fracture group may mask the suppression of IGF-1 caused by an energy deficiency due to the stimulatory effect of exercise on IGF-1 production.

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CHAPTER 1

1.1 Introduction: Bone is an active tissue, undergoing continuous cycles of remodeling to maintain a healthy state [1]. Although frequent and dynamic loading of the bones achieved through physical activity is beneficial for bone health, repetitive loading of the bones without appropriate rest periods may lead to an accumulation of microcracks within the bone tissue [1]. These microcracks are normally repaired through bone remodeling; however, when there is inadequate time for repair between exercise sessions, the microcracks may accumulate, increasing the risk of sustaining a stress fracture [2]. Stress fractures are most commonly seen in long distance runners and primarily affects the lower extremities, with involvement of the tibia in 49%, the tarsals in 25%, and the metatarsals in 8.8% of cases [3]. A femoral neck stress fracture is a rare injury, occurring in about 1% of stress fractures [3].

Recent studies have shown that exercising women who develop an energy deficiency, menstrual dysfunction, and low bone mass, the so-called "female athlete triad," are at a greater risk for stress fractures [4]. For example, results from a case study of a female runner who presented with the female athlete triad and suffered a femoral neck fracture revealed that she had reduced secretion of luteinizing hormone (LH) and follicular stimulating hormone (FSH) from the pituitary gland [3]. Low concentrations of these reproductive hormones may have led to ovarian suppression, and subsequently, hypoestrogenism and amenorrhea. It has been reported that a long-term hypoestrogenic state due to untreated amenorrhea decreases bone mineral density, which can predispose an athlete to stress fractures of the femoral neck and vertebral bodies [5]. Therefore, in the aforementioned athlete, excessive training and inadequate caloric intake to compensate for energy expenditure may have led to an energy deficient state as well as amenorrhea that, in turn, contributed to the development of a stress

fracture.

Among exercising women with an energy deficiency, physiological adaptations occur to conserve energy, including suppression in resting energy expenditure (REE) and decreased circulating concentrations of total triiodothyronine (TT3) and insulin-like growth factor-1 (IGF-1), two metabolic hormones that serve as markers of energy status [6]. It is known that IGF-1 promotes type 1 collagen production, which is essential for bone formation [7] Energy deficiency has also been linked with suppression of bone formation markers, such as pro-collagen type 1 amino-terminal propetide (P1NP) and bone specific alkaline phosphatase (BSAP), thereby potentially contributing to altered bone turnover and an increased risk of stress fracture [5]. De Souza [8] showed that an energy deficient and hypoestrogenic environment was associated with suppression of osteoblast activity and elevation of osteoclast activity, as evidenced by suppressed levels of serum P1NP and elevated levels of U-CTX-1, a marker of bone resoprtion. The impaired bone formation and elevated bone resorption that may occur as a result of an energy deficiency has the potential to place bones at serious risk for damage. It is currently unknown, however, whether metabolic markers are indicators of the risk of stress fracture among exercising women.

1.2 Purpose: The purpose of this study is to determine whether exercising women with stress fractures have suppressed resting energy expenditure (REE) and metabolic hormones, such as total triiodothyronine (TT3) and insulin-like growth factor-1 (IGF-1).

1.3 Objective #1: To compare energy status, defined as the ratio of measured REE compared to the predicted REE (REE/pREE), between exercising women suspected of lower extremity bone injury and exercising women without a bone injury.

1.3.1 Hypotheses: It is hypothesized that the most robust improvements in energy status, reproductive function, and bone health will be observed among the women who gained the most weight. Specifically, resting energy expenditure, circulating TT_3 and leptin concentrations and urinary estrogen, progesterone, and luteinizing hormone concentrations will increase after 6 and 12 month of the intervention. Circulating ghrelin concentrations will decrease after 6 and 12 month of the intervention. Small increases in bone mineral density will also be observed after 12 months of the intervention.

1.3.2 Rationale: The body's 24-hour caloric requirement is a combination of resting energy expenditure (REE), the thermic effect of food (TEF), and physical activity [9]. Resting energy expenditure accounts for 65-70% of the total 24-hour energy expenditure [9]. Two methods are commonly used to calculate REE. The first is through prediction equations, and one such equation is the Harris-Benedict equation. This equation takes height, weight, age, and sex into account [10]. The second is a measurement of REE through indirect calorimetry. This test involves the measurement of oxygen consumption and carbon dioxide production.

Energy status has previously been assessed using the ratio of measured REE compared to the Harris-Benedict predicted REE (REE/pREE) [8]. In fact, an energy deficiency has been operationally defined as a REE/pREE of less than .9 [6]. As a result of energy intake that is inadequate to compensate for energy expenditure, exercising women, particularly those with menstrual disturbances, have been observed to be in an energy deficient state as evidenced by a REE/pREE <0.9 (Scheid et al., 2009).

We expect REE to be reduced in women suffering from a stress fracture because they

may be presenting with an energy deficiency. REE could be an indicator that negative alterations associated with an energy deficiency are occurring in the body that could lead to compromised bone health. The ratio of REE/pREE has been shown to be a significant predictor of PINP concentrations among exercising women, explaining 19.3% of the variance [6]. Thus, a low ratio of REE/pREE may be associated with low PINP levels and reduced bone formation, indicating that women with suppressed REE may be at greater risk of bone injury.

1.4 Objective #2: To compare circulating concentrations of total triiodothyronine (TT3) between exercising women suspected of a lower extremity bone injury and exercising women without a bone injury.

1.4.1 Hypothesis: It is hypothesized that women with a bone injury will have suppressed circulating concentrations ofTT3 compared to exercising women without a lower extremity bone injury.

1.4.2 Rationale: Triiodothyronine (T3) is an active metabolic hormone whose status is maintained within a normal range by the hypothalamic-pituitary-thyroid (HPT) axis [11]. The release of active T3 begins with the hypothalamus secreting thyroid-releasing hormone (TRH) into the portal circulation. TRH acts on pituitary thyrotropes to stimulate the synthesis and secretion of thyroid-stimulating hormone (TSH). TSH acts on thyroid follicular cells to stimulate growth of the gland and secretion of thyroid hormones T4, and its active form T3.

Long bones, which include the femora, tibiae and fibulae in the leg, form via endochondral ossification. Progression of endochondral ossification and rate of linear growth are tightly regulated by multiple systemic hormones, including T3 [9]. Also, the initiation and duration of the bone remodeling cycle is regulated by thyroid hormone. T3 stimulates osteoblast differentiation and bone matrix synthesis, modification and mineralization [9]. Studies have demonstrated that hypothyroidism results in reduced bone turnover with prolongation of the bone remodeling cycle [12]. Therefore, inadequate bone turnover to repair microcracks induced by repetitive loading may be another mechanism by which exercising women with an energy deficiency are at increased risk for stress fracture. In fact, hypothyroidism has been shown to be associated with a 2- to 3-fold increased risk of fracture in large population studies [13]. Because exercising women with an energy deficiency may have suppressed TT3 levels [6], they may also be at increased risk for a bone injury.

1.5 Objective #3: To compare circulating concentrations of insulin-like growth factor-1 (IGF-1) between exercising women diagnosed with a lower extremity bone injury and exercising women without a bone injury.

1.5.1 Hypothesis: IGF-1 promotes type 1 collagen production, which is essential for bone formation. Therefore, it is hypothesized that women with a bone injury will have suppressed circulating concentrations of IGF-1 compared to exercising women without a bone injury.

1.5.2 Rationale: Insulin-like growth factor I (IGF-1), also known as somatomedin-C, is a systemic growth hormone-dependent polypeptide known to stimulate skeletal growth [14]. *In vivo*, IGF-I stimulates long bone linear growth by effects on the epiphyseal cartilage [15]. IGF-I is also made by a variety of cells found in bone and cartilage, so that it can act as a local regulator of cell replication and tissue growth [16].

IGF-I is essential for proper bone formation due to its role in promoting type I collagen production.. A study conducted with postmenopausal Japanese women, demonstrated an age- and body mass index- independent positive correlation between serum IGF-I level and bone mineral density of the lumbar spine, femoral neck, and mid-radius [17]. Furthermore, the study suggested that serum levels of IGF-I would be clinically important predictors of vertebral fracture risk because they were significantly lower in subjects with vertebral fractures than those without fractures [15]. Zanker and Swaine (2008) observed that the lowest levels of bone formation markers were apparent in amenorrheic athletes with the lowest TT3 and IGF-1 levels. Also, it has been reported that an adaptation to low energy availability includes decreased IGF-I levels (ref). Loucks and Thuma [18] demonstrated that IGF-1 was suppressed in regularly menstruating women who experienced a restriction in energy availability under tightly controlled conditions. As such, it's plausible that the exercising women who are energy deficient will present with suppression in circulating IGF-1 concentrations. Changes in IGF-1 levels may be linked to fluctuations of bone formation markers [19], thereby explaining another mechanism by which exercising women with an energy deficiency may be at greater risk for stress fractures.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction: A stress fracture is a skeletal overuse injury that is caused by the accumulation of strain damage from repetitive load cycles [1] . Stress fractures commonly occur from vigorous exercise, especially that involving repetitive, weight-bearing loads, like running or marching [2]. Bone is an active tissue, constantly undergoing a cycle of bone resorption and formation, which collectively has been termed bone remodeling [3]. This cycle of bone remodeling allows for the repair of microcracks that occur when forces are exerted on bone [3]. These microcracks are a natural and normal component of the bone remodeling process; however, they can develop into larger cracks that are not characteristic of healthy bone [3]. In the event of excessive and repetitive loading of bone as occurs in exercise such as running, bone may not be able to adequately repair the microcracks, leading to stress fractures [4].

It has been established that stress fractures are the most common overuse injury in athletes [1]. This injury can disrupt training and quickly terminate sports careers [1]. Numerous studies have been conducted to assess the frequency of this injury [1, 5-9]. Reports from five studies among collegiate athletes participating in varied sports including, but not limited to, track, football, lacrosse, basketball, volleyball and soccer, reveal that the proportion of athletes with stress fractures ranges from 1.0% to 2.6% [10, 11]. In addition, it has been reported that 0.5% to 15.6% of recreational or competitive athletes who visit a healthcare provider due to an injury complaint present with a stress fracture [12]. Results from these investigations provide evidence that running is a causal factor for stress fracture in athletes that sustained an injury. Furthermore, other investigators have reported cumulative

annual incidence of stress fractures to be 8.7% and 21.1% among track and field athletes [1, 13]. As seen in the Brubaker study [12], the incidence of these fractures provide support for the notion that the repetitive impact and loading component of running are likely major contributors to stress fracture risk. It is therefore essential to understand the reasons for the high stress fracture incidence among athletes and to be aware of who is at risk for injury.

In addition to athletes, military recruits experience a high incidence of stress fractures [14]. This injury impacts the health of our service members and imposes a large financial burden on the military by delaying the training of new recruits [14]. Stress fractures commonly occur during military basic training, with a significantly higher incidence in female recruits compared to male recruits [15, 16]. When comparing stress fracture incidence by gender, investigators estimate that injury rates in military women during basic training range from 1-20%, while rates in military men range from 0.2 to 9% [15, 17-23].

Many risk factors have been proposed to explain why some recruits suffer a stress fracture and others do not [7-9]. These include irregular menstruation; poor skeletal alignment; narrow tibial cross-sectional area; low bone mineral density (BMD); reduced muscle size; strength and mass; and reduced overall fitness [7-9]. Factors often attributable to female athletes that may create a greater likelihood for incurring stress fractures include a small calf circumference, menarche at a late age, menstrual dysfunction, low BMD, an abnormal gait and a current eating disorder [3, 6, 10-12, 24, 25]. Despite these findings, there are inconsistencies in reported studies. For example, Snyder et al. [5] reported that low BMD is associated with increased risk of stress fracture in some cases but not others. Discrepancies in the relation between these factors and stress fracture risk, have prompted our investigation on the importance of an adequate energy status for reduced fracture risk,

which has not been evaluated to date.

Among athletic women suffering a stress fracture, irregular menses, late age of menarche, and menstrual dysfunction are frequently observed [4]. All factors are likely caused by an energy deficiency where energy intake is inadequate to meet the needs of energy expenditure [24]. It has often been reported that exercising women are susceptible to an energy deficiency [9]; therefore, it is plausible that energy deficiency may provide a potential explanation for stress fracture occurrence [26]. In response to an energy deficiency, metabolic adaptations occur to include suppressed REE and decreased concentrations of insulin-like growth factor-1 (IGF-1) [27], and total triiodothyronine (TT3) which may impact bone health [28].Resting energy expenditure (REE), also referred to as resting metabolic rate, can be measured to evaluate energy status [24]. REE is typically suppressed in a state of energy deficiency as the body attempts to conserve energy; therefore, a ratio of REE to predicted REE (pREE) of less than 0.9 has been used to indicate an energy deficiency [24]. Previous reports have revealed that exercising women with menstrual disturbances present with suppressed REE and a ratio of REE/pREE <0.9, indicating an energy-deficient state [24, 25]. In addition, REE has been observed to be positively associated with BMD [11], therefore REE may be an indicator of stress fracture risk.

Insulin-like growth factor-1 (IGF-1) is an important growth factor in the human body. Growth hormone (GH) acts as a tropic factor on IGF-1 and stimulates about 75% of the production of IGF-1 in the liver [29]. In an energy deficient state the GH receptors on the liver may become less responsive to GH, leading to GH resistance. This, in turn, causes a decline in circulating concentrations of IGF-1 [30]. As such, GH concentrations are often elevated among energy-deficient women due to loss of negative feedback from IGF-1 [30].

Thus, elevated GH concomitant with suppressed IGF-1 concentrations is a hormonal profile characteristic of energy deficient women. Therefore, this energy-related decline in IGF-1 concentrations makes it an ideal serum marker to identify women in an energy deficient state.

Thyroid hormone helps the body convert food into energy and heat (metabolism), and regulates body temperature and heart rate [31]. Triiodothyronine (TT3) is the active form and is derived from thyroxine (T4) [32]. Low levels of thyroid hormone, called hypothyroidism, has been shown to be associated with diminished metabolism [31]. Therefore, a negative change in metabolism, as evident by a suppressed resting metabolic rate in energy deficient women, possibly explains the decrease in TT3 levels. For example, De Souza et al [33] showed that TT3 levels were lower ($p=0.007$) in women who had an REE/pREE ratio less than 0.9, indicative of an energy deficiency, compared to women with a ratio of greater than 0.9. The alterations observed in circulating TT3 concentrations with changes in energy status demonstrates its ability to be an ideal marker of energy deficiency.

Metabolic hormones, such as TT3 and IGF-1, also play a role in bone health. Long bones, including the femur, tibia and fibula in the leg, form via endochondral ossification ref. Progression of endochondral ossification and rate of linear growth are tightly regulated by multiple systemic hormones, including TT3 [9]. Also, the initiation and duration of the bone remodeling cycle is regulated by thyroid hormone. TT3 stimulates osteoblast differentiation and bone matrix synthesis, modification and mineralization [9]. In addition to TT3, IGF-I is essential for proper bone formation due to its role in promoting type I collagen production ref. A study conducted in postmenopausal Japanese women, demonstrated an age- and body mass index- independent positive correlation between serum IGF-I level and BMD of the lumbar spine, femoral neck, and mid-radius [34]. In light of the impact TT3 and IGF-1 have

on bone health, it is not surprising that ultimately these hormones influence bone turnover.

Bone turnover is essential to maintaining healthy bone. It is comprised of harmonious cycles of bone formation and bone resorption [3]. To date, investigators have not observed a direct relationship between bone turnover changes and the likelihood of stress fracture in athletes [35]. However, numerous investigators have found a relationship between markers of energy status, such as IGF-1 and TT3, and markers of bone turnover [6, 36], thus providing additional support to the speculation that energy status impacts stress fracture occurance. Zanker and Swaine [37] conducted a study examining bone turnover among both amenorrheic and eumenorrheic female distance runners and sedentary women. They observed that TT3 and IGF-1 concentrations were significantly lower ($P<0.05$) among the amenorrheic runners compared to the eumenorrheic runners and sedentary women, coinciding with suppressed concentrations of bone formation markers; osteocalcin (OC), bone-specific alkaline phosphatase (BAP) and propeptide of type 1 procollage (P1CP) and bone resorption markers; pyridinoline (Pyr) and deoxypyridinoline (Dpyr) [37]. Additionally, studies have demonstrated that hypothyroidism results in reduced bone formation and resorption rates causing a prolongation of the bone remodeling cycle [38]. Therefore, energy deficient exercising women who experience suppressed metabolic hormones such as IGF-1 and TT3, may be at greater risk for stress fractures because of the significant role these hormones have in the bone remodeling process.

The balance between energy intake and energy expenditure is important in maintaining healthy bone. Energy deficiency leads to physiological adaptations including suppressed REE and alterations in metabolic hormones that can negatively impact bone turnover. As a result, the presence of an energy deficiency may contribute to an increased

risk for stress fracture. Thus, the purpose of this review is to explore what is currently known and to highlight what remains unknown about the association between energy deficiency and stress fractures in exercising women.

2.2 Resting Energy Expenditure: Resting energy expenditure (REE), also referred to as resting metabolic rate, can be measured to evaluate energy status [24]. The REE value represents the amount of kilocalories used by the body while at rest during a 24-hour period REF. It accounts for the energy needed to perform the basic physiological and cellular functions for survival [39]. REE contributes approximately 60-75% of a body's total energy expenditure (TEE) [39]. Of the remaining 40%, about 5-10% of the body's total energy expenditure is used for digestion, while approximately 15-30% is used for movement [39].

REE is commonly measured using indirect calorimetry – a technique that measures the amount of O_2 consumed and the amount of CO_2 produced while at rest. REE values can be calculated as well as predicted. By using the Weir equation, O2 and CO2 values can be determined [40]. In addition, the Harris-Benedict equation can be used as a predictor value of REE [41]. This ratio can then be applied in order to gauge energy status [24, 25, 33]. A decreased ratio of REE/pREE of 0.6-0.8 was described in studies when there was severely diminished body weight, such as that observed in anorexic women. Therefore, energy deficiency has been defined as an REE/pREE ratio less than 0.9 in exercising women given that their energy deficit may not be as severe as that observed among women with anorexia [24, 25, 33].

When energy intake is inadequate, the body repartitions and prioritizes its energy expenditure [4]. Often in an effort to conserve energy and maintain processes necessary for

survival, energy is repartitioned toward essential functions (cellular maintenance and repair, thermoregulation, and locomotion) and away from functions not necessary for survival, such as growth and reproduction [42]. In an effort to conserve energy and maintain processes for survival, the body then decreases REE, and subsequently reduces the REE/pREE ratio [24]. For example De Souza el al. [33] have reported REE/pREE to be below 0.9 in exercising women with a high drive for thinness (DT) and a ratio above 0.9 in sedentary women with a normal DT. A higher DT has been associated with women who are likely diagnosed with an eating disorder [33]. Therefore, This study supports the notion that REE, and thus the ratio, decreases when the body is placed in an energy deficient state. Based on these observations, De Souza et al. [24] operationally defined energy deficient as a ratio of REE/pREE < 0.90 and energy replete as a ratio of REE/pREE > 0.90. This definition of an energy deficit has been used in other reports [4, 24, 33].

The presence of an energy deficiency can negatively impact bone health [4]. Among exercising women that are grouped according to energy and estrogen levels, energy deficient women (REE/pREE ratio < 0.9) had a significantly lower concentration of osteocalcin, a marker of bone formation, compared to energy replete women [24]. In addition, when comparing energy and estrogen deficient women to energy and estrogen replete ones, the following was found. The exercising women with deficiencies in energy and estrogen displayed significantly lower N-terminal of propetide of Type 1 collagen (P1NP), which is another marker of bone formation, and greater urinary C-terminal telopeptide (UCTX-1), a marker of bone resorption [24], These findings demonstrate that exercising women who present with an energy deficiency, as defined by a reduced REE/pREE ratio, display an unfavorable alteration in bone turnover. As such, it appears that an energy deficiency may

negatively impact bone metabolism and a low REE/pREE ratio may be associated with suppressed bone formation.

BMD is an important indicator of bone health and fracture risk [4]. Many factors have been found to be associated with BMD, and investigators have demonstrated that REE may be one such factor [43]. A study performed in a population of Caucasian men and women showed that fat mass, lean body mass (LBM), weight, height, body mass index (BMI), waist and hip girth, grip strength and REE were all positively correlated with hip, spine, and total body BMD [43]. In women, the strongest correlation was observed between REE and BMD at all sites, with a significant association at the lumbar spine (r=0.34), total hip (r=0.56) and total body (r=0.56) [43]. This strong association suggests that a decrease in REE, as seen by a reduced REE/pREE ratio, may contribute to a decrease in BMD. A study conducted by Kaufmann et al. [44] among elite osteopenic ballet dancers, further demonstrated an association between REE and BMD. Significant correlations were observed between REE and arm BMD (r=.7; $P<0.001$), spine BMD (r=.59, $P < 0.01$), leg BMD (r=.52, $P < 0.05$) and total body bone mineral content (r=.83; $P < 0.001$) among ballet dancers aged 20-30 years [44]. This link between REE and BMD in women who are osteopenic suggests that REE is an important indicator of bone health [44].

2.3 Triiodothyronine (TT_3): Triiodothyronine (TT3) is the most active form of the thyroid hormone and affects virtually every organ system in the body. The circulation of the hormone is maintained through the hypothalamic-pituitary-thyroid (HPT) system [45]. It is known that thyroid hormone affects energy balance [46]. One of the first studies on the action of thyroid hormones on energy metabolism showed that the administration of TT3 to

hypothyroid rats induced an increase in basal metabolic rate [47]. This hormone stimulates the rate of metabolism by accelerating a number of metabolic anabolic and catabolic pathways. When this occurs, other body processes that require energy for functioning have greater demands placed upon them [36]. Therefore, TT3 plays a role REE and is a marker of metabolism.

Thyroid hormones play an important role in the development and maintenance of muscle [48]. TT3 is believed to increase the production of "uncoupling proteins" [48]. These proteins, located in the inner membrane of the mitochondria, act to uncouple ATP synthesis [49]. Since ATP provides fuel for the body, alternative substrates must be burned to counter this deficit. The alternative ATP production comes at the expense of protein loss from muscle [48]. With increased muscle strength, there is the potential for the reduction of the mechanical load on bone [50]. Markey [51] suggested that muscle mass will disperse forces that are transmitted to the bone. The metabolism of muscle protein leads to a decrease in muscle mass, which in turn puts bones at risk. With decreased ability to disperse forces, the bone becomes more susceptible to a fracture. Maintaining proper TT3 levels is therefore essential for ensuring healthy human bone. In addition to its impact on muscle mass, TT3 also stimulates the growth and development of bones by activating the bone remodeling cycle [52]. *In vitro* studies have shown that TT3 stimulates osteoblast differentiation and bone matrix synthesis, modification and mineralization [45]. In addition, investigators have reported that TT3 directly regulates collagen synthesis and collagen cross-linking, both of which are processes essential for the maintenance of healthy bone [53].

Low concentrations of TT3 and bone markers have been observed simultaneously in female runners [54]. A study performed among amenorrheic and eumenorrheic female

distance runners showed the amenorrheic runners had significantly lower levels of TT3 compared to eumenorrheic runners [54]. In addition, the amenorrheic women had lower concentrations of the bone formation markers, osteocalcin (OC) and bone-specific alkaline phosphatase (BAP) [54] compared to the eumenorrheic runners. The finding that TT3 was significantly lower among the amenorrheic runners who also displayed suppressed concentrations of bone formation markers supports the assumption that TT3 concentrations serve as a reliable indicator of energy status and may be involved in the regulation of bone mass, either directly through its effects on bone tissue or, indirectly, as a marker of nutritional status. Little has been reported however, regarding the association between TT3 concentrations and bone mass in humans; therefore more research is necessary to clarify the role of TT3 in bone metabolism among humans.

TT3 concentrations have also been observed to parallel changes in energy status in both the animal and human model. Williams et al [55] altered the energy status in 8 adult monkeys and monitored the concentration of TT3. It was found that the energy state was strongly associated with circulating TT3 levels [55]. Circulating TT3 concentrations were about 20% lower during the amenorrheic period that was induced by increases in energy expenditure without compensatory increases in energy intake than during the sedentary period [55]. During refeeding of the monkeys, TT3 concentrations increased significantly ($p<0.05$ vs. late amenorrhea period) [55]. Similarly, within humans, De Souza et al [33] showed that TT3 levels were lower ($p=.007$) in women who had an REE/pREE ratio less than 0.9, indicative of an energy deficiency, compared to women with a ratio of greater than .9.

Other investigators exploring the relationship between energy status and metabolic hormones observed that regularly-menstruating sedentary women with energy availability (EA), defined as energy intake minus exercise energy expenditure divided by LBM (EI-EEE/LBM), of approximately 30 kcal/kg LBM·d, had significantly suppressed T3 concentrations ($P<0.05$) [56]. T3 was further reduced when energy availability decreased to 20 kcal/kg LBM·d ($P < 0.01$), but there was no additional suppression in concentration when energy availability was lowered to 10 kcal/kg LBM·d. Subsequently, Loucks and Ihle [57] investigated the relationship of energy availability on markers of bone turnover. Interestingly, the results of this study demonstrated that the decline in OC concentrations with decreasing EA was similar to the decline observed for TT3 with decreasing EA, such that both OC and TT3 concentrations declined predominantly between 20 and 30 kcal/kgLBM/d, and plateaued between 10 and 20 kcal/kgLBM/day [57]. These similarities suggest that TT3 may play a role in mediating the influence of energy availability on the bone remodeling cycle. However, it is currently unclear if TT3 plays a direct role via an influence on bone cells, or an indirect role via the effects of an energy deficiency on bone metabolism.

2.4 Insulin-like Growth Factor 1 (IGF-1): IGF-1, also called somatomedin C, is a single-chain polypeptide protein [58]. Its structure consists of 70 amino acids and is similar to that of insulin [58]. IGF-1 regulates the proliferation and differentiation of a multitude of cell types and is capable of exerting insulin-like metabolic effects [58].

The high levels of IGF-1 that can be extracted from bone matrix have led researchers to believe that IGF-1 is involved in the bone remodeling process [59]. Bone remodeling

constitutes an ongoing lifelong renewal of old bone with new bone [3]. It involves the sequential operation of osteoclastic bone resorption followed by osteoblastic bone formation [3]. Both cell types seem to be affected by IGFs [60]. Johansson et al. [61] performed a study on IGF-1 action by administering IGF-1 (160 μg $kg^{-1}day^{-1}$ for 7 days) to a man with idiopathic osteoporosis and low IGF-1. After one week, the changes observed included increased formative markers [alkaline phosphatase (140%), osteocalcin (OC, 150%) and C-terminal propeptide of type 1 procollagen (PICP (200%) and increased resorption markers [hydroxyproline-creatinine ratio (210%) and carboxyterminal telopeptide domain of type 1 collagen (200%)]. Despite changes observed in both formation and resorption markers, it was suggested that IGF-1 possibly plays a larger role in bone formation than bone resorption [62]. Among humans, investigators have observed that a decrease in serum P1NP, a bone formation marker, was strongly correlated to a decrease in IGF-1 concentrations [54]; however, it was not shown that IGF-1 concentrations correlated with the concentrations of bone resorption markers. Similarly, Grinspoon et al. [63] reported that IGF-1 administration among fasting women increased bone formation markers, but had no influence on bone resorption markers. Following IGF-1 administration, serum OC levels increased fivefold and procollagen I carboxyterminal propeptide (PICP) concentrations increased threefold; whereas, no change was seen in osteoclast activation and collagen degradation markers [63].

The IGF-1 mechanism pathway begins with growth hormone (GH) production, which acts as a tropic factor on IGF-1 [64]. The stimuli for GH secretion are integrated in the hypothalamus, which secretes two neuropeptides, growth-hormone releasing hormone and growth-hormone inhibiting hormone (GHRH and GHIH) into the hypothalamic-hypophyseal portal system [64]. GHRH stimulates GH release into the blood stream from cells within the

anterior pituitary gland [64]. GH then acts on the liver and other tissues to produce IGF-1 [64]. IGF-1 is produced primarily by the liver as an endocrine hormone as well as in target tissues such as the skin, lungs, and kidney via a paracrine and/or autocrine mechanisms [58]. Various factors can influence the production of IGF-1 to include GH insensitivity, poor nutrition, an absence of GH receptors or disruptions in the GH-IGF-1 signaling pathway [59].

The primary action of IGF-1 is mediated by the binding of this growth factor to its specific receptor, the insulin-like growth factor 1 receptor (IGF1R) [65]. The binding of IGF-1 to its receptor initiates the AKT signaling pathway, which then leads to cell growth and proliferation [59]. Investigators have shown the growth-promoting effects of IGF-1 on almost every cell in the body, especially skeletal muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs [66].

Metabolic hormones like IGF-1 play an important role in energy balance [56]. Changes in IGF-1 levels have been observed in exercising women with an energy deficiency [27]. Several investigations have been conducted to study the effects of acute and chronic energy deficiencies on IGF-1 concentrations [56, 67]. In a study conducted by Loucks et al. [56], IGF-1 decreased about 25% with an acute decrease in EA from 30 kcal/kg LBM/d to 10 kcal/kg LBM/d. Likewise, Clemmons et al [68] showed 10 days of fasting in obese male subjects was sufficient to decrease serum IGF-1 levels by 75%. Smith et al. [69] performed a study on fourteen children (aged 8-11) and sixteen adult subjects (aged 22-40) to assess changes in IGF-1 concentrations during 6 days of calorie restriction (50% reduction intake). IGF-1 concentrations declined by 12.7% and 24% in adults and children, respectively, throughout the interval of calorie restriction[69]. During three days of refeeding, there was a slight increase in IGF-1 in both groups, but it did not return to baseline [69]. Similar results have

been observed among adolescents with anorexia nervosa, a disorder that creates a severe state of chronic energy deficiency. An investigation of 22 adolescent girls with anorexia nervosa (AN) and 20 healthy controls was performed to assess adaptations that occur in response to a severe energy deficiency [67]. It was observed that mean IGF-I concentrations were significantly lower in girls with AN than in the controls, and that the circulating IGF-1 concentrations among the AN group were reduced by almost 30% [67].

Since observations have indicated that serum IGF-1 undergoes changes in response to alterations in diet and EA, then it would further follow that IGF-1 concentrations might serve as a marker of energy status [56, 67]. Therefore, the presence of an energy deficiency could be determined based on IGF-1 concentration levels. Metabolic hormones like IGF-1 are altered in exercising women with chronic energy deficiency. This change involves a decrease in concentrations of IGF-1 [33]. For example, Zanker and Swaine [54] performed a study looking at changes in IGF-1 levels in endurance athletes in an energy balanced state (consuming 100% of their estimated daily energy requirement) and energy restriction (consuming 50% of their estimated daily energy requirement). The athletes in a restricted energy state showed a 17% decline in IGF-1 concentrations, whereas the athletes in a balanced energy state maintained their IGF-1 concentrations. Limited studies have tracked changes of IGF-1 levels in energy deficient exercising women; however, several investigators have shown suppression of TT3 levels (also a marker of energy status) in such athletes [70] [71].

2.5 Stress Fracture and Energy Status: As previously described, optimal energy status is important for the maintenance of bone health [72]. Because energy expenditure is a key

component of the energy balance equation, excessive exercise can contribute to a negative energy balance, i.e. an energy deficit [73]. Kadel et al [74] demonstrated that ballet performers who danced more than 5 hours per day suffered more stress fractures compared to those who danced less than or equal to 5 hours each day. Of those who danced less than 5 hours, 13% suffered a stress fracture. However, of those who danced more than 5 hours per day, 50% suffered a stress fracture. This demonstrates the importance of energy status on stress fracture risk. To date, few direct connections have been made between energy status, markers of energy status such as REE, IGF-1 and TT3, and stress fracture occurrence. However, several risk factors associated with stress fractures may be indicators of energy status.

Frequently, exercising women in an energy deficient state, caused by inadequate dietary intake or excessive exercise, developmenstrual disturbances [70, 75]. Williams et al. [55] demonstrated this causal relationship in exercising monkeys. By altering the energy availability in monkeys through increased energy expenditure without compensatory increases in energy intake, Williams induced menstrual dysfunction in the monkeys, then reestablished menstrual cyclicity with the addition of supplemental kilocalories. Menstrual disturbances such as those observed among energy-deficient, exercising women have been reported to be a risk factor for stress fracture occurrence [4]. When menstrual disturbances are caused by an energy deficit, bone loss occurs [75], leading to low BMD which is, another risk factor for stress fracture [75]. For example, West et al [24] observed a significantly lower BMD at both the total body and lumbar spine sites as well as a lower lumbar spine L2-L4 BMD Z-score among energy-deficient, exercising women with amenorrhea (REE/pREE ratio <0.9) compared to exercising, regularly-menstruating women (REE/pREE ratio > 0.9).

These results demonstrate that BMD, a risk factor for stress fractures [24], may be low among energy-deficient women. Low BMD and a menstrual dysfunction are two possible factors for increased stress fracture risk, and both are often found in exercising women with an energy deficiency.

An energy-deficient state leaves less energy available for physiological processes such as reproduction and growth. Because the bone remodeling cycle is an integral component of growth, it is one physiological process that is typically affected by an energy deficit. Loucks and Ihle [57] altered the energy availability in regularly-menstruating, sedentary women for 9 days and analyzed changes in the bone resorption marker, N-telopeptides of type 1 collagen (NTX), and two bone formation markers, PICP and OC. Restricted energy availability treatments at 10, 20, and 30 kcal/kgLBM/day reduced plasma OC concentration by 28%, 32%, and 11%, respectively. The restricted energy availability treatments at 10, 20, and 30 kcal/kgLBM/day also reduced serum PICP concentrations by 26%, 19%, and 12%, respectively. By contrast, restricted energy availability treatment at 10 kcal/kgLBM/day raised NTX concentrations by 34%, whereas the treatments at 20 and 30 kcal/kgLBM/day had no effect [57]. These results clearly demonstrate that acute reductions in energy availability lead to an unfavorable uncoupling of bone turnover as evidenced by suppressed bone formation and elevated bone resorption. Likewise, other investigators have reported that an environment characterized by a chronic energy deficiency is associated with suppression of osteoblast activity and elevation of osteoclast activity, as evidenced by suppressed levels of serum PINP and elevated levels of U-CTX-I [24]. It is unclear whether changes in bone remodeling are involved in the occurrence of stress fractures [35]. However, it has been shown that suppressed bone resorption and a reduced activation of bone

remodeling are associated with increased microdamage accumulation [76]. It was previously stated that accumulation of microdamage causes stress fractures [4], therefore changes in the bone remodeling cycle caused by an energy deficiency is a plausible explanation for stress fracture risk.

As such, excessive exercise, menstrual disturbances, low BMD, and alterations in bone metabolism have been shown to be indicators of an energy deficit [55, 57, 74, 77]. Furthermore, it has been suggested that these are also risk factors for stress fracture [8]. Therefore, our novel study seeks to determine if there is a direct relationship between an energy deficiency and stress fracture.

Investigators have assessed markers of energy status, such as IGF-1, in the stress fracture population. Recently, an investigator measured IGF-1 concentrations, before and after basic training, among military women who suffered a stress fracture compared to those of uninjured recruits [78]. This novel study demonstrated that the stress fracture group exhibited a 68% decrease in bioavailable IGF-1; whereas, the non-stress fracture group demonstrated a 20% increase in bioavailable IGF-1 from pre- to post-basic training [78]. These results demonstrate the importance of IGF-1 in attaining optimal bone quality and preventing stress fractures. However, no studies to date have examined the difference in TT3 concentrations and REE between stress fracture populations and uninjured controls.

2.6 Conclusion: The purpose of this review was to explore what is currently known and identify what is unknown about the association between energy deficiency and stress fracture risk. Stress fractures are a significant health problem for both competitive athletes and recreational exercisers. Monitoring the intensity and duration of exercise, along with

practicing proper rest and nutrition, is important in preventing this injury. An energy deficiency is one factor that can contribute to a bone injury due to its impact on bone metabolism, i.e. suppression of bone formation which then leads to an uncoupling of bone turnover. This disturbance in bone remodeling far too often may lead to a stress fracture due to the inadequate repair of microcracks created during repetitive loading, as is typical during physical activity. Consequences associated with an energy deficiency include suppressed levels of TT3, IGF-1 and REE; therefore, these markers of energy deficiency may also be associated with stress fracture risk.

Healthy bone is maintained through a tightly regulated cycle of bone formation and resorption. Changes in this cycle are believed to be an underlying cause of a stress fracture. Research has shown that TT3, IGF-1 and REE levels can negatively affect bone metabolism markers. However, a direct relation between these physiological markers and stress fractures has yet to be confirmed. Assessing bone health is impossible through the naked eye; therefore, finding a connection between markers of energy deficiency and stress fracture occurrence will provide a measurable diagnostic indicator for the risk of stress fractures. For this reason, further research is needed to evaluate markers of energy deficiency, i.e. REE and circulating concentrations of TT3 and IGF-1, in women with and without a stress fracture.

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CHAPTER 3

METHODS

3.1 Study Design: Exercising women with a lower extremity bone injury (n=15, SFx group) including a stress reaction, medial tibial stress syndrome, or stress fracture were recruited with the help of local sports medicine physicians. After diagnosis of a bone injury confirmed by imaging techniques such as MRI, the physicians informed potential participants about the study and provided study personnel with the contact information of interested women. Exercising women without lower extremity bone injuries (n=13, NSFx group) were also recruited from the community to serve as a control group. Inclusion criteria for this study were as follows: 1) female, 2) aged 18-35 years, 3) presenting with (SFx group) or without (NSFx group) lower extremity bone injury (stress reaction, medial tibial stress syndrome or a shin splint, or stress fracture), 4) participating in at least 2 hours of exercise per week, and 5) free of chronic illness impacting bone metabolism. All participants were informed of the purpose, procedures, and potential risks of participation in the study and signed an informed consent approved by the Institutional Review Board at the Pennsylvania State University.

Participants reported to the lab for two visits that were scheduled about 1 week apart. We attempted to schedule the first visit for the injured subjects within a week of diagnosis of the bone injury. Non-injured controls in the study were brought into lab on a random day. The first visit involved measurement of height and weight and completion of questionnaires to include: 1) Health, Exercise and Nutrition Survey to obtain information about demographics and medical, exercise, menstrual, nutrition, and bone health and injury history, 2) Stress and Recovery Survey, 3) Eating Disorder Inventory-3, and 4) Three-Factor Eating Questionnaire. For assessment of energy status, REE was also measured followed by

collection of a blood sample for the measurement of metabolic hormones indicative of energy status, i.e., TT3 and IGF-1. During the second visit, body composition and bone mineral density were assessed using dual-energy x-ray absorptiometry (DXA).

3.2 Anthropometrics: Total body weight was measured in the morning by a digital scale in the laboratory to the nearest 0.01 kg with subjects wearing t-shirt and gym shorts. Height was measured to the nearest 0.1 cm without shoes, and BMI was calculated as a ratio of weight to height (kg/m^2).

3.3 Body Composition and Bone Mineral Density: Body composition and BMD was assessed using DXA (GE Lunar iDXA, Madison, WI). A total body scan was performed to measure percent body fat, lean mass, fat mass, and total body BMD. Areal BMD of the lumbar spine L1-L4, femoral neck, and total hip was obtained from anteroposterior lumbar spine and dual femur scans. All scans were performed and analyzed by the same ISCD-certified DXA technician. Coefficient of variations (%) of BMD measurements were calculated as 0.53%, 0.70%, 0.48% for L1-L4, femoral neck, and total hip BMD, respectively.

3.4 Resting Energy Expenditure: REE was determined by indirect calorimetry (Sensormedics Vmax metabolic cart, Yorba Linda, CA). Participants reported to the lab in the morning fasting for 12 hours and refraining from exercise and caffeine for 24 hours. After a 30-minute rest period, a ventilated hood was placed on the participants, and REE was measured for 30-45 minutes. Oxygen consumption (VO_2) and carbon dioxide production

(VCO_2) were collected every 30 seconds. To calculate REE, data for VO_2 and VCO_2 were only used if at least 10 minutes of steady state were attained. Steady state was achieved when the volume of expired air and VO_2 were not varying by more than 10% and when the respiratory quotient was not varying by more than 5%. Consecutive data points that met these criteria were then averaged, and REE was calculated using the Weir equation [1].

Predicted REE was also calculated using the Harris Benedict equation [2]. 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 [3-5]. We have previously published data using a ratio of REE/pREE less than 0.90 as the operational definition of an energy deficiency [6-9]. As such, in this study, a ratio <0.90 was used to discriminate between being energy deficient and energy replete.

3.5 Blood Sampling: Blood was collected after an overnight fast immediately following measurement of REE in a subset of the women (n=16). The blood sample was obtained via venipuncture by a General Clinical Research Center (GCRC) nurse. Samples were allowed to clot for at least 30 minutes at room temperature. Samples were then spun in a centrifuge at 4^o 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^o Celsius until analysis.

3.6 Serum Hormone Analysis: For a subset of the study sample (n=16), metabolic hormones TT3 and IGF-1 were measured in the serum. TT3 was analyzed using a chemiluminescence-based immunoassay analyzer (Diagnostic Products Corporation, Los

Angeles, CA). Analytical sensitivity for the TT3 assay was 35 ng/dl. The intra-assay and inter-assay coefficients of variation were 13.2% and 15.6%, respectively. 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. 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.

3.7 Statistical Analysis: Prior to analysis, the data were screened for outliers. Independent t-tests were performed to determine group differences for demographic, energy status, and BMD variables. The women were grouped according to exercise volume, using the sample median (712.5 min/wk) as the cutoff between high exercise volume and normal exercise volume. Chi-square tests were conducted to determine if the proportion of women participating in high exercise volume and normal exercise volume differed between groups. Analyses were performed using SPSS software (version 19.0; Chicago, IL), and data were reported as mean ± standard error mean (SEM). A significance level of 0.05 was used to detect differences between groups.

3.8 References:

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CHAPTER 4

MANUSCRIPT

4.1 Abstract:

Stress fractures are a common injury among exercising women and often interrupt training and hinder athletic performance. Furthermore, the presence of a stress fracture may indicate other underlying health problems in female athletes such as an energy deficiency, menstrual disturbances, or low bone mass. An energy deficiency has been shown to impact hormone concentration and, in turn, bone health through suppressed bone formation or low bone mass. As such, the purpose of this paper is to determine whether exercising women with stress fractures have suppressed resting energy expenditure (REE) and metabolic hormones, such as total triiodothyronine (TT3) and insulin-like growth factor-1 (IGF-1). Exercising women with a lower limb stress injury (SFx) and healthy exercising controls (NSFx) were recruited. As indicators of energy status, resting energy expenditure was assessed using indirect calorimetry, and a blood sample was collected for measurement of circulating TT3 and IGF-1 concentrations. SFx (n=15) and NSFx (n=13) women aged 21.1 and 20.6 respectively, differed significantly in height and lean mass. Serum concentrations of TT3 and IGF-1 did not differ between groups. Similarly, REE and the ratio of REE/pREE were not different between the SFx and NSFx women. However, the SFx group demonstrated a trend toward a lower REE/kg LBM (p=0.083) and higher exercise volume (p=0.093) compared to the NSFx group. Assessment of exercise volume as high (cutoff) or normal (cutoff) revealed that a greater proportion of SFx women (73%) participated in a high volume of exercise compared to the NSFx women (23%). When categorizing the women based on exercise volume, the high exercise volume group (add in here the cutoff)

demonstrated a higher concentration of IGF-1 ($p<0.001$) compared to the normal exercise volume group (add in cutoff). Energy status, as assessed by REE and circulating concentrations of IGF-1 and TT3, was unable to discriminate between exercising women with and without a lower limb bone injury. However, the high volume of exercise seen in the stress fracture group may mask the suppression of IGF-1 caused by an energy deficiency due to the stimulatory effect of exercise on IGF-1 production.

4.2 Introduction: Exercise has numerous health benefits. For example, aerobic activity increases exercise capacity and plays a part in primary and secondary prevention of cardiovascular disease [1]. However, a mismatch between energy expenditure and energy intake can result in physiological adaptations that negatively affect health and well-being. It has been reported that many female athletes as well as recreationally-active women fail to consume adequate calories to match energy expenditure; thereby inducing an energy-deficient state [2, 3]. In an effort to conserve energy and maintain processes needed for survival, energy is directed to essential functions like cellular maintenance and repair, thermoregulation, and locomotion, and diverted away from functions unnecessary for survival, such as growth and reproduction [4]. As such, energy deficiency in exercising women can cause musculoskeletal injuries and reproductive dysfunction [5]. Recently, reports have acknowledged energy deficiency as a key factor in the Female Athlete Triad [6], a syndrome of interrelated conditions involving disordered eating, low bone mass, and amenorrhea in physically active women [6].

In response to an energy deficiency, metabolic adaptations occur which include suppressed resting energy expenditure (REE) and decreased concentrations of insulin-like

growth factor-1 (IGF-1) and total triiodothyronine (TT3) [7]. REE is used to assess energy status, as it represents the amount of kilocalories used by the body while at rest during a 24-hour period [8]. In a deprived energy state, REE is typically suppressed. As such, an energy deficiency has been operationally defined as a ratio of measured REE to predicted REE (REE/pREE) < 0.9 [5]. Both IGF-1 and TT3 are metabolic hormones that play an important role in growth [9, 10]. In addition, TT3 has a significant function in the regulation of body temperature, heart rate and metabolism. Energy status has been shown to be associated with reduced circulating concentrations of IGF-1 and TT3 [7]. Therefore, IGF-1, TT3, and REE are considered reliable markers of energy status.

In addition to the aforementioned metabolic changes, bone quality is also impacted by energy deficiency. Healthy bone is maintained through a bone remodeling process [11] known as bone turnover which consists of balanced cycles of bone formation and bone resorption [11]. This process is essential in physically-active women who experience high-impact and repetitive mechanical loading during exercise. Loading on the bone causes microcracks to accumulate [12]. These microcracks need to be repaired with healthy bone, especially in the case of exercising women who are frequently exposing their bones to high-impact or repetitive mechanical loads. Investigators have observed energy-deficient women, as evidenced by suppressed IGF-1 and TT3 concentrations, to also have suppressed concentrations of bone formation and bone resorption markers [13]. Therefore, energy-deficient exercising women may be at risk for a bone injury, such as a stress fracture, since the ability to repair skeletal microdamage may be impaired. As such, microcracks may accumulate, leading to subsequent fracture. Stress fractures are considered the most common overuse injury in athletes [14]. Among track and field athletes, investigators have reported

the cumulative annual incidence of stress fractures to range from 8.7% to 21.1% [14, 15]. Furthermore, stress fractures are also a problem for the military population; reports among U.S. military recruits demonstrated that 1 to 7% of female recruits suffered a lower extremity stress fracture [16].

Many risk factors have been linked to stress fractures [14]. Some factors have been shown to be associated with one another, and if present, can cause additive effects [14]. For example, exercising women frequently experience menstrual disturbances [17]. As a result of the menstrual disturbance, they often experience bone loss [18] and present with low BMD [18]. West et al. [5] observed a relationship between low BMD and a ratio of REE/pREE < 0.9 in exercising women with amenorrhea. Low BMD and menstrual dysfunction are two possible factors that increase stress fracture risk, and, in turn, both of these factors are often found in exercising women with an energy deficiency [18].

To date, only one study has explored the direct link between markers of energy status and stress fracture occurrence. Strohback et al. [19] measured IGF-1 concentrations, before and after basic training, among military women who suffered a stress fracture compared to those who remained uninjured. The stress fracture group exhibited a 68% decrease in bioavailable IGF-1; whereas, the non-stress fracture group demonstrated a 20% increase in bioavailable IGF-1 from pre- to post-basic training [19]. This study demonstrates the impact that IGF-1 concentrations may have on bone quality and stress fracture occurrence.

Few studies have examined direct connections between energy status; markers of energy status such as REE, IGF-1 and TT3; and stress fracture occurrence. Therefore, the purpose of this study is to determine whether exercising women with stress fractures have evidence of energy deficiency, including suppressed REE and metabolic hormones such as

TT3 and IGF-1. It is hypothesized that women with a stress fracture will have lower REE/pREE ratio, and reduced circulating concentrations of TT3 and IGF-1 compared to the non-injured exercising women.

4.3 Methods:

4.3a Study Design: Exercising women with a lower extremity bone injury (n=15, SFx group) including a stress reaction, medial tibial stress syndrome, or stress fracture were recruited with the help of local sports medicine physicians. After diagnosis of a bone injury confirmed by imaging techniques such as MRI, the physicians informed potential participants about the study and provided study personnel with the contact information of interested women. Exercising women without lower extremity bone injuries (n=13, NSFx group) were also recruited from the community to serve as a control group. Inclusion criteria for this study were as follows: 1) female, 2) aged 18-35 years, 3) presenting with (SFx group) or without (NSFx group) lower extremity bone injury (stress reaction, medial tibial stress syndrome or a shin splint, or stress fracture), 4) participating in at least 2 hours of exercise per week, and 5) free of chronic illness impacting bone metabolism. All participants were informed of the purpose, procedures, and potential risks of participation in the study and signed an informed consent approved by the Institutional Review Board at the Pennsylvania State University.

Participants reported to the lab for two visits that were scheduled about 1 week apart. We attempted to schedule the first visit for the injured subjects within a week of diagnosis of the bone injury. Non-injured controls in the study were brought into lab on a random day. The first visit involved measurement of height and weight and completion of questionnaires

to include: 1) Health, Exercise and Nutrition Survey to obtain information about demographics and medical, exercise, menstrual, nutrition, and bone health and injury history, 2) Stress and Recovery Survey, 3) Eating Disorder Inventory-3, and 4) Three-Factor Eating Questionnaire. For assessment of energy status, REE was also measured followed by collection of a blood sample for the measurement of metabolic hormones indicative of energy status, i.e., TT3 and IGF-1. During the second visit, body composition and bone mineral density were assessed using dual-energy x-ray absorptiometry (DXA).

4.3b Anthropometrics: Total body weight was measured in the morning by a digital scale in the laboratory to the nearest 0.01 kg with subjects wearing t-shirt and gym shorts. Height was measured to the nearest 0.1 cm without shoes, and BMI was calculated as a ratio of weight to height (kg/m^2).

4.3c Body Composition and Bone Mineral Density: Body composition and BMD was assessed using DXA (GE Lunar iDXA, Madison, WI). A total body scan was performed to measure percent body fat, lean mass, fat mass, and total body BMD. Areal BMD of the lumbar spine L1-L4, femoral neck, and total hip was obtained from anteroposterior lumbar spine and dual femur scans. All scans were performed and analyzed by the same ISCD-certified DXA technician. Coefficient of variations (%) of BMD measurements were calculated as 0.53%, 0.70%, 0.48% for L1-L4, femoral neck, and total hip BMD, respectively.

4.3d Resting Energy Expenditure: REE was determined by indirect calorimetry

(Sensormedics Vmax metabolic cart, Yorba Linda, CA). Participants reported to the lab in the morning fasting for 12 hours and refraining from exercise and caffeine for 24 hours. After a 30-minute rest period, a ventilated hood was placed on the participants, and REE was measured for 30-45 minutes. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were collected every 30 seconds. To calculate REE, data for VO_2 and VCO_2 were only used if at least 10 minutes of steady state were attained. Steady state was achieved when the volume of expired air and VO_2 were not varying by more than 10% and when the respiratory quotient was not varying by more than 5%. Consecutive data points that met these criteria were then averaged, and REE was calculated using the Weir equation [20].

Predicted REE was also calculated using the Harris Benedict equation [21]. 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 [22-24]. We have previously published data using a ratio of REE/pREE less than 0.90 as the operational definition of an energy deficiency [5, 7, 25, 26]. As such, in this study, a ratio <0.90 was used to discriminate between being energy deficient and energy replete.

4.3e Blood Sampling: Blood was collected after an overnight fast immediately following measurement of REE in a subset of the women (n=16). The blood sample was obtained via venipuncture by a General Clinical Research Center (GCRC) nurse. Samples were allowed to clot for at least 30 minutes at room temperature. Samples were then spun in a centrifuge at 4^{o} 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^{o} Celsius until analysis.

4.3f Serum Hormone Analysis: For a subset of the study sample (n=16), metabolic hormones TT3 and IGF-1 were measured in the serum. TT3 was analyzed using a chemiluminescence-based immunoassay analyzer (Diagnostic Products Corporation, Los Angeles, CA). Analytical sensitivity for the TT3 assay was 35 ng/dl. The intra-assay and inter-assay coefficients of variation were 13.2% and 15.6%, respectively. 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. 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.

4.3g Statistical Analysis: Prior to analysis, the data were screened for outliers. Independent t-tests were performed to determine group differences for demographic, energy status, and BMD variables. The women were grouped according to exercise volume, using the sample median (712.5 min/wk) as the cutoff between high exercise volume and normal exercise volume. Chi-square tests were conducted to determine if the proportion of women participating in high exercise volume and normal exercise volume differed between groups. Analyses were performed using SPSS software (version 19.0; Chicago, IL), and data were reported as mean ± standard error mean (SEM). A significance level of 0.05 was used to detect differences between groups.

4.4 Results: Demographic characteristics for the participants are provided in Table 1. A total of 33 women participated in the study; however, 5 women were excluded from analysis due to missing data (n=1), invalid results (n=2), and not meeting the exercise criteria (n=2). As such, 15 exercising women with stress fractures (SFx) and 13 exercising control women (NSFx) were included in the analysis. The groups did not differ in age, body mass, BMI, percent body fat, or fat mass. However, the SFx group was taller and had greater lean mass than the NSFx group. Self-reported habitual physical activity did not significantly differ between groups; however, there was a trend (p=0.093) toward the SFx women engaging in more minutes of exercise each week compared to the NSFx women. The sports that the athletes particpated in encompassed a range of weight-bearing activities to include field hockey, volleyball, long and short distance running, softball, and dance. Based on a classification system by Nikander (Femoral neck structure…2005) the majority of women in both groups participated in repetitive low-impact loading activities (SFx 67% and NSFx 77%). In the SFx group 13.3% of the women participated in high impact activities and 20% participated in odd impact activities. For the NSFx group, 7.7% participated in either high impact or odd impact exercise.

Table 1. Demographic characteristics of the stress fracture vs. control group

	SFx (n = 15)	NSFx (n = 13)	P-value
Demographic Characteristics			
Age (yr)	21.1±0.7	20.6±0.4	.535
Height (cm)	169.1±2.1	163.5±1.0	.026
Weight (kg)	63.0±2.1	58.3±1.9	.116
BMI (kg/m^2)	22.0±0.6	22.0±0.7	.977
Body Fat (%)	24.3±1.6	24.9±1.5	.772
Fat Mass (kg)	15.3±1.3	14.7±1.4	.734
Lean Mass (kg)	45.1±1.4	40.9±2.2	.018
Training Characteristics			
Physical Activity (min/wk)*	900.1±94.5	642.0±115.9	.093

*Self-reported exercise (past 6 months)

Table 2 provides the results of energy variables. The measured REE (kcal/day) and REE/pREE ratio did not differ between the SFx and NSFx women. However, there was a trend ($p=0.083$) toward a lower REE/kgLBM (lean body mass) in the SFx group compared to the NSFx group. Similarly, serum concentrations of TT3 and IGF-1 were not significantly different between groups.

BMD data of the women are provided in table 3. The SFx and NSFx women did not differ in absolute BMD measurements or Z-scores at the total body, L1-L4 spine, femoral neck, or total hip.

Figure 4 depicts the proportion of women in the SFx group (Figure 4A) and NSFx group (Figure 4B) who engaged in a high exercise volume (> 712.5 min/wk) or normal exercise volume (≤712.5 min/wk). Within the stress fracture group (n = 11) 73% of the women participated in a high volume of exercise; whereas, 27% of the women participated in a normal volume of exercise. On the contrary, 77% of the women in the NSFx group (n=10) engaged in a normal volume of exercise compared to 23% of the women who participated in a high volume of exercise. As such, significantly more women in the SFx group engaged in a high volume of exercise compared to the NSFx group ($p=.008$).

Figure 5 represents the serum concentrations of TT3 and IGF-1 in the high exercise volume group (>712.5 min/wk) and the normal exercise volume group (≤712.5 min/wk). No significant difference was observed between the exercise volume groups for TT3 concentration. . However, the high exercise volume group demonstrated a significantly greater ($p<0.001$) IGF-1 concentration compared to the normal exercise volume group.

Table 2. Resting metabolic rate (REE) and metabolic hormone (IGF-1 and T3) concentrations of the stress fracture vs. control groups.

	SFx (n=15)	NSFx (n=13)	P-value
REE			
Resting energy expenditure (kcal/day)	1343.5±41.9	1309.4±45.9	.587
REE/kgLBM (kcal/day*LBM)	29.9±0.7	32.1±1.0	.083
REE:pREE (actual/predicted)	0.91±0.02	0.93±0.03	.698
Metabolic Hormones TT3 (ng/dl)	95.6±10.55	79.1±9.28	.275
IGF-1 (pg/mL)	79.2±5.94	65.3±6.03	.130

Table 3. Bone mineral density (BMD) measurements for stress fracture vs. control groups

	SFx (n=15)	**NSFx (n=13)**	**P-value**
Bone Mineral Density			
Total Body z-score	0.55±0.3	0.61±0.3	.892
Total Body BMD (g/cm^2)	1.134±0.03	1.163±0.05	.591
Lumbar Spine L1-L4 z-score	0.11±0.3	0.01±0.4	.841
Lumbar Spine L1-L4 BMD (g/cm^2)	1.188±0.04	1.158±0.04	.600
Femur neck z-score*	0.61±0.3	1.12±0.3	.245
Femur neck BMD (g/cm^2)	1.106±0.04	1.089±0.04	.753
Total Hip z-score*	0.50±0.3	1.10±0.3	.138
Total Hip BMD (g/cm^2)	1.104±0.04	1.101±0.03	.947

*SFX, n=12; NSFx, n= 11

Figure 4A.

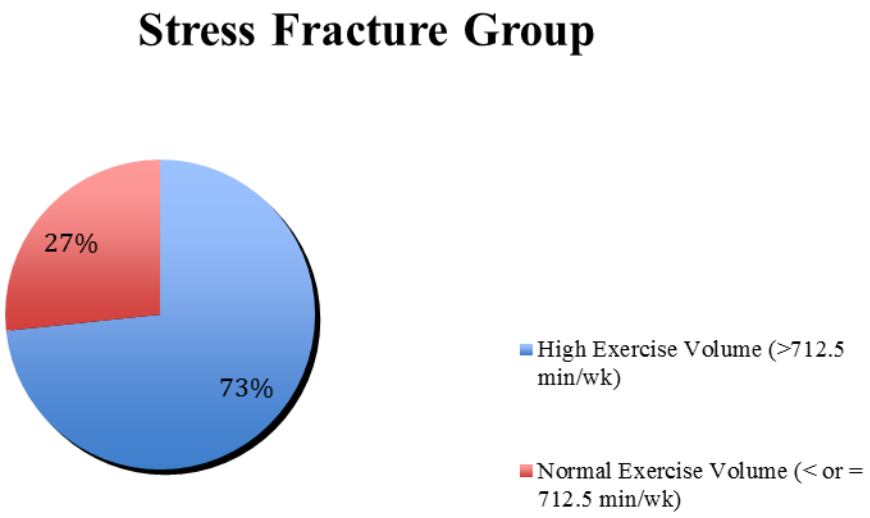

Figure 4A depicts the percentage of women within the stress fracture group (n=14) who took part in high volume of exercise or normal volume of exercise based on a median cut-off of 712.5 min/wk.. Within the stress fracture group, 27% of the women engaged in normal exercise volume (n = 4) and 73% of the women participated in high exercise volume (n = 11).

Figure 4B.

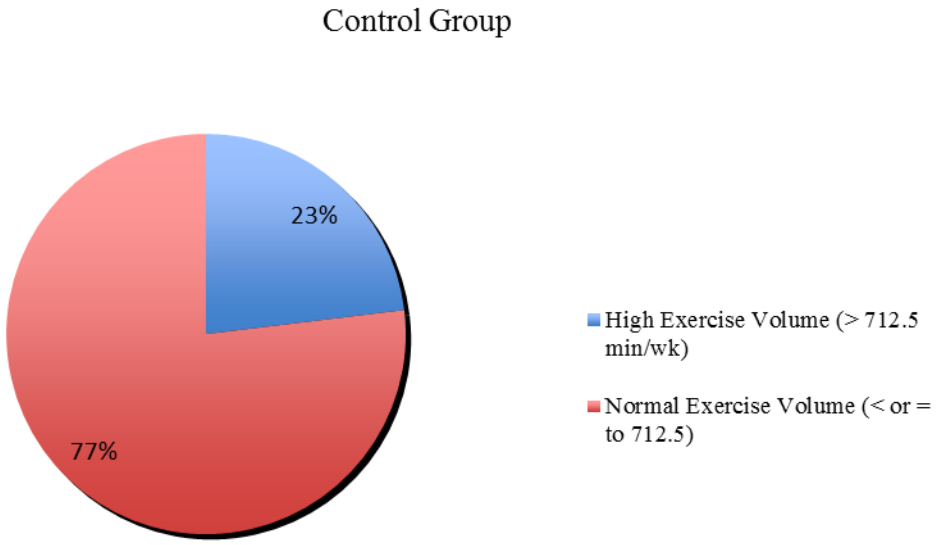

Figure 4B depicts the percentage of women within the control group (n=13) who took part in high volume of exercise or normal volume of exercise based on a median cut-off of 712.5 min/wk. Within the healthy control group, 77% of the women engaged in normal volume exercisers (n = 10) and 23% of the women participated in high exerciser volume (n = 3).

Figure 5.

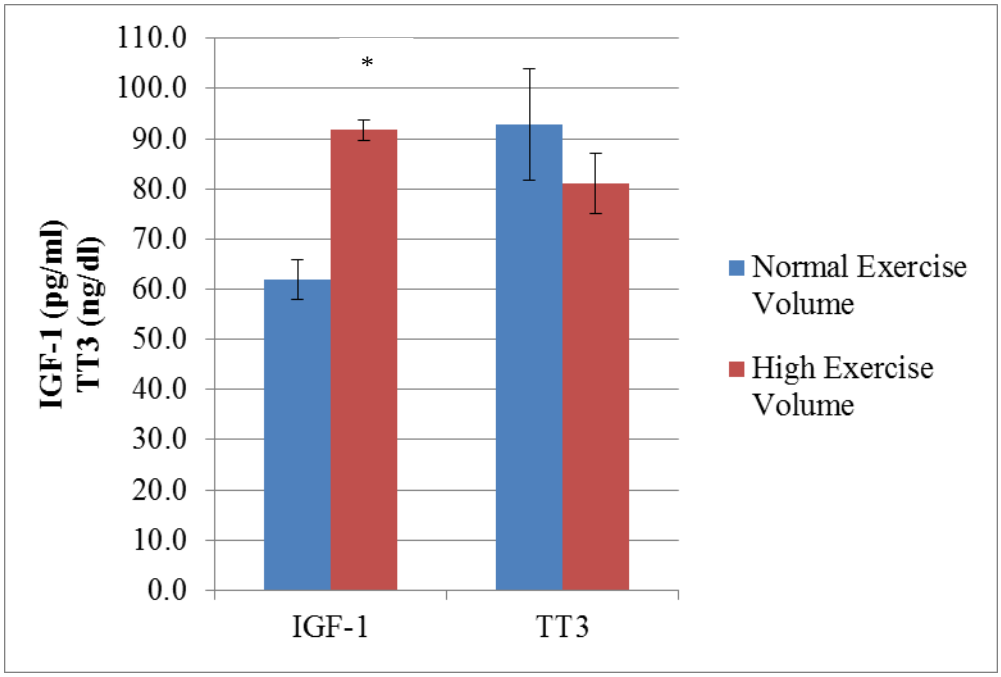

Figure 6 compares the concentrations of IGF-1 (pg/ml) and TT3 (ng/dl) in the normal exercise volume group (cutoff) to the high exercise volume group (cutoff). For the normal volume group, IGF-1 concentration was 62.0 pg/ml and TT3 concentration was 92.8 ng/dl. For the high volume group, IGF-1 concentration was 91.7 pg/ml and TT3 concentration was 81.0 ng/dl. IGF-1 was significantly greater in the high exercise volume group. * $p < 0.001$. IGF-1: insulin-like growth factor 1; TT3: total triiodothyronine

4.5 Discussion:

This study evaluated energy status in female athletes suffering a lower extremity bone injury. Contrary to our hypothesis, no significant differences in energy status as assessed by REE and the metabolic hormones TT3 and IGF-1were observed between female athletes with and without a lower limb bone injury; however there was a trend toward a lower REE/kgLBM in the SFx group compared to the NSFx group. Furthermore, there was also a trend toward the SFx women engaging in more minutes of exercise each week compared to the NSFx women.

These findings suggest that when normalized for lean body mass, REE in female athletes with a lower limb bone injury may be suppressed, indicating a physiological response to energy deficiency. However, according to the REE/pREE ratio (REE/pREE > 0.90) and serum concentrations of TT3 and IGF-1, both groups appeared to be energy replete with a similar metabolic profile. The small sample size is a limitation of the study and may have contributed to the null results. In addition, our sample represented female athletes from many different types of activities. The sports that the athletes particpated in encompassed a range of weight-bearing activities to include field hockey, volleyball, long and short distance running, softball, and dance. Selection was based solely on whether the athlete suffered an injury or not; therefore a mix of both high impact/low impact and lean sports/non-lean sports athletes was obtained. For example, a female softball player, who is considered a non-lean sport athlete, would have a different body composition compared to a long distance runner, who is considered to be a lean sport athlete. Additionally, the type of impact experienced between the two would differ. A runner undergoes repetitive, low impact loading whereas a softball player experiences odd impact and heterogenous loading. This could possibly lead to

a vastly different bone profile between the two athletes. The runner may accumulate more microdamage due to the repetitive loading; whereas, the softball player may actually have greater bone strength due to the uncustomary loading that is experienced during participation in the activity. Recruiting controls and stress-fracture women with similar exercise type would help to minimize the heterogeneity in the sample that exists due to non-similar sports type and that potentially introduces confounding factors.

The timing in which we collected our data could provide another explanation for the lack of difference in energy status observed between injured and non-injured female athletes. Although we attempted to assess energy status of the athletes soon after diagnosis, there was often still a lapse of time before injury onset and diagnosis. During this time, athletes may have reduced training. The metabolic environment is very sensitive to changes in energy status; therefore, indicators of metabolic status may have already normalized by the time of metabolic assessment.

Notably, exercise volume differed among female athletes grouped according to the presence of a lower limb bone injury. Among the stress fracture group, a significantly greater proportion of athletes (73%) participated in a high volume of exercise compared to the control group (23%) based on the median of exercise minutes per week. These results suggest that high exercise volume may be a risk factor for stress fractures. Other investigators have identified exercise volume as a possible risk factor. Brukner [27] stated that contributions of each training component (volume, intensity, frequency, surface, and footwear) are possible factors in the occurrence of an injury, as they influence characteristics of the mechanical stress. Exercise volume is directly related to the number of repetitive loads on the lower extremities. The loads create microcracks in the bone. If the body is given

sufficient time to heal, new healthy bone will replace the cracks. Stress fractures occur due to the accumulation of microcracks from repetitive loading. Therefore, high exercise volume with inadequate rest to allow for bone healing places an athlete at risk for a stress fracture. Our finding is in agreement with those observed by other investigators. Results from a study conducted among female dancers showed that for those dancing <5 hours per day, only 31% sustained a stress fracture. Of the females who danced > 5 hours per day, 50% suffered fractures [28]. In addition, a study conducted on female adolescent runners showed the prevalence of stress fracture was approximately 2% to 3% for those who engaged in physical activity < 16 hours per week, but for girls who participated in ≥ 16 hours per week about 5% had a history of stress fractures [29].

Exercise volume may not be an indicator of energy status, but it may be a better indicator of stress fracture risk than energy status according to our results. Understanding the exercise volume that is associated with greater stress fracture risk may provide valuable information about the amount of repetitive loading that represents inadequate time for bone repair and thus leads to injury. Determining a threshold quantity of activity at which the risk of stress fracture increases would be a valuable tool for clinicians, coaches, athletes, and trainers.

No significant difference in IGF-1 and TT3 concentrations were observed between the SFx group and NSFx group. However, when assessing metabolic hormones concentrations among women grouped according to exercise volume, the high exercise volume group demonstrated a significantly higher IGF-1 concentration compared to the normal volume group. Investigators have reported that exercise stimulates IGF-1 production. Bamman et al. [30] observed that IGF-1 mRNA concentrations increased 62% ($p<0.05$) 48

hours after eccentric muscle action. . Additionally, a study conducted among college women with varying activity profiles (sedentary women, aerobic exercisers, and muscle-building exercisers), showed that aerobic and muscle-building exercisers had higher concentrations of IGF-1 compared to the sedentary group [31]. As such, IGF-1 production appears to increase post-exercise which may explain the higher IGF-1 concentrations that we observed in the high exercise volume group. Furthermore, because we observed that a significantly greater proportion of SFx women participated in a high volume of exercise, the lack of a difference in IGF-1 concentrations that we observed between the SFx and NSFx women may be because the SFx women were undergoing a bone healing process. IGF-1 has been demonstrated to play an important role in fracture healing. Andrew et al. [32] experimentally determined that IGF-1 mRNA was expressed in osteoblasts at the time of bone tissue and cartilage formation during fracture healing. Therefore, because we observed the SFx women during the healing process, IGF-1 concentrations may have elevated due to the synergistic effects of exercise-induced and healing-induced increases in IGF-1 production.

4.6 Conclusion:

In conclusion, energy status as assessed by REE and circulating concentrations of IGF-1 and TT3 do not appear to definitively discriminate between exercising women with and without a lower limb bone injury. However, women with a stress injury participate in a high volume of exercise which may mask the suppression of IGF-1 caused by an energy deficiency due to the stimulatory effect of exercise on IGF-1 production. More research is needed in a larger sample and in athletes of specific sport types to provide further clarity regarding the role of energy deficiency in stress fracture occurrence.

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CHAPTER 5

SUMMARY, CONCLUSION, AND IMPLICATIONS FOR PRACTICE

Adaptations to low energy availability tend to be seen in energy deficient women, including suppressed resting energy expenditure (REE), decreased triiodothyronine (TT3) and decreased insulin-like growth factor-1 (IGF-1) [1]. The female athlete is at risk for an energy deficiency when energy expenditure is high and caloric intake is restricted or inadequate to compensate for energy expenditure. At this point, a stress fracture is possible, as bone health has been shown to be negatively impacted by an energy deficient state [2].

Bone loading creates microcracks in bone tissue. These microcracks are normally repaired through bone remodeling; however, if inadequate time is given for repair then the microcracks may accumulate and the risk of obtaining a stress fracture increases [3]. It has been demonstrated both IGF-1 and T3 play a role in the bone remodeling process [4, 5]. IGF-1 promotes type 1 collagen production, which is essential for bone formation, and T3 stimulates osteoblast production [4, 6]. Chronic energy deficiency is associated with low levels of IGF-1 and T3; therefore, the suppression of these metabolic hormones may be one mechanism by which an energy deficiency impairs bone remodeling by reducing bone formation. To date, the association between chronic energy deficiency and stress fracture occurrence has been thoroughly explored. Thus, the purpose of this study was to determine whether exercising women with lower limb stress injury have evidence of energy deficiency, including suppressed REE and metabolic hormones such as TT3 and IGF-1.

Contrary to our hypotheses, no significant differences were observed for REE, TT3 and IGF-1 in the women with the bone injury (SFx group) compared to the control group (NSFx group). Both groups were considered energy replete, as indicated by the REE/pREE

ratio > 0.90. Furthermore, no significant differences were seen in BMD measurements between the two groups. However, when REE was normalized for lean body mass (LBM), there was a trend toward a lower REE/kgLBM in the SFx group compared to the NSFx group, indicating that the women with the bone injury may have been moderately energy deficient compared to the NSFx group.

In a subanalysis exploring exercise volume, two groups were created based on median exercise volume, a high exercise volume (min/wk>712.5) group and a normal exercise volume (min/wk<712.5) group. Interestingly, most of the women who suffered an injury were considered high volume exercisers; whereas, the majority of control women participated in normal volume of exercise. Additionally, the high exercise volume group had significantly greater concentrations of IGF-1 compared to the normal exercise volume group, possibly indicating the role of IGF-1 in the bone remodeling process both post-exercise and during healing.

Determining the factors related to a lower leg bone injury, and specifically the effect of energy deficiency on bone strength and bone injury, is important for athletes, especially the female athlete. Many females suffer from the condition known as the Female Athlete Triad, which is an interrelationship between disordered eating, menstrual irregularities, and low bone mass [7]. These factors are considered interconnected, as an energy deficiency often leads to a menstrual disturbance, and both consequently lead to bone loss. Exercising women at risk for the triad are also increasingly at risk for sustaining a stress fracture, as bone is weaker and may lose its ability to withstand a high level of stress when exposed to the environment of metabolic and reproductive suppression that is typically observed in energy deficient, exercising women [7]. Monitoring factors of energy status, like

metabolic hormones and REE, could possibly identify those athletes that are most at risk for sustaining an energy-related injury.

Our results demonstrated that high exercise volume is a common factor in women who suffered a stress fracture. Further understanding of the relationship between exercise volume and energy status markers like REE, TT3, and IGF-I would be beneficial in future studies. Furthermore, it is important to promote awareness of the consequences associated with an energy deficiency among exercising women in an effort to encourage and motivate them to adequately fuel themselves for the volume of physical activity that they habitually engage in. Through a proper diet, exercising women can protect themselves from the health consequences associated with an energy deficiency.

The primary exercise type for many of the women in the high volume exercise group was running. The sport of running is known for its emphasis on leanness; therefore, these women may be most at risk for the triad and possibly a bone injury. Although our results do not definitively indicate that there is a difference in energy status between women with and without a bone stress injury, it cannot be concluded that such a difference does not exist. The trend toward lower REE/kg LBM in the SFx group indicates that with a greater sample size, such a difference may be present. As such, future studies should be conducted with a larger sample that contains women women from the same running/track team and assessing those that suffer an injury compared to uninjured teammates.

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ACADEMIC VITA

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EDUCATION

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PROFESSIONAL EXPERIENCES

Research Experiences:

Undergraduate Research Assistant, Honors Thesis Student (Women's Health and Exercise Lab): 2010 – Present

- Aided in research under the supervision of Dr. Mary Jane De Souza and Dr. Nancy Williams concerning energy balance, exercise associated amenorrhea, bone health, and menstrual status
- Completed honors research thesis on stress fractures and energy deficiency in premenopausal exercising women
- Processed blood and urine samples in the wet lab to include specialized processing for gut peptide assays
- Participated in journal club discussions
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Research Technician, UPMC Children's Hospital (Neonatology Lab): Summer 2011

- Participated in nationally funded research using the *C. E.egans* model to explore newborn diseases.
- Conducted a summer long research project constructing various proteins tagged with a fluorescent marker that were injected into the *C. Elegans*.
- Acquired the following skills: PCR, Cloning, Fluorescent Microscopy, and DNA Sequencing

LEADERSHIP EXPERIENCES

President and PR Chair, Alpha Epsilon Delta - National Health Pre-professional Honor Society: 2010-Present

- Oversaw operations of the 150-member honor society
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Head Swim Coach and Lifeguard, Community Swim Club: 2006-2011

- Coached over 120 participants of the Community Swim Club swim team.
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