The Effects of Low Energy Availability and High-Impact Exercise on Markers of Bone and Body Composition

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ABSTRACT

Low energy availability (LEA) has been identified as the underlying etiology of the Female Athlete Triad and Relative Energy Deficiency in Sport (REDs) syndrome. The term energy availability (EA) describes the amount of dietary energy intake (EI) that is remaining to support physiological function after accounting for the energy cost of exercise. Exposure to LEA stimulates metabolic adaptations that may disrupt certain biological systems, such as endocrine function, and impair sports performance. Controlled laboratory research has shown suppression of bone formation biomarkers with accelerated rates of bone resorption after only three to five days of LEA in active females. Correcting LEA by increasing EI or decreasing exercise energy expenditure (EEE) may not be feasible for all athletes and additional approaches for protecting bone health during LEA require further investigation. Recent evidence suggests that brief bouts of high-impact exercise attenuate the increased rate of bone resorption in females with diet-induced LEA. However, it is unknown whether similar exercises have a protective effect on bone health when LEA is induced through a combination of dietary restriction and exercise. A gap also remains in the understanding of how EA fluctuates throughout the athletic season and what potential effect that has on body composition and performance outcomes. To address these gaps, we conducted two studies to investigate the interactions of EA, bone health, and body composition. The first investigation employed a randomized crossover design in which female runners underwent two, five-day experimental conditions of LEA consisting of dietary restriction and daily running (EA = 15 kcal·kg FFM^{-1}·day^{-1}). During one of the experimental conditions, participants also completed a bout of 50 jumping exercises daily. Serum markers of bone resorption (C-terminal cross-linking telopeptide of type 1 collagen [CTX-I]), bone formation (N-terminal propeptide of type 1 procollagen [PINP]), and hormonal profiles were compared between baseline and post-intervention using linear mixed effects modeling. We hypothesized that daily high-

impact exercise would have a positive effect on bone by attenuating the rise in bone resorption. In contrast to our hypothesis, bone resorption marker CTX-I increased following both LEA conditions (+12%, *P*=0.004) with no difference in the response between the jumping and non-jumping conditions. Bone formation was not suppressed following either LEA condition. Concentrations of free triiodothyronine (T_3), insulin-like growth factor-1, leptin, and insulin decreased in response to five days of LEA independent of condition (*P*<0.05); however, when taking into account condition, the decrease in free T_3 was only statistically significant following the LEA condition without jumping (-27%, *P*=0.022, *Cohen's d*=0.87). Our findings suggest that high-impact jumping exercises are not an effective countermeasure to protect bone health during short-term LEA in female runners who continue to run routinely. In a second study, we conducted a longitudinal, observational study in collegiate male soccer players to investigate seasonal changes in EA and body composition. Measurements of EA, body composition, and sports performance were assessed at the start and end of the non-championship Spring athletic season. We hypothesized that EA would be positively associated with changes in body composition at the end of the three-month season. Despite most athletes reporting desires to gain total and/or lean body mass, no changes in EA or body composition were detected at the end of the season compared to the start. Furthermore, sports performance and bone density improved across the season regardless of individual changes in EA. These results indicate EA of collegiate male soccer players during the Spring season is sufficient to maintain current body composition and improve sports performance, but insufficient to support total and/or lean body mass gains.

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GENERAL AUDIENCE ABSTRACT

Adequate energy intake (EI) is essential for fueling athletic performance and supporting general health. Low energy availability (LEA) occurs when EI is insufficient to meet the energy demands of both exercise and basic health functions. Athletes with LEA may experience various repercussions such as suppressed metabolism, hormonal changes, and impaired bone health. Training adaptations may also be impaired by LEA, thereby affecting athletic performance. Daily high-impact jumping exercises have been shown to have a positive effect on bone health in women, even during periods of LEA caused by dietary restriction. However, this type of exercise intervention has not been tested in combination with other forms of daily exercise in women exposed to a controlled period of LEA. The purpose of these studies was to examine how exercise and EA affect bone health and body composition in recreational and competitive athletes. The first study investigated the effects of daily jumping exercises on markers of bone formation and breakdown during five days of LEA in female runners. The completion of 50 jumping exercises each day along with running on a treadmill was not shown to provide additional bone-protective benefits during LEA compared to running alone, as shown by similar rates of bone breakdown observed under both conditions. The second study investigated whether changes occur in EA or body composition in male collegiate soccer players over an athletic season. Despite most of the athletes reporting desires to gain weight or muscle during the season, there were no differences in body composition or EA at the end of the season compared to the start. However, there were significant improvements in aerobic fitness, relative strength, and bone density throughout the season.

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ATTRIBUTION

Chapter III: Enette Larson-Meyer contributed to the overall study design and methodology. Elaina Marinik assisted with study coordination. Anna Morozov, Janet Rhinehart, Jamie Martin-Long, Jessica McDonough, and Natalie Slagel assisted with data collection. Jiarui Liang assisted with food preparation and weighing food residuals in the metabolic kitchen. Ryan McMillan processed blood assays (ELISA) at The Metabolism Core.

Chapter IV: Enette Larson-Meyer and Lucas Mason contributed to the overall study design and methodology.

CHAPTER I

Introduction

Maintaining adequate energy intake (EI) is essential for optimizing athletic performance and general health. Yet, many athletes find themselves in a state of energy deficiency, also known as low energy availability (LEA), where EI is insufficient to meet these demands. The term energy availability (EA) refers to the amount of energy remaining from dietary EI to support general health and body functions after accounting for exercise energy expenditure (EEE) and is expressed relative to fat-free mass as kilocalories per kilogram of fat-free mass (FFM) per day (kcal·kg FFM^{-1}·day^{-1}) (1). According to the Life History Theory, the human body will adapt to conserve energy under conditions of biological stress, such as energy restriction and LEA, by downregulating biological processes that are less essential for immediate survival (2). The consequences of energy restriction on athlete health were first highlighted in the early 2000s by the female athlete triad (Triad), a condition involving the relationship between EA, menstrual function, and bone mineral density (BMD) (3). In 2014, the International Olympic Committee expanded upon the Triad to include other performance and health consequences of LEA, which occur in both male and female athletes; this was termed Relative Energy Deficiency in Sport (REDs) syndrome (1). In 2023, a further update was made to the REDs model to distinguish between “adaptive” and “problematic” LEA (4). Adaptable LEA is typically mild and transient with little to no negative impact on long-term health and sports performance. Problematic LEA, on the other hand, is more severe and has the potential to disrupt the health and well-being of an athlete. Athletes suffering from REDs and problematic LEA may experience several interrelated health consequences involving endocrine function, reproduction, and bone health. Problematic LEA has been associated with an increased risk for bone stress injuries and low BMD (5–8), and the suppression of certain bone-regulating hormones including estrogen, insulin, triiodothyronine (T_3), and insulin-like growth factor (IGF-1) (9). Certain athletes may be at higher risk for LEA such as women, athletes competing in weight-sensitive sports, and endurance athletes (10).

Quantifications of EA can be calculated using methods to estimate daily EI and EEE. However, the methodologies for these assessments vary across the literature and are limited by their reliance on self-reported data. Thus, surrogate markers for EA have been proposed such as leptin, insulin, IGF-1, and T_3 (11, 12) concentrations and suppressed resting metabolic rate (RMR) (13). Clinicians can screen for and diagnose REDs using the International Olympic Committee REDs Clinical Assessment Tool-Version 2 (IOC REDs CAT2) (4). The IOC REDs CAT2 is a three-step procedure that involves screening with a population-specific questionnaire or clinical interview, conducting a severity/risk assessment, and diagnosis by an expert physician. Registered dietitians and other health professionals may also be able to identify high-risk athletes who present with signs and symptoms of REDs, such as decreased athletic performance, stagnant or decreased strength, mood disturbances, and inability to maintain or gain weight (1). However, unlike the Triad, research on REDs is relatively new in the last decade. Therefore, further investigation is needed on the newly identified signs and symptoms beyond menstrual function and bone health to justify their use as clinical indicators of LEA risk.

One of the limitations of calculating EA at a single timepoint is that it fails to account for seasonal fluctuations in EA (14, 15). It is not fully known whether athletes have a compensatory change in EI in response to training periodization and competition. In general, most competitive athletes have training periods that consist of an off-season, pre-season, competitive season, and post-season. The athletic season for National Collegiate Athletic Association (NCAA) Division I athletes consists of a championship and non-championship segment each year. For soccer athletes, the traditional championship (regular) segment of the season is played in the Fall and the non-championship season is in the Spring. Athletes have significantly fewer mandatory training sessions and matches during the non-championship seasons compared to the championship seasons in the Fall. This is an important consideration when evaluating LEA risk because the level of EA may be different in a championship versus non-championship season. Increased muscle strength and hypertrophy are emphasized more during the non-championship season,

while conditioning and field play are the focus during the regular season. Some athletes, predominately men, also desire positive gains in total and/or lean body mass (LBM) (16). Most existing studies have examined at changes in EA, body composition, and BMD of athletes that occur during off-season (i.e., no supervised training) and championship seasons. However, to our knowledge, researchers have not examined these changes in collegiate male soccer players during a non-championship season.

The recommended treatment for LEA is to correct the energy deficiency by increasing EI, decreasing EEE, or a combination of the two (17). However, this approach is not as straightforward as it might appear. During certain training phases, short-term LEA may be desirable to reduce body fat and improve body composition for performance (18). Athletes competing in weight-sensitive sports may also have periods of unavoidable LEA leading up to competition. Additionally, not all athletes with LEA purposefully restrict their dietary intake (19). Unintentional LEA may occur due to factors such as inadequate knowledge of fueling recommendations, decreased appetite, lack of time, or low food security (20). While the primary treatment for LEA should be to either increase EI or reduce the energy expended through training, not all athletes may be able or willing to achieve this goal.

The potential for impaired bone health leading to low BMD is one of the most significant repercussions of chronic energy deficiency because there is limited evidence that it can be reversed in all cases (21). It is speculated that problematic LEA left untreated could potentially impair bone formation and accrual during adolescence, decrease BMD in adults, and increase the risk of injury and stress fracture (22). Conversely, bone responds favorably to high-intensity mechanical loading that is applied at a high rate and low frequency. It has been proposed that high-impact exercise may be a suitable intervention for protecting against the negative effects of LEA on bone health (23), however, only one study to date has investigated this theory during LEA without additional exercise (24). High-impact exercise (e.g., brief jumping) may be an effective intervention strategy to protect bone health during short-term LEA for several reasons. First, the intervention does not significantly increase daily EEE and thus has minimal

impact on further lowering EA. Secondly, there is evidence of a threshold to which bone cells will eventually become desensitized or unresponsive (or “deaf”) to a stimulus (25). Brief high-impact exercises are quick and do not require any equipment, which gives athletes the flexibility to complete these exercises at home or on the go with a sufficient rest period between training sessions for bone cell recovery. Finally, this intervention is realistic for athletes to incorporate into their training schedules with minimal disruptions. Thus, the benefits of brief bouts of high-impact exercise such as jumping as a possible intervention to protect against accelerated rates of bone resorption during LEA should be explored.

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CHAPTER II

Literature Review

1. Energy Availability Background

1.1 Energy Metabolism, Energy Balance, and Energy Availability

Growth and maintenance of the human body rely on sufficient dietary intake to meet required energy needs. A person's energy requirement is dependent on age, sex, body composition, and level of physical activity (1). Resting energy expenditure (REE), also known as resting metabolic rate (RMR), is the energy expended to maintain basic body functions. REE accounts for approximately 60-70% of a person's daily total energy expenditure (TEE) and is typically higher in individuals with greater total and lean body mass (LBM) (2). There is an additional energy cost for the digestion, absorption, and metabolism of nutrients that is known as the thermic effect of food (TEF) or diet-induced thermogenesis. TEF is highest immediately following food intake and contributes roughly 10% of TEE. The remaining 20-30% of TEE is attributed to activity thermogenesis which is the energy expended for activities of daily living and exercise. Activity thermogenesis is broken into two categories: exercise energy expenditure (EEE) and non-exercise activity thermogenesis (NEAT). Energy expended for planned exercise or training is considered EEE, whereas NEAT refers to the energy cost of daily living activities. For highly active individuals, REE and TEF may account for a lower percentage of TEE given the higher EEE compared to the general population. An imbalance between energy intake (EI) and TEE will result in a state of energy deficiency, also referred to as negative energy balance (EB).

The concept of EB is based on the first law of thermodynamics which states energy is constant and cannot be created nor destroyed, only converted between systems (3). EB is calculated by subtracting TEE from EI. An optimal state of EB is achieved when dietary EI is equal to TEE (EI – TEE = 0 kcal), and an imbalance on either side of the equation would theoretically alter total body mass (TBM). However, there is evidence the body will adapt to conserve energy under conditions of chronic deficiency by

downregulating certain metabolic processes resulting in a lower TEE (4). Therefore, the severity of an energy deficiency may be masked using the energy balance equation (EI – TEE) based on the TEE adaptation. This was demonstrated in a study of eight untrained men who were placed in a state of energy deficiency for one week in a room calorimeter (5). Despite EI and EEE remaining constant over the seven days, average daily EB increased by 90 kcal·d^{-1} due to a progressive reduction in TEE. Therefore, EB is not the recommended method for assessing dietary intake of athletes, especially those at risk for energy deficiency (6). Energy availability (EA), on the other hand, refers to the amount of energy remaining for biological processes after accounting for EEE. EA was first reported relative to body weight (BW) (7) but later updated to kcal·kg LBM^{-1} (8), given the higher metabolic activity of lean tissue compared to adipose tissue. However, it should be noted that the first studies to report kcal·kg LBM^{-1}·day^{-1} assessed body composition using hydrostatic weighing which only provides information on fat mass (FM) and fat-free mass (FFM) (which includes bone, muscle, and organ tissue). Although many studies use the terms LBM and FFM interchangeably, they are distinctly different anthropometric measurements. FFM represents all non-lipid tissue including bone, while LBM represents non-lipid tissue in addition to small amounts of essential fat. Additionally, LBM obtained using DXA is typically reported without bone content. Therefore, EA units will be reported as *kcal·kg FFM^{-1}·day^{-1}* unless otherwise specified.

Energy Balance (EB) = Energy Intake – Total Energy Expenditure

Energy Availability (EA) = (Energy Intake – Exercise Energy Expenditure) / Fat-Free Mass

Athletes are often categorized as having optimal, reduced, or low EA (LEA) based on the calculation of EA from EI and EEE (9). The concept of a LEA threshold was first proposed in the late 1990s. In a study by Loucks and colleagues (10), 27 sedentary women were randomized to one of four EA conditions (40, 25, 19, and 10 kcal·kg FFM^{-1}·day^{-1}). In all conditions, exercise was performed on a treadmill

or cycle ergometer, and energy expenditure was standardized at 30 kcal·kg FFM^{-1}·day^{-1}. Total triiodothyronine (T_3) and free T_3 (fT_3) were reduced at the two lower EA conditions, but not at 25 or 40 kcal·kg FFM^{-1}·day^{-1}, suggesting that thyroid function becomes affected at a threshold between 19 and 25 kcal·kg FFM^{-1}·day^{-1}. In a subsequent study, Loucks and colleagues (11) investigated the effects of three restricted EA conditions (10, 20, and 30 kcal·kgFFM^{-1}·day^{-1}) on LH pulsatility and metabolic hormones compared to balanced EA (45 kcal·kg FFM^{-1}·day^{-1}). LH pulse frequency and amplitude were only affected when EA was equal to 20 kcal·kg FFM^{-1}·day^{-1} or less. However, changes were observed in the concentrations of insulin, insulin-like growth factor-I (IGF-1), total T_3, leptin, and cortisol beginning at 30 kcal·kg FFM^{-1}·day^{-1}. Ihle and Loucks (12) also reported suppression of bone formation and remodeling biomarkers in exercising women when EA was ≤ 30 kcal·kg FFM^{-1}·day^{-1}. Based on these findings, a majority of studies moving forward have used 30 kcal·kg FFM^{-1}·day^{-1} as the cutoff value for LEA in athletes (13–15). However, as the understanding of EA has evolved, it appears that a single cutoff value cannot be used in all circumstances and may depend on several factors including the specific health outcome of interest, severity of energy restriction, body size, and sex (9, 16). Reed and colleagues (17) assessed EA in 91 recreationally active women who were categorized as eumenorrheic (n=41), oligomenorrheic (n=20), and amenorrheic (n=30) based on self-reported menstrual history and daily measurements of urinary estrone-1-glucoronide (E1G), pregnanediol glucuronide (PdG), and mid-cycle luteinizing hormone (LH) profiles. Self-reported eumenorrheic athletes were monitored for 1-3 menstrual cycles to further identify any subclinical menstrual disturbances. Eumenorrheic athletes were then sub-grouped based on their menstrual profiles as ovulatory (n=20), inconsistent menstrual disturbances (n=13), and anovulatory (n=8). Estimation of EA was conducted using 3-day diet records and 7-day physical activity logs with heart rate sensors. Unsurprisingly, EA was lower in the subjects with amenorrhea (30.9 ± 2.4 kcal·kg LBM^{-1}; n=30) compared to eumenorrheic subjects (36.9 ± 1.7 kcal·kg LBM^{-1}; n=41). However, when the eumenorrheic subgroups were assessed, EA was not different between the ovulatory athletes and the

subjects with subclinical menstrual disturbances (p=0.297). In fact, average EA trended lower in athletes with normal ovulation (35.5 ± 2.4 kcal·kg LBM^{-1}) than subjects reporting anovulation (40.1 ± 3.9 kcal·kg LBM^{-1}) and inconsistent menstrual cycles (37.1 ± 3.0 kcal·kg LBM^{-1}). Oligomenorrheic athletes reported a similar EA to ovulatory subjects (35.4 ± 3.2 kcal·kg LBM^{-1}) (17). These findings suggest EA may be useful in identifying athletes with amenorrhea, but it fails to detect subtle menstrual disturbances.

The foundational studies on EA were primarily conducted in women, given the close relationship between EA and menstrual function. More recent evidence suggests male athletes can also be affected by LEA (18); however, men may be more resilient to the negative effects of energy restriction (19). Thus, if there is a LEA threshold value in men, it is likely lower than the threshold in women, with one recent randomized control trial suggesting a value between 9 and 25 kcal·kg FFM^{-1} (20). Furthermore, the consequences of LEA may depend on the severity and duration of energy restriction, referred to as the EA "dose" (21). However, experimental trials in a laboratory setting are often acute, single exposures to severely reduced EA, which may not accurately reflect LEA scenarios in real-world settings. For example, athletes may experience mild, yet consistent energy deficiency over a prolonged period, or EA may fluctuate intermittently throughout the year based on training periodization (22). Therefore, reference standards for LEA require further investigation based on target health outcomes, sex differences, and application outside of a laboratory setting.

In 2023, the IOC proposed assessing LEA exposures on a continuum ranging from *adaptable* to *problematic* (23). Adaptable LEA describes a period where EA is reduced but does not pose a significant risk to the athlete's long-term health or performance. Perturbations in biological processes are typically mild and/or quickly reversible with adaptable LEA. Problematic LEA, on the other hand, presents with signs and/or symptoms indicative of disturbances in health, well-being, and/or sports performance. Signs and symptoms used to identify problematic LEA are described in Section 2.

1.2 The Athlete Triad and Relative Energy Deficiency in Sport

Problematic LEA is a medical concern for active individuals that can lead to severe consequences such as endocrine disruptions, cognitive disturbances, increased injury risk, and osteoporosis. The relationship between disordered eating, amenorrhea, and low bone density in female athletes was first identified in the 1980s (24). The syndrome became known as the Female Athlete Triad (Triad) and was officially recognized by the American College of Sports Medicine (ACSM) in 1997 (25). Ten years later, the clinical definition of the Triad was updated to reflect LEA with or without disordered eating as the underlying cause (26). It was also acknowledged that athletes move along a continuous spectrum of EA, menstrual function, and bone health. Increasing EA directly improves Triad outcomes by restoring menstrual function and improving bone density.

As research in this area continued to progress, it was evident that the condition resulting from LEA was not limited to consequences in menstrual function and bone metabolism. In fact, there are several other biological systems and aspects of health that are potentially affected by energy deficiency including cardiovascular, endocrine, metabolic, psychological, and gastrointestinal functions (18). Due to the involvement in systems other than bone and reproduction, the IOC introduced the term Relative Energy Deficiency in Sport (REDs) which expanded upon the Female Athlete Triad to include additional symptoms and male athletes (27). Unlike the Triad which focuses on three clinical conditions, REDs encompasses all possible health and performance impairments caused by energy deficiency. However, given that it is a relatively new area of research, it has been criticized for a lack of clarity and scientific rigor (28). In 2021, The Female and Male Athlete Triad Coalition proposed a model for the Male Athlete Triad which is defined as "a syndrome of 3 interrelated conditions, including energy deficiency/LEA, impaired bone health, and suppression of the hypothalamic-pituitary gonadal (HPG) axis" (28). In this model, LEA is determined by indicators of metabolic adaptations that occur in response to prolonged energy deficiency such as changes in body composition, endocrine biomarkers (e.g., T_3 and leptin), and

suppressed RMR. Similar to the REDs model, the Male Athlete Triad requires further investigation to clearly define the clinical diagnosis and outcomes of these conditions.

Despite the current debate regarding these three models, they all share the same goal, which is to protect and improve athlete health. It is agreed that prolonged inadequate energy intake is the underlying cause for the Female and Male Athlete Triads and REDs. As scientific understanding of energy deficiency moves forward, it may be advantageous to bridge the gap between these overlapping conditions to avoid confusion among athletes, coaches, and clinicians.

1.3 Prevalence and Etiology of Low Energy Availability

The prevalence of LEA is difficult to report given the varying assessment methods used across studies and the potential for EA fluctuation over time. However, recent literature estimates that approximately 22% to 58% of athletes are at risk for LEA, with a high prevalence in women and endurance sports (29). The reported prevalence is even higher in competitive female endurance athletes, ranging from 65% (30) to approximately 80% (31). Athletes and recreationally active individuals are not the only populations at risk. LEA has also been reported in military personnel (32), especially during intense periods of training (33).

Athletes may intentionally restrict food intake to alter body composition because they believe having a leaner physique will benefit sports performance or improve social acceptance (34). The prevalence of disordered eating (DE) and eating disorders (ED) are higher in women compared to men (35), which may put female athletes at higher risk for intentional energy restriction. Reported DE and ED ranges between 4% to 76% in female high school athletes (36, 37), and can persist into adulthood. Elite female runners who participated in the 2020 U.S. Olympic Team Trials Marathon were surveyed to assess self-reported eating behavior (38). Of the 146 athletes who completed the survey, 67.1% (n=98) reported intentional restriction of energy intake and 32.9% (n=48) reported current or previous history of a clinically

diagnosed ED. Despite an average BMI of 19.41 ± 1.42 kg/m^2, nearly half of respondents (44.5%) reported feelings of weight dissatisfaction.

Restrictive eating and pressures to alter body composition can be largely influenced by the athlete's social environment and team culture (39). While coaches may not intentionally encourage DE in their athletes, coaching style (40) and negative comments (41) can contribute to body image anxiety. In a survey of 128 elite female athletes, 64.8% reported feeling pressured by a coach "to maintain low body fat percentage and/or low body weight" (42). Female athletes also report experiencing pressure from teammates related to body weight and appearance (43). Reed et al. (44) found that teammates contributed more to perceived body weight pressure in Division I female athletes than performance-related concerns. The excessive use of social media may also negatively influence athletes' self-perception if they are comparing themselves to others (45). One study found that high-profile athletes are more likely to post photos of themselves in non-athletic settings and receive the greatest amount of likes on photos that are sexually suggestive (46). Additionally, comparison to other athletes on social media may trigger feelings of anxiety and insecurity, and athletes may be motivated to alter their body composition for aesthetic purposes at the detriment of their athletic performance. Therefore, team culture and social perceptions of body image must be addressed to support athlete well-being (47).

While much of the LEA literature has focused on competitive and elite athletes, recreational runners are also at risk for energy deficiency and symptoms of ED (48). In a study of 524 recreational and competitive female runners, 47.3% (n=248) screened positive for risk of LEA (49). This study found no differences based on competitive level, however, there was a higher prevalence of LEA in athletes between the ages of 18-24 (73%) compared to older athletes (25-30 years, 51%; 31-40 years, 39%; 40+ years, 39%). Risk of DE and ED in all athletes were 40% (n=209) and 9.4% (n=49), respectively, with a higher risk of ED in the 18-24 age group (19%) compared to all other age categories. Folscher et al. (50) reported

a similar prevalence of LEA (44.1%) in older female ultramarathon runners (n=306; age 39.45 ± 7.97), with 32% at risk for DE and 67.4% expressing concern that weight gain would impair performance.

Transgender athletes may also be at risk for intentional LEA due to weight concerns and body dissatisfaction (51). In a survey of 442 transgender adults, significant eating disorder risk was identified in 23% of transgender women and 22% of transgender men (52). Furthermore, transmasculine spectrum athletes may use restrictive eating behavior to intentionally suppress menstrual function and induce amenorrhea (52). One case study documented a high school transgender cross-country runner (they/them) who presented with a restrictive eating disorder and secondary amenorrhea (53). This athlete identified as male but did not want to transition hormonally until after their collegiate running career. Menstruating was an emotional burden for them because it reminded them of their female gender assigned at birth. Health professionals on the sports medicine team need to consider the unique challenges faced by transgender athletes when providing counseling and medical care.

Not all athletes who present with LEA exhibit symptoms related to DE/ED or body dissatisfaction (54). In a sample of high school female athletes, 36% reported reduced EA (< 45 kcal·kg LBM^{-1}) but only 4% screened positive for symptoms of DE (36). Some of the possible reasons for unintentional LEA are inadequate knowledge of fueling recommendations, decreased appetite, lack of time, or low food security (55). Observational studies have found a general lack of sports nutrition knowledge and poor dietary habits in athletes competing in the National Collegiate Athletic Association (NCAA) (56). Since the NCAA regulations on nutrition changed in 2014, there has been an increase in the number of sports dietitians working in collegiate athletics. This is a major benefit because access to a sports dietitian is associated with better diet quality and adherence to sports nutrition guidelines (57). Nutrition counseling by a sports dietitian has also been shown to improve energy intake (58) and Triad outcomes (59) in female athletes. Unfortunately, not all athletes have access to a sports dietitian. While many of the larger Division I programs have a full-time dietitian, this is not the case for most smaller colleges, lower divisions, and

programs outside of the NCAA. This leaves thousands of student-athletes to make dietary choices without guidance from a nutrition expert.

Amateur athletes and highly active individuals may also lack proper nutrition knowledge. In a study of recreational athletes (n=409), their knowledge of total and general nutrition was higher than their knowledge of sports nutrition. Men scored higher on sports nutrition knowledge compared to women but lower on total nutrition (60). The discrepancy may partially be attributed to where recreational athletes seek nutrition information. In a sample of ultra-endurance athletes, over 70% of athletes reported getting nutrition information from magazines and other athletes compared to only 8% from a dietitian (61).

Athletes may also experience involuntary appetite suppression in response to exercise. While the exact mechanism is not fully understood, it appears that changes in appetite-regulating hormones are associated with reduced appetite and subsequent energy intake post-exercise (62). Immediately after a workout, the appetite-stimulating hormone, ghrelin, is shown to decrease and the satiety hormones, peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), are elevated (63, 64). Despite the increased energy expenditure from exercise, it does not appear this corresponds with increased appetite ratings or energy intake following the activity (65). Altered concentrations of appetite-regulating hormones and suppressed appetite are most significant immediately following exercise (66) but may persist for up to 2 to 10 hours (64). Inadvertent energy deficiency may occur if athletes do not compensate with additional food intake when their appetites return. Sports dietitians may need to encourage athletes to consume easily tolerated foods and beverages including “liquid calories” immediately after exercise such as smoothies, whole-fat milk, or other energy-dense beverages if they have difficulty eating “solid” foods.

2. Assessment of Energy Availability

2.1 Calculated Estimations

Calculating EA involves estimated measurements of EI, EEE, and body composition (specifically FFM). While this may appear straightforward, the assessment of EA outside of a controlled laboratory setting is challenging. Several methods can be used to evaluate each component, many of which rely on self-reporting. Additionally, it is unknown how the lack of a standardized protocol for the measurement of EA affects risk identification. This section reviews the various methods and limitations to assess each component of the EA calculation.

2.1.1 Energy Intake

Several methods can be used to evaluate dietary intake such as food records/diaries, 24-h dietary recalls, diet histories, and food frequency questionnaires (FFQ). Food records are the most used method in the field to assess EI in the calculation of EA (16). Recording typically occurs on three to seven consecutive days (ideally including one weekend day) when all foods and beverages are weighed, measured, or estimated by the athlete. However, EA calculations can be affected by errors of under-reporting. A 2017 meta-analysis study found that athletes tend to under-report EI by 19% (0.4-36%) compared to energy expenditure measured by doubly-labeled water (67). Using weighed food records over longer periods may improve the accuracy of this method but is more burdensome on the athlete (68). With all dietary assessment methods, including food records, athletes may also intentionally avoid or omit foods that they view as “unhealthy” or “bad” when they know they are being monitored (16). Errors in estimated EI can also be introduced by the persons responsible for coding and analyzing the food record. Despite advanced training in dietary assessment methods, Brakkhuis et al. (69) found significant variability in estimated EI from food records that were analyzed by experienced sports dietitians.

Studies evaluating EA do not typically use single 24-h recalls or diet histories to assess EI. Unlike food records, 24-h recalls provide information about only one day of food consumption. Therefore, multiple 24-h recalls would need to be obtained to accurately assess longer periods of energy status. The athletes’ training and competition schedules would also need to be considered when deciding which

day(s) to conduct the recall. Dietary habits may differ from day to day depending on rest, training, and competition (70), especially in weight-sensitive sports. However, one of the benefits of obtaining a 24-h recall is that recalls are typically administered by a registered dietitian or highly trained professional. During the recall, the interviewer uses a five-step multiple-pass method to help the participant remember any forgotten food items and obtain further details regarding preparation methods and portion sizes (71). Additionally, the 24-h recall is a low-burden procedure and does not require the athletes to consciously record the foods consumed. One of the limitations of food records is that athletes may modify their eating behaviors in response to being monitored. However, the risk for this type of limitation is reduced when the 24-h recall is conducted unannounced. The inclusion of a 24-h recall with food records may improve the accuracy of the dietary assessment (67). The diet history method also involves a dietary recall, in addition to completing a FFQ and food record. The purpose of including a diet history is to assess longer periods of habitual intake. However, this method is time consuming and may not be practical in the assessment of EA in free-living athletes (72). Finally, FFQs can provide helpful information when assessing overall diet quality but they lack the specificity to calculate changes in EA. They may also inaccurately assess EI of athletes from diverse ethnic backgrounds who do not commonly consume the foods listed on the FFQ. Furthermore, it would be difficult to estimate the EEE for the same period when calculating EA using a FFQ.

2.1.2 Exercise Energy Expenditure

The measurement of EEE can be estimated using indirect calorimetry (IC), heart rate (HR) sensors, wearable devices, self-reported activity logs, or a combination of methods. Energy expenditure estimated by IC utilized measured oxygen uptake (VO_2) and carbon dioxide production (VCO_2) (73). IC is commonly measured using a stationary system, also known as a “metabolic cart”. However, athletes are connected to the stationary system by a mouthpiece or mask and tubing thereby limiting their movement within a certain radius of the IC cart. Therefore, this system would not be able to assess EEE of activities that are

performed outdoors or those requiring a large range of motion. Newer portable and mobile IC devices have been developed to improve the ability to measure EEE in free-living athletes. Some studies have shown similar measurements of VO_2 and VCO_2 by portable devices compared to stationary carts at various exercise intensities (74, 75). However, Perez-Suarez and colleagues (76) found that during high-intensity exercise, the portable COSMED K5 device underestimated VO_2 by 6.6% when it sampled air breath-by-breath and overestimated VO_2 by 12.3% in the mixing-chamber mode. This corresponded to a 6.6% underestimation and 11.5% overestimation of energy expenditure in the breath-by-breath and mixing chamber modes, respectively. Another limitation to estimating EEE using only pulmonary gas exchange is the inability of IC to detect energy contributions from anaerobic glycolysis. During intermittent exercise, such as high-intensity interval training (HIIT), athletes may be working at or above their maximal aerobic capacity (VO_{2max}) indicating energy contribution from anaerobic pathways not captured by IC. In addition to VO_2 and VCO_2, the measurement of blood lactate has been suggested for a more accurate estimation of energy expenditure during intermittent exercise (77). Additionally, although IC can provide fairly accurate estimates of EEE, these devices are expensive and require trained operators. Therefore, this method may not be accessible for many athletes.

Indirect assessment of energy expenditure can also be estimated based on the linear relationship between VO_2 and HR. The validity of this method can be improved by first obtaining the athlete's VO_{2max} and max heart rate (HR_{max}) and calculating an individualized HR/VO_2 regression equation. Although there is some evidence that HR can predict VO_2 during intermittent exercise (78), HR is not an accurate predictor of VO_2 during high-intensity anaerobic exercise. A study of competitive male sprinters and distance runners found that the estimated VO_2 predicted by HR was 7.2% lower than VO_2 measured by portable IC during a track workout (79). Thus, this method may be limited to moderate or vigorous steady-state activity (80).

Wearable activity trackers such as accelerometers, GPS devices, and smartwatches are commonly used to estimate EEE. However, compared to IC these devices have been shown to underestimate energy expenditure during high-intensity exercise (81, 82). In a recent systematic review, the validity of nine commercial wearable devices were measured with no brand found to accurately predict energy expenditure. Expenditure was underestimated by the Garmin devices while overestimated with Apple and Polar (83). It is unclear how these commercial devices estimate energy expenditure given the technology is considered proprietary information.

Finally, exercise logs are frequently used to estimate EEE using Metabolic Equivalent of Tasks (METs) (84). While this method is far less expensive than wearable devices and IC, it is considered less accurate and more burdensome for the athlete (16). Until a standard assessment method is determined for measuring EEE in the EA literature, using a combination of methods may help improve accuracy.

2.1.3 Body Composition

To calculate EA, dual-energy X-ray absorptiometry (DXA), air displacement plethysmography, and bioelectrical impedance are the most common methods used to assess FFM in research and applied practice. Hydro-densitometry (i.e., underwater weighing) was previously the preferred method for estimating body composition; However, this method has largely been replaced by the advancement of new technology. DXA, which was originally developed for clinical use to measure bone mineral density (BMD), is now one of the most popular and preferred methods to assess body composition in athletes (85). DXA is a three-compartment model, which separates the body analysis into FM, FFM, and bone mineral content (BMC). Although DXA can provide a relatively accurate estimate of FFM (86), there are several limitations that should be considered. In addition to needing a trained technician, DXA scanners are costly and many athletes may not have access to one. For athletes who do have access to a DXA scanner, failure to follow a standardized protocol can influence the accuracy of the results. Testing should be conducted after an overnight fast and in a state of euhydration. Bladders should be voided, and fluid

intake restricted before DXA scanning (87). Additionally, larger athletes may not fit within the scan regions of the DXA machine, resulting in omission of the head or feet from the assessment. Since each DXA scan exposes athletes to a small amount of radiation, there are ethical considerations for how often an athlete can undergo repeated measures and pregnant athletes should avoid radiation exposure of any amount (88). Finally, DXA measurements cannot be compared between different models and brands. Therefore, if an athlete chooses to have a DXA scan using another machine, comparing changes in FFM and EA is limited.

Air displacement plethysmography is a two-compartment model that asses only FM and FFM. The BOD POD is commonly used equipment in sports practice that estimates body composition using air displacement. While the BOD POD is less expensive than a DXA scanner, it is still costly, which again limits access. Additionally, the accuracy of this method can be affected by body hair, excessively moist skin, changes in temperature, and clothing worn (86).

Bioelectric impedance devices may be more accessible and familiar to athletes and active individuals. Body composition is indirectly assessed in these devices via small electrical currents for total body water volume and conductivity measurements for FM and FFM. Compared to FM, more water is stored within FFM, making it less resistant to the electrical current. Bioelectrical impendence analysis (BIA) refers to devices that measure single points of contact such as hand-held devices or BIA scales (without hand contact). On the other hand, bioelectrical impendence spectroscopy (BIS) uses multiple points of contact (e.g., InBody Professional Body Composition Analyzer) and is considered the superior bioelectrical impedance method (88). Despite the affordability and accessibility of these devices, bioelectrical impendence is not a preferred method for assessing FFM. BIA and BIS measurements are highly sensitive to hydration status which requires abstinence from exercise several hours before the measurement, which may not be possible for athletes at certain times of the season or training cycle. Additionally, it is

not recommended to assess changes in FFM unless the measurements were done under identical conditions including an objective measurement of hydration (e.g., urine specific gravity) (88, 89).

Finally, surface anthropometry (i.e., skinfold measurements) is an inexpensive and convenient technique for assessing body composition changes in applied practice. However, estimates of body fat are calculated indirectly using regression equations (88). Subsequently, estimated FFM derived from skinfold measurements would be a doubly indirect method. Even if a predictive equation was validated to estimate FFM in athletes, consideration should be given to the psychological effect this type of measurement may have on an athlete with body image anxiety. Therefore, the use of skinfold measurements in the calculation of EA should be used with caution.

Although estimations of FFM are necessary to calculate EA, careful consideration should be given to whether the assessment of body composition is appropriate for an individual athlete. Current best practice recommendations for body composition assessments include limiting measurements to no more than six times per year and screening athletes for ED/DE and body image anxiety before any assessments (90). Given that some athletes with LEA may also experience DE/ED behaviors, alternative approaches to assessing EA other than calculations with FFM is an important area to investigate.

2.2 Surrogate Markers

The assessment of EA is challenging based on the lack of a standardized protocol and methodology concerns described previously. Therefore, surrogate metabolic and endocrine markers are being used more often in the assessment of EA (91). Thyroid hormones, leptin, and IGF-1 are the most feasible biochemical markers to measure in clinical practice (21), but other hormones can be affected by LEA such as hepcidin, LH pulsatility, and testosterone. Additionally, suppressed RMR has been proposed as an indicator of metabolic adaptations in response to energy deficiency (91) in men and women (14, 92). Suppression of RMR is assessed by comparing RMR measured by indirect calorimetry to expected RMR using a predictive equation such as Cunningham or Mifflin-St. Jeor. This method is commonly referred to

in the literature as *RMR ratio*. However, the sensitivity of RMR ratio to accurately identify LEA is dependent on the predictive equation selected (93). Selecting a predictive equation that is appropriate for the specific athlete population is important to prevent over or under-estimation of LEA risk. A recent study of high-level male and female athletes (n=241) reported a wide range of low RMR ratio prevalence when ten different predictive equations were applied (94). Using the Harris-Benedict equation for predicated RMR produced the lowest prevalence of low RMR ratio (3% and 5% for women and men, respectively), while the Jagim equation showed the highest prevalence (46% and 52% for women and men, respectively). Interestingly, although LEA is more common in female athletes, this study consistently showed a higher prevalence of low RMR ratio in male athletes, regardless of the predictive equation selected. Future research on athlete-specific RMR equations and RMR ratio cutoffs for LEA are warranted. See Sterringer and Larson-Meyer (95) for a full review on the use of RMR ratio as a surrogate indicator of LEA.

Thyroid hormones have an important role in the regulation of energy expenditure, body weight, and lipid metabolism. Thyrotropin (TSH) is secreted from the anterior pituitary and stimulates the thyroid gland to synthesize thyroxine (T_4) and a modest amount of the active form T_3. The majority of circulating T_3 is produced by the peripheral deiodination of T_4. Under normal conditions, approximately 80% of T_4 undergoes deiodination, with 35% converted into T_3 and 45% metabolized into inactive reverse T_3 (rT_3) (96). In circulation, over 99.95% of T_4 and 99.5% of T_3 are bound to the transport proteins thyroxine-binding globulin, transthyretin, and albumin, rendering them inactive (97). The small remaining unbound or free T_4 (fT_4) and fT_3 are the biologically active forms of thyroid hormones that can enter cells and interact with nuclear receptors (98). Thus, serum fT_4 and fT_3 are the preferred assays when assessing the metabolic activity of thyroid hormones. In women with LEA, the thyroid gland has been reported to respond by reducing total T_3, fT_3, and fT_4 (7). Elevated concentration of rT_3 has also been reported during caloric restriction related to increased activity of type 3 5'-deiodinase, which is the enzyme responsible

for metabolizing T_4 into rT_3 (99). One of the first studies to investigate the effect of LEA on exercising women found that T_3 concentration was reduced within just two days of LEA (7). Thyroid function was further impaired by day four, with an overall reduction of -18% in fT_3 and -15% in total T_3. The effects of LEA on thyroid hormone concentrations were independent of exercise. While a similar response in total and free T_3 was shown in a case series of four trained men (100), other studies have found no changes in T_3 in male athletes with LEA (101). Total T_4 is observed to decrease in men but findings on the female response have been mixed (102). Although not commonly tested, an observed increase in metabolically inactive rT_3 has also been shown in energy-restricted women (7). Despite these changes in thyroid hormone conversion and metabolism, TSH does not appear to be acutely affected by LEA (102).

Leptin is an appetite-regulating hormone secreted by adipocytes, including those found in bone marrow. The regulatory role of leptin in energy metabolism is to promote satiety. Leptin has also been reported to positively correlate with BMD at the lumbar spine, total hip, and total body sites in premenopausal women (103). In response to energy deficiency, leptin production is suppressed in both men and women (104). Hilton and colleagues (105) conducted a study wherein nine habitually sedentary women were exposed to four days of optimal EA (45 kcal·kg FFM^{-1}·day^{-1}) and LEA (10 kcal·kg FFM^{-1}·day^{-1}) induced through a combination of diet and exercise. In both conditions, exercise was controlled to achieve an energy expenditure of 30 kcal·kg FFM^{-1}·day^{-1} during a treadmill walk at 70% VO_{2max}. During the LEA condition, leptin concentration decreased by 53±3% and amplitude of diurnal rhythm was suppressed by 58±6%. A more recent study in recreational runners found similar results with reported leptin concentrations 61% lower after three days of EA restricted to 15 kcal·kg FFM^{-1}·day^{-1} (106). At that same level of EA, leptin concentration was also reported to decrease over 50% in exercising men (107, 108). In addition to its role in appetite regulation, leptin also indirectly stimulates the pulsatility of gonadotropin-releasing hormone (GnRH) from the hypothalamus (109). GnRH stimulates the production of LH and follicle-stimulating hormone (FSH) in the anterior pituitary (110). Theoretically, reduced leptin in response

to LEA could decrease GnRH secretion and the production of LH and FSH. However, the relationship between leptin and sex hormones is not clear and caution should be taken when using leptin as an indicator of menstrual function. Corr and colleagues (111) compared leptin concentration in recreationally active women with ovulatory menstrual cycles and amenorrhea. Mean leptin concentration was lower in the amenorrheic women, however, after adjusting for body fat the difference was no longer significant. Therefore, the assessment of EA using leptin as a biomarker is limited to its role in body weight and appetite regulation.

Nesfatin-1 is a novel appetite-regulating hormone that is closely related to leptin. Nesfatin-1 was first identified less than 20 years ago in rodents. After continuous injections of nesfatin-1, food intake and body weight gain in the rats were lower compared to control rats indicating anorexigenic properties of nesfatin-1 (112). Although nesfatin-1 and leptin are closely related in the energy regulatory pathway, their functions appear to be independent (112). The evidence supporting the actions of nesfatin-1 to decrease food intake and increase satiety are almost exclusively from animal studies (113). However, one study in young women with anorexia nervosa found that nesfatin-1 concentration was 65% higher in women with high anxiety scores compared to those with low anxiety. The correlation between nestfin-1 and anxiety suggests it may be involved in the expression of anxiety symptoms and subsequent food intake in women with anorexia nervosa (114). However, this hypothesis has not been tested to our knowledge. More research is needed to elucidate the effects of nesfatin-1 on energy and food intake regulation in humans.

Growth hormone (GH) is an important anabolic peptide that increases protein synthesis and affects lipid and carbohydrate metabolism (115). The synthesis and secretion of GH from the anterior pituitary is stimulated by growth hormone-releasing hormone (GHRH). IGF-1 produced by the liver indirectly mediates some of the growth-enhancing effects of GH (116). IGF-1 also plays a role in bone metabolism through its effects on vitamin D. In the kidneys, 25-hydroxyvitamin D is converted to the active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) by the enzyme α-1-hydroxylase (117). There is

substantial evidence that IGF-1 enhances the synthesis and secretion of α-1-hydroxylase (118). During periods of energy restriction, GH concentration is reported to increase in men (102), with the highest peak immediately following exercise (119). Although EA was not assessed, Kyröläinen and colleagues (120) investigated the effect of energy deficiency on GH concentrations in male soldiers during a 20-day field exercise. The field exercise was broken into three phases with consistent EI but varied levels of exercise intensity. The fluctuation in EEE resulted in energy deficits of 4,000, 450, and 1,000 kcal·d^{-1} in phases I (5 days), II (10 days), and III (5 days), respectively. GH concentration was 616% higher after 5 days but returned to baseline within three days when EEE and the energy deficit were reduced. No increase in GH was observed during phase III despite an energy deficiency of 1,000 kcal·d^{-1}, suggesting GH may only be affected during severe energy deficiency (120). Additionally, no changes in IGF-1 concentrations were found in men when EA was restricted to 15 kcal·kg FFM^{-1}·day^{-1} (19, 107, 108). This contrasts with studies in women which have consistently shown increased GH and decreased IGF-1 in response to LEA (11, 121). Within three days of EA restricted to 15 kcal·kg FFM^{-1}·day^{-1}, Papageorgiou and colleagues (122) reported a 23% reduction in IGF-1 concentration in exercising women. More research on the use of IGF-1 as a surrogate marker for LEA in men and women is warranted.

Insulin is an anabolic hormone secreted from the beta cells of the pancreas that regulates energy storage by promoting glycogenesis, triglyceride storage, and protein synthesis (123). Insulin also supports bone growth by promoting osteoblastogenesis (i.e., creation of cells responsible for bone formation) (124). Osteoblasts secrete the hormone, osteocalcin, which is decarboxylated during bone resorption into uncarboxylated osteocalcin (unOC). The uncarboxylated form of osteocalcin is secreted into circulation and may affect glucose metabolism by stimulating insulin secretion and sensitivity (125, 126). Although there have been several animal studies conducted, the relationship between unOC and glucose metabolism is not clearly understood in humans and has not been explored in energy-restricted athletes.

On the other hand, insulin is shown to decrease in response to LEA (11, 107, 121). This downregulation of insulin is likely an adaptive response to decreased substrate availability during restricted intake.

Iron status may also be affected by LEA due to increased hepcidin activity and insufficient micronutrient intake. Hepcidin is considered the master regulatory hormone for iron and is elevated during periods of inflammation and energy deficiency (127). Hepcidin, which is stimulated by the cytokine interleukin-6 (IL-6) and secreted by the liver, controls iron availability in the blood by inactivating ferroportin, the transmembrane protein responsible for exporting iron. Ferroportin channels are found on intestinal enterocytes, hepatocytes, and macrophages (128). The binding of hepcidin to ferroportin stimulates cellular internalization and degradation of the iron exporter. Downregulation of ferroportin activity prevents iron release into circulation by inhibiting dietary iron absorption in the duodenum, preventing the release of stored iron from hepatocytes, and impairing iron recycling by macrophages (129). Unlike iron deficiency, ferritin concentration will increase in response to hepcidin as iron is sequestered by the cells. Although IL-6 is shown to directly stimulate hepcidin production, LEA may upregulate its activity through a non-inflammatory pathway. Ishibashi and colleagues (130) assessed hepcidin and IL-6 in well-trained male distance runners under three-day conditions of LEA (20 kcal·kg FFM^{-1}·day^{-1}) and optimal EA (45 kcal·kg FFM^{-1}·day^{-1}) with exercise. Observed resting concentrations of hepcidin were higher after three days of LEA compared to optimal EA. Serum hepcidin was also elevated three hours post-exercise on Day 3 in both conditions. Interestingly, the increase in hepcidin was not different between the two conditions despite the higher IL-6 reported in the LEA condition immediately post-exercise (130). Considering that hepcidin was elevated at rest in the LEA condition but the response to exercise was similar even in the presence of elevated IL-6, these results suggest a direct effect of LEA on hepcidin that is independent of IL-6. In addition to hepcidin activity, iron status can be affected by inadequate dietary intake. Iron deficiency has been reported in approximately 15-35% of female athletes and 5-11% of male cohorts (131). Iron status is assessed clinically using biochemical markers which include

ferritin (stored iron), hemoglobin (Hb), transferrin saturation, and total iron binding capacity (TIBC). In stage 1 of iron depletion, serum ferritin is reduced (< 35 $\mu g \cdot L^{-1}$) but Hb, transferrin saturation, and TIBC are unaffected. The second stage, known as iron-deficient non-anemia, is characterized by decreased ferritin (< 20 $\mu g \cdot L^{-1}$) and transferrin saturation (< 16%), and increased TIBC (132). Once iron nears depletion, ferritin is dramatically reduced (< 12 $\mu g \cdot L^{-1}$) and Hb production is impaired (< 130 $g \cdot L^{-1}$ men and < 120 $g \cdot L^{-1}$ women) resulting in iron deficiency anemia (131). The use of more than one biomarker is important when assessing iron status to distinguish between iron deficiency related to dietary intake or increased hepcidin activity.

Adrenocorticotropic hormone (ACTH) from the anterior pituitary stimulates the adrenal gland to produce and secrete cortisol in response to stress. ACTH production is stimulated by hypothalamic release of corticotropin-releasing hormone (CRH). This pathway is known as the hypothalamic-pituitary-axis (HPA) and is controlled by negative feedback such that increases in cortisol concentration inhibit CRH secretion from the hypothalamus, thus lowering ACTH release and cortisol production (133). The secretion of cortisol follows a diurnal pattern with a concentration peak upon waking and nadir close to the onset of sleep at night. However, this pattern can be interrupted when CRH is released in response to a stressor. The main function of cortisol in energy regulation is the promotion of gluconeogenesis from stored fatty acids and proteins. Under normal conditions, cortisol secretion is important for managing inflammation and modulating the body's stress response. However, when cortisol concentration is chronically elevated it can have detrimental effects on bone formation and cardiovascular health (133). Ackerman and colleagues (134) compared the secretory pattern of cortisol in amenorrheic exercisers to eumenorrheic exercisers and sedentary controls. Blood was sampled every 10 minutes from 23:00 to 7:00 the following morning. The secretion and pulse amplitude of cortisol were higher in the amenorrheic compared to eumenorrheic exercisers and sedentary controls. Cortisol was also inversely associated with leptin and LH. Interestingly, EI and EEE were not different between the amenorrheic and eumenorrheic participants

suggesting a similar level of EA. However, LEA has been shown to affect cortisol and LH in women with normal menstruation (8, 121). In a study by Loucks and colleagues (11), endocrine response was examined in exercising women at four levels of EA (10, 20, 30, and 45 kcal·kg FFM^{-1}·day^{-1}). Cortisol was most affected at the lowest EA level with elevated concentrations reported during both feeding and fasting periods of the day. During the EA treatment of 20 kcal·kg FFM^{-1}·day^{-1}, LH pulse frequency decreased by 16% and pulse amplitude increased by 21%. Cortisol and LH pulsatility were not affected when EA was ≥ 30 kcal·kg FFM^{-1}·day^{-1}. Although there is evidence that energy deficiency is associated with increased serum cortisol independent of the stress from exercise (121), elevated cortisol concentrations are not specific to LEA. Therefore, additional biomarkers should be measured with cortisol when assessing EA risk in athletes.

2.3 Questionnaires

Questionnaires are often used in clinical practice and research studies, in addition to clinical interviews, as an initial screening tool to identify athletes at risk for LEA. While there are several questionnaires to choose from, not all have been validated and some have only been validated in certain athlete populations (135). In applied practice, the current recommendation is to use the International Olympic Committee Relative Energy Deficiency in Sport Clinical Assessment Tool-Version 2 (IOC REDs CAT2) to guide clinical evaluation and diagnosis (23). The IOC REDs CAT2 was developed by REDs experts and involves a three-step process for screening, risk assessment, and diagnosis. Clinicians are advised to first screen their athletes with validated, population-specific questionnaires or clinical interviews. Athletes that screen positive then undergo a severity/risk assessment that evaluates the presence of primary and secondary REDs indicators. The risk assessment model outlined in the IOC REDs CAT2 is a traffic-light categorization system consisting of Green (healthy), Yellow (mild), Orange (moderate), and Red (severe) Light classifications. If an athlete meets the criteria for the Yellow, Orange, or Red Lights, medical treatment and supervision are recommended. Clearance for sports participation is not recommended for

most athletes in the Red Light category. Diagnosis of REDs can only be made by a physician using the information collected by the medical sports team.

The Low Energy Availability in Females Questionnaire (LEAF-Q) is a commonly used screening tool that has been validated in female endurance athletes (136). The LEAF-Q is a 29-item screening tool to identify symptoms of LEA related to menstrual function, injury, illness, and gastrointestinal function. This questionnaire was validated in a study of 45 endurance athletes (age 26.6 ± 5.4 yr). Sensitivity and specificity to correctly classify risk level was 78% and 90%, respectively, for total scores ≥ 8. Athletes classified as high risk were shown to have lower leptin and T_3 concentrations compared to those classified as low risk. Cortisol concentrations were not different between the groups (136). The LEAF-Q was not developed to assess disordered eating behavior, however, a study in ultra-marathon female runners reported more DE/ED symptoms in the athletes with high LEAF-Q scores compared to what was expected (50). The LEAF-Q has also been used in male athletes with omission of menstrual-related questions (31); however, this approach has not been validated. Furthermore, although the LEAF-Q is one of the most widely used screening questionnaires for REDs, it was developed prior to the IOC defining REDs in 2014 and thus, does not address all possible REDs symptoms that have been reported in recent years.

Besides the LEAF-Q, there have been very few questionnaires validated in athlete-specific populations. The Brief Eating Disorder in Athletes Questionnaire (BEDA-Q) (137) and female version of the RED-S Specific Screening Tool (RST) (138) have been validated in adolescent female athletes, but the male version of the RST has not been validated in any population. The only validated questionnaire in male athletes is the Sport-specific Energy Availability Questionnaire and Interview (SEAQ-I) which was validated in a sample of 50 male competitive road cycles. Lundy and colleagues (139) attempted to validate a male version of the LEAF-Q (LEAM-Q); however, the questionnaire was not able to accurately distinguish the athletes at risk for LEA.

Given the close relationship between LEA and restrictive eating behavior, DE/ED screening questionnaires are also useful assessment tools. Two of the most frequently used questionnaires in athlete studies are the Eating Disorder Examination Questionnaire (EDE-Q) and the Drive for Thinness score from the Eating Disorder Inventory (EDI). The EDE-Q consists of four subcategories including dietary restraint, eating concern, shape concern, and weight concern, and has been validated in non-active men and women. The EDI has been validated only in women (135). Using a combination of LEA and DE/ED questionnaires may improve screening efforts and early identification of high-risk athletes.

3. Energy Availability and Bone Health

Early identification and treatment of LEA is important to prevent long-term health consequences. Although some aspects of the Triad can be corrected with dietary and lifestyle interventions (140–142), it may not be possible to completely reverse the negative effect of LEA on bone health (143). To understand how energy deficiency interacts with bone, we must first review the basic structure and factors that influence its metabolism.

3.1 Bone Anatomy and Composition

Bone is classified into two macroscopic categories: cortical bone (aka compact) and trabecular (aka cancellous or spongy) bone. Cortical bone makes up about 80% of the skeleton and is found on the outermost part of the bone between the periosteum and endosteum (144). The periosteum is a double-layer membrane on the bone surface that consists mostly of dense irregular connective tissue, in addition to nerve fibers and blood vessels. The endosteum is the inner layer of connective tissue that covers the trabecular bone, which is comprised of a meshwork of bony trabeculae and bone marrow between the trabecular spaces. Trabecular bone has a much higher surface-to-volume ratio than cortical bone leading to a higher rate of remodeling and bone loss during periods of accelerated bone turnover (145).

There are four types of cells in bone tissue: osteogenic cells, osteoblasts, osteocytes, and osteoclasts. Bone consists of approximately 90-95% osteocytes, 4-6% osteoblasts, and 1-2% osteoclasts. Osteoblasts and osteoclasts are found in the periosteum and endosteum of bone, while the osteocytes are in small cavities within cortical and trabecular bone called lacunae (146). Osteogenic cells (also known as osteoprogenitor cells) are mesenchymal stem cells that differentiate into bone-building osteoblasts (147). The major function of osteoblasts is to produce and secrete proteins for the formation of extracellular bone matrix. Type I collagen is the main protein produced by osteoblasts and makes up over 90% of the bone matrix. Other proteins secreted by osteoblasts include bone alkaline phosphatase (BAP) and gamma carboxylate glutamic acids such as osteocalcin (OC). Osteoblasts are also responsible for the mineralization of bone by producing hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], which is a calcium and phosphate-containing salt. Mature osteoblasts survive for approximately two weeks before they undergo apoptosis, become flattened bone lining cells on the bone surface, or become embedded within the bone matrix (144). Osteoblasts that become trapped in the calcified bone matrix develop into osteocytes which are sensory cells that regulate bone formation and resorption. Osteocytes secrete sclerostin, which is a protein that downregulates bone formation by inhibiting the Wnt/β-catenin pathway and osteoblast differentiation (148). When mechanical strain is sensed by osteocytes, signals are released to stimulate bone resorption by osteoclasts. Receptor activator of nuclear factor-kappa β ligand (RANKL) is a cytokine expressed by osteocytes during cell death that regulates osteoclast activation. RANKL expression and osteoclastogenesis are upregulated by pro-inflammatory cytokines, parathyroid hormone (PTH), sex-steroid deficiency, and during chronic conditions such as diabetes (149). Osteoblasts can regulate osteoclast activity by producing osteoprotegerin (OPG), a decoy receptor for RANKL. The binding of RANKL to OPG inactivates RANKL and prevents osteoclastogenesis (144).

3.2 Bone Remodeling

Bone remodeling is the carefully balanced cycle of bone activation, resorption, reversal, and formation by bone cells (also known as the basic multicellular unit) that maintains bone homeostasis (Figure 1). At any given time, approximately one to two percent of the human skeleton is being remodeled through resorption and formation of bone tissue (133). Bone resorption occurs relatively quickly within a few weeks, as opposed to bone formation which can take several months until mineralization is complete (150). The activation phase is initiated by mechanical stress/strain on the bone, hormones, or other metabolic signaling. Osteoblasts respond by recruiting osteoclast precursors (pre-osteoclasts) and increasing expression of RANKL to begin the resorption process (151). Pre-osteoclasts differentiate into osteoclasts that bind to the targeted bone resorption site through the $\alpha_v\beta_3$ integrin, a cell-surface receptor that mediates cell-matrix interaction (152). After the osteoclast has adhered to the bone surface, it forms a tight sealing zone that separates the enclosed space between the osteoclasts and bone matrix from the surrounding extracellular space. The osteoclast then undergoes polarization and releases hydrochloric acid and proteolytic enzymes such as cathepsin K into the enclosed area. The acidic environment mobilizes hydroxyapatite crystals by separating the link between hydroxyapatite and the collagen contained within the organic bone matrix. The hydroxyapatite crystals are dissolved into calcium and phosphate ions which are released into blood circulation. Removal of hydroxyapatite consequently exposes the organic bone matrix which is comprised of approximately 90% type I collagen fibers that are then degraded by cathepsin K (144). During the reversal phase, mononucleated reversal cells colonize on the degraded surface and prepare for bone formation (153). Protein fragments remaining from the collagen breakdown are removed and transported to the cell surface to be released into the surrounding intracellular fluid. At the resorption site, osteoprogenitor cells then differentiate into osteoblasts and the formation phase begins. Osteoblasts build the bone matrix by producing type I collagen and other non-collagenous proteins. Mineralization is the final step for newly formed bone through the incorporation of hydroxyapatite. The

mature osteoblast then undergoes apoptosis, becomes flattened into a bone lining cell on the non-mineralized surface, or is incorporated into the bone matrix as an osteocyte (151).

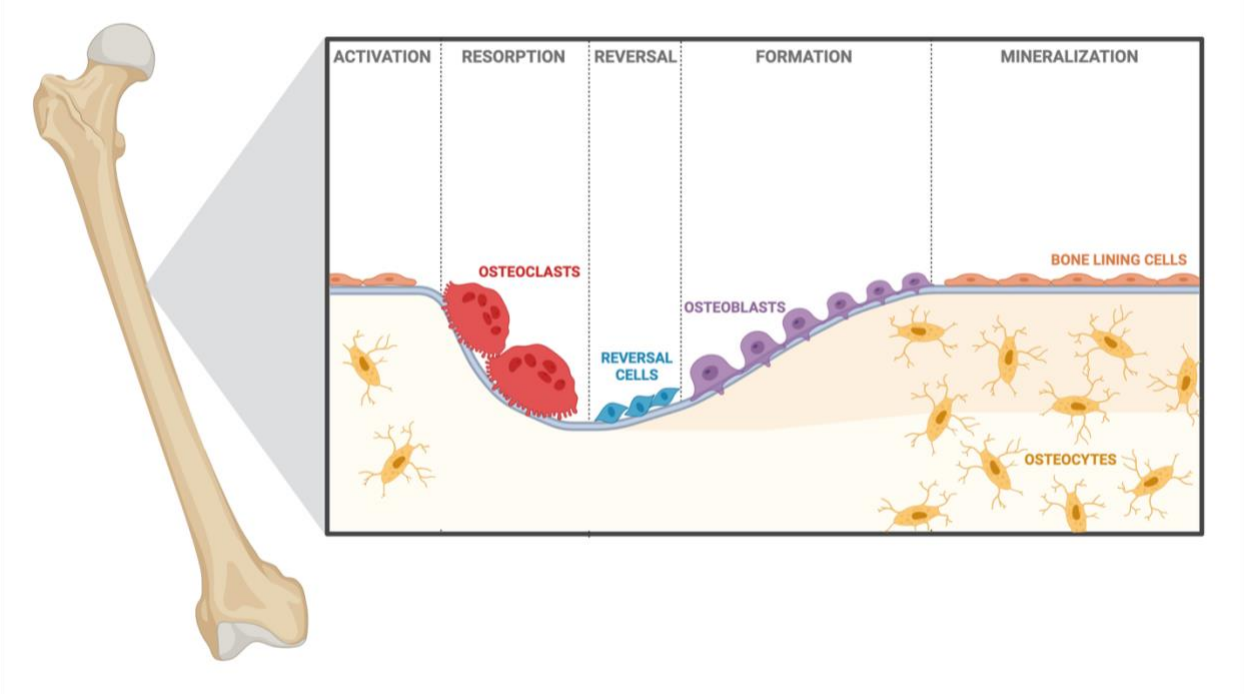

Figure 1. Bone Remodeling Cycle. *Created with BioRender.com*

3.3 Bone Assessment Methods

Bone quality can be assessed by measuring its mechanical properties, geometry, and composition. BMD is one of the most commonly used measurements to assess bone strength, although other factors such as bone size and composition are also important to consider. Since it can take several months for the completion of a bone remodeling cycle, changes in bone microarchitecture cannot be measured acutely. Therefore, surrogate markers of bone remodeling are recommended to assess short-term changes in bone metabolism in research and clinical practice (e.g., response to medications in osteoporosis treatment) (154). This review will focus on bone imaging methods and markers of bone (re)modeling.

3.3.1 Imaging Techniques

Imaging techniques are used to measure chronic changes in bone geometry and microarchitecture and for the clinical diagnosis of osteoporosis. The patient population, resources available, and intended purpose of the scan are taken into consideration when selecting an imaging technique.

The diagnostic criteria for osteoporosis in the United States typically require BMD testing using DXA unless there is a history of certain fracture types or an elevated Fracture Risk Assessment Tool (FRAX) score (155). In recent years, DXA has also become more common in applied sports practice and research to assess bone mass, in addition to body composition. DXA measures the attenuation of photons in an X-ray beam as they are passed through the body. To distinguish between soft-tissue and bone density, the DXA uses two low-dose X-ray beams of different energies. Denser tissue will attenuate more energy resulting in a weaker beam that exists the tissue. The type of beams used in DXA acquisition varies by manufacturer including pencil, wide fan, and narrow fan beams. The wide fan beams move in a longitudinal direction and can acquire high-resolution images in a relatively quick amount of time. The narrow fan beam scans in a longitudinal and traverse motion and is less expensive than wide fan beam systems but has a slower acquisition time.

Radiation exposure from DXA scans is relatively small. Depending on the number of sites and scans performed, a patient is exposed to approximately 1-10 microSieverts (μSv) during a routine scan (156). According to the United States National Council on Radiation Protection and Measurements, the average American is exposed to approximately 5-8 μSv of background radiation each day (157). Despite the relatively low dose of radiation, DXA scans should not be performed during pregnancy.

The procedure for DXA scan acquisition is important to consider when interpreting results. Soft-tissue measurements are more susceptible to error based on factors such as the participant's fasting status, level of hydration, most recent bout of exercise, and clothing worn (85). Additionally, bone density measurements can be impacted based on participant positioning by the technician (158). Thurlow and

colleagues (159) examined the effects of hand positioning on regional and total body bone density estimates in 23 physically active men using the General Electric Lunar iDXA in the standard scanning mode. Each participant was scanned four consecutive times, twice with hands in the prone position as recommended by the International Society for Clinical Densitometry (ISCD) and twice with hands in the mid-prone position as recommended by the Lunar iDXA. In the mid-prone position, the radius was aligned directly above the ulna resulting in a smaller arm bone area and subsequent higher arm and total body BMD compared to the prone position. The increase in total body BMD also resulted in a higher z-score. These results highlight the importance of following consistent positioning protocols when assessing changes in BMD over time. It is also critical to note that the National Health and Nutrition Examination Survey (NHANES) in the United States is one of the major densitometry reference databases that follows the ISCD protocol with scans acquired in the prone position. Therefore, caution should be taken when comparing total body densitometry in the mid-prone position to NHANES reference data.

Computed tomography (CT) is another X-ray attenuation method that can be used to assess volumetric BMD. Quantitative CT (QCT) produces a three-dimensional image of bone geometry that distinguishes between cortical and trabecular bone. The advantage of QCT is the ability to measure volumetric BMD at the spine and hip. However, the effective radiation dose for QCT is higher than DXA with patients exposed to 100-300 µSv per scan (146). Peripheral sites such as the distal radius can be assessed using high-resolution peripheral QCT (HR-pQCT). The radiation dose of HR-pQCT is similar to DXA with an exposure of less than 5 µSv for a standard scan (146).

High-resolution magnetic resonance imaging (HR-MRI) is an imaging technique for assessing trabecular bone at peripheral sites. Unlike DXA and CT-based imaging, HR-MRI does not use ionizing radiation. Instead, it uses a magnetic field and radio waves to capture an image of the hydrogen ions of water stored within the bone. This technique provides a contrasted image of the trabecular bone structure

because very little water is found within bone tissue. Therefore, the bone appears darker in the image while the surrounding soft tissue is bright (160).

3.3.2 Bone Remodeling Markers

Changes in bone structure and density develop slowly over time. Therefore, markers of bone (re)modeling are used to assess acute and short-term changes in bone metabolism. These biomarkers include byproducts from type 1 collagen synthesis and degradation, along with enzymes and other proteins that are produced during bone (re)modeling (Figure 2). Unlike bone density, bone biomarkers change rapidly in response to physiological and environmental stimuli such as circadian rhythm, exercise, nutrient intake, medications, and inflammation (161). During acute studies or interventions, these markers can provide relatively quick feedback on the rate of bone resorption and formation. The National Bone Health Alliance recommends using N-terminal propeptide of type I procollagen (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX-I) as the standard reference markers for bone formation and resorption, respectively (154).

Over 90% of the organic bone matrix is comprised of type I collagen which is synthesized from procollagen type I by osteoblasts. The N-terminal and C-terminal propeptide extensions of procollagen type I (PINP and PICP, respectively) are cleaved during the conversion into type I collagen. As type I collagen is incorporated into the bone matrix, PINP and PICP are released into circulation. Although studies have reported a correlation between PICP and bone formation, serum PICP has a short half-life of approximately 6-8 minutes (162). Therefore, serum PINP is the recommended biomarker of type I collagen formation.

Osteocalcin (OC) is a non-collagenous protein secreted by osteoblasts and has also been used in research studies as a marker of bone formation (12, 163, 164). However, there are two forms of OC found in circulation that differ based on carboxylation and subsequent roles within the body. Fully carboxylated OC is the inactive form that binds to hydroxyapatite in the bone matrix. Uncarboxylated (unOC) or

undercarboxylated OC is the active form and does not bind to hydroxyapatite. The carboxylation of OC is vitamin K-dependent, therefore, higher concentrations of unOC reflect lower vitamin K availability (165). Approximately 40-60% of OC released into circulation is in the inactive (unOC) form (166). Since unOC does not bind to hydroxyapatite, concentration is inversely related to bone formation. In other words, an increase in unOC reflects lower rates of bone formation. Therefore, total OC reflects the entire bone (re)modeling process, while unOC is a more specific measure of bone formation.

Bone-specific alkaline phosphatase (BALP) is an enzyme marker of osteoblast activity and bone formation. BALP does not respond acutely to food intake and is relatively stable with a serum half-life of one to two days (167). Alkaline phosphatase (ALP) is produced by most tissues including the liver, bone, muscle, and kidneys. Mineralization of bone matrix requires BALP to hydrolyze pyrophosphate into phosphate for the synthesis of hydroxyapatite (168). Approximately half of the ALP in circulation is synthesized by bone, however, advanced techniques are required to distinguish between ALP produced by the liver and bones (162).

Sclerostin is a protein secreted by osteocytes to downregulate bone formation by inhibiting the Wnt signaling (or Wnt/β-catenin) pathway which is required for osteoblast synthesis. The Wnt/β-catenin pathway involves a complex of Frizzled G protein-coupled receptors and low-density lipoprotein receptor-related protein 5 or 6 (LRP5 or LRP6) receptors on the cell surface (169). Within the cell, there is a "degradation complex" in the cytosol comprised of glycogen synthase kinase-3 beta (GSK-3β), casein kinase 1 (CK1), axin inhibition protein (AXIN), adenomatous polyposis coli (APC) protein, and β-catenin. When there is no Wnt signaling, β-catenin is phosphorylated by GSK-3β and CK1 in the degradation complex, which then activates β-catenin degradation. However, when Wnt signaling is present, the components of the degradation complex are recruited to the plasma membrane and inactivated. β-catenin then moves into the nucleus and binds with T cell factor/lymphocyte enhancer factor-1 (TCF/LEF) to regulate gene expression. Without β-catenin, Wnt gene expression is inhibited. Sclerostin

downregulates the Wnt/β-catenin pathway by binding to LRP6, which prevents Wnt binding and gene expression. Thus, elevated concentration of sclerostin is a marker of reduced bone formation (148).

When the bone matrix is degraded during resorption, cathepsin K cleaves the N-terminal and C-terminal telopeptides of type I collagen (NTX-I and CTX-I, respectively) which are then released into circulation. The preferred assays for measuring bone resorption are serum (or plasma) CTX-I or urinary NTX-I. Although CTX-I can be measured in the urine, the daily variation is much higher in urinary CTX-I compared to the serum form. Furthermore, beta-CTX-I (β-CTX-I), which reflects the degradation of mature collagen, is preferred over the alpha form in healthy adults and cases of osteoporosis (168). Although urinary NTX-I is commonly used in LEA research (12, 170, 171), it is currently recommended to measure CTX-I when assessing bone resorption.

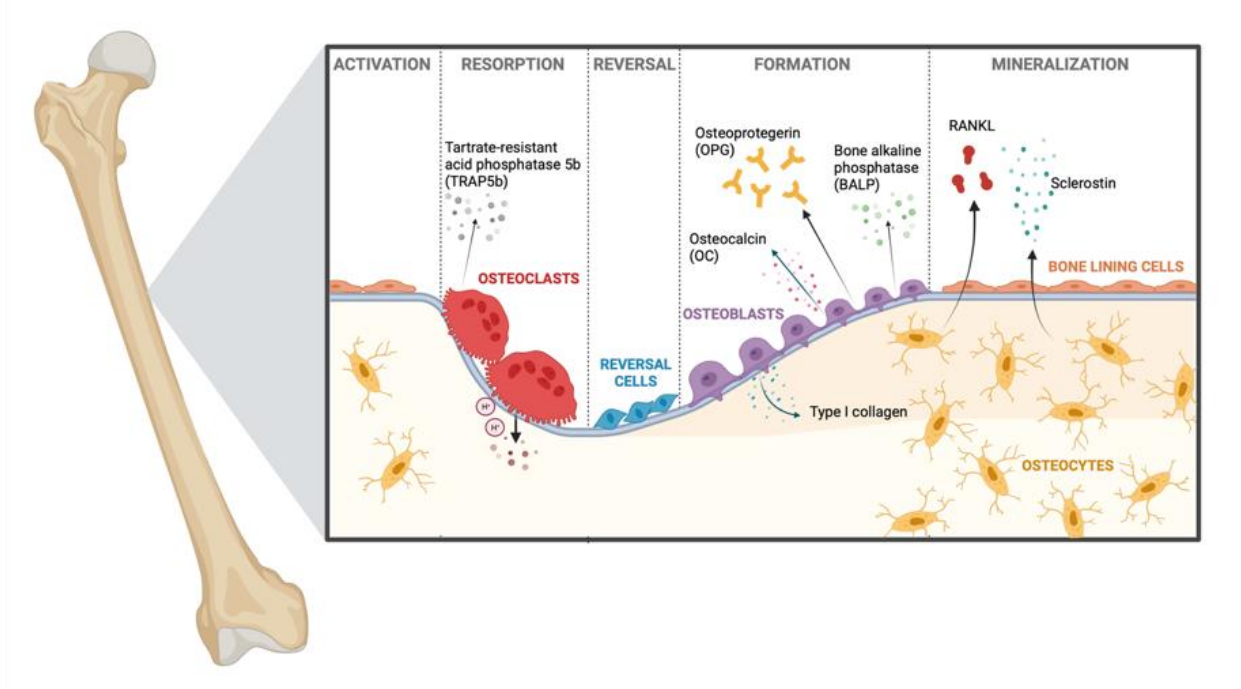

Figure 2. Bone Remodeling Markers. *Created with BioRender.com*

Another commonly used marker of bone resorption is tartrate-resistant acid phosphatase 5b (TRAP5b), which is an enzyme secreted by osteoclasts. However, TRAP5b is considered an indicator of

osteoclast number and volume rather than osteoclast activity (150). Changes in TRAP5b also tend to be less than the ones observed in serum CTX-I (172). Therefore, CTX-I would be a better marker of bone resorption during a short-term intervention.

Although biomarkers can provide some insight into the current state of bone (re)modeling, careful consideration should be taken when interpreting the results. The time of sample collection is critical for CTX-I given its circadian variability and decrease in response to food intake. Concentration of CTX-I peaks in the early morning hours before waking and is lowest in the late morning/early afternoon (154). Therefore, CTX-I sample collection is recommended in the morning after an overnight fast (173). Expression of PINP, on the other hand, does not follow a circadian pattern and is less sensitive to food intake. Since bone resorption occurs before formation in the bone remodeling cycle, expression of CTX-I is likely to occur before there is a detectable change in PINP. Rantalainen and colleagues (174) demonstrated this by measuring bone (re)modeling markers in young men after a single bout of high-intensity jumping exercises. PINP and CTX were measured at baseline and immediately post-exercise with three follow-up samples collected post-exercise after 2 hours, 1 day, and 2 days after. Changes in CTX were not observed until two days post-exercise with an average increase of 32% from baseline. PINP was not different from baseline at any time point. However, participants were not asked to fast prior to the follow-up visits which may have influenced the change observed in CTX. Bone (re)modeling markers have also been shown to respond differently when carbohydrate is ingested during the exercise. In a study by Sale and colleagues (175), ten active men completed two moderate-intensity runs at 70% VO_{2max} for 120 minutes. Participants received either a carbohydrate-containing beverage or a placebo beverage without carbohydrates during each run. Immediate and short-term changes in serum markers of bone (re)modeling were measured over the following 72 hours. The immediate change in β-CTX and PINP was smaller in the two hours post-exercise when participants consumed the carbohydrate beverage compared to the placebo. However, short-term responses measured on the following 3 days were not different

between the two trials. In both conditions, β-CTX and PINP were significantly higher than baseline on the follow-up days. Based on these findings, carbohydrate availability during exercise attenuates the rise in β-CTX and PINP only in the hours immediately post-exercise but not on the subsequent days. Similar to the rise in CTX observed by Rantalainen et al. after two days post-exercise, the highest concentrations of β-CTX and PINP were observed on the third day of follow-up. Therefore, there may be a delay or 'lag' in the observed changes in remodeling makers relative to the intervention or stimulus. Consideration of the time elapsed between an intervention and blood sampling should be considered when interpreting study findings.

Another limitation when using bone (re)modeling markers is the inability to draw conclusions regarding long-term implications. Most studies collect follow-up measurements for only a few days after the intervention, therefore it is uncertain how these biomarkers will continue to respond over time and how that translates to structural changes in bone. Additionally, bone (re)modeling markers are not site-specific and can only provide insight into overall bone (re)modeling. Despite these limitations, bone biomarkers are currently the best option for assessing bone (re)modeling in LEA intervention studies. It would be unethical for a research study to chronically restrict energy intake with the intention of causing detrimental effects on BMD.

3.4 Regulation of Bone Metabolism

3.4.1 Endocrine Regulation

Bone is the primary location for calcium storage in humans, with 99% of all calcium being stored within hydroxyapatite. Only a small amount (~1%) is found in blood with 45% bound to albumin and 45% in the free or ionized (active) form (144). Ionized calcium in the blood is tightly regulated by the bone, intestine, and kidneys to a concentration between 4.65 to 5.25 $mg \cdot dL^{-1}$ (144). Parathyroid hormone (PTH), vitamin D, and fibroblast growth factor 23 (FGF23) are the key hormones that work together to regulate blood calcium (144). Any small decrease in ionized calcium will stimulate the synthesis and secretion of

PTH by the chief cells of the parathyroid gland. PTH increases calcium by binding to the PTH/PTHrP receptor (PTH1R) on bone and in the kidney to stimulate RANKL release and bone resorption. Calcium and phosphate are then released as the hydroxyapatite crystals in the bone matrix are degraded. PTH also reduces renal calcium loss by stimulating calcium reabsorption in the distal convoluted tubule of the kidneys (176). Additionally, PTH indirectly affects intestinal calcium absorption by stimulating the secretion of 25(OH) vitamin D 1-αhydroxylase, the enzyme involved in the renal conversion of 25(OH)D to the active form $1,25(OH)_2D$. Increased $1,25(OH)_2D$ enhances calcium absorption and mobilizes calcium from the bones (144). Similar to PTH, $1,25(OH)_2D$ promotes bone resorption by stimulating RANKL expression and a decrease in OPG (177). Synthesis of PTH and $1,25(OH)_2D$ are inhibited by calcium and FGF23 in a negative feedback loop as calcium concentrations rise and return to normal (178).

The importance of estrogen for bone homeostasis is evidenced by estrogen deficiency bone loss in functional hypothalamic amenorrhea (FHA) (179) and postmenopausal women (180). Estrogen indirectly inhibits osteoclast activity by inhibiting RANKL activity (181) and increasing the expression of OPG (182). Inactivating RANKL prevents osteoclast differentiation and bone resorption. Estrogen may also reduce the expression of sclerostin (148), thereby upregulating osteoblast activity and bone formation. Hormone replacement therapy (HRT) has been shown to attenuate the rate of bone resorption post-menopause in active women. Tomkinson and colleagues (183) measured BMD changes in female runners over the age of 40 years. Participants completed two testing sessions with measurements approximately four years apart. Of the 35 participants at follow-up, five remained premenopausal, 15 were postmenopausal without HRT, two were premenopausal and started HRT, and 13 were postmenopausal on HRT. BMD was maintained in the premenopausal women and those who took HRT during the study compared to significant bone loss in the women not on HRT. The rate of bone loss in postmenopausal women reported in this study was about the same as findings in sedentary postmenopausal women despite engaging in high-impact running. Additionally, Fahrenholtz and colleagues (184) reported an

inverse association between the number of hours spent in negative energy balance and estrogen concentration in female endurance athletes. Unlike EA assessments which are normally reported as a daily average, this study assessed hourly energy intake and expenditure to estimate within-day energy balance (WDEB). The number of hours with WDEB < 0 kcal and < -300 kcal were negatively associated with estrogen concentration despite average daily EA reported greater than 30 kcal·kg FFM^{-1}·day^{-1}. Loucks and colleagues (11) also reported suppressed 24-h mean estrogen concentration (-15%) in exercising women when EA was restricted to 10 kcal·kg FFM^{-1}·day^{-1} during a 5-day randomized controlled trial. However, estrogen was not affected in the women in the 20 kcal·kg FFM^{-1} EA condition suggesting acute changes in estrogen concentration may not occur until EA is severely restricted.

In addition to energy regulation, thyroid hormones have direct and indirect roles in skeletal health. Osteoblasts and chondrocytes express thyroid hormone receptors, TRα and TRβ (185). The actions of T_3 on bone are primarily mediated through TRα1, which involves bone mineralization and osteoblast activity (186). T_3 also acts indirectly on osteoblast and chondrocyte differentiation by stimulating IGF-1 production (186). Abnormalities in T_3 concentration may influence bone health by altering the balance between bone formation and resorption in the remodeling cycle. Thyroid hormone excess is associated with bone mineral loss and increased fracture risk related to accelerated bone turnover (96). On the other hand, menstrual abnormalities (e.g., prolonged and heavier menses, anovulation) are common among women with hypothyroidism, indicating altered sex hormones (96). In a cross-sectional study of male and female elite cyclists (n=93), T_3 was positively associated with total body, lumbar spine, hip, and femoral neck BMD z-scores (187). These results suggest athletes with low T_3 may also be at risk for low BMD.

GH and IGF-1 are important anabolic hormones involved in bone metabolism. Numerous animal studies have shown impaired skeletal growth and bone acquisition in mice with deficiencies in GH or IGF-1 (188). In addition to initial bone development, GH and IGF-1 are also required for coupling bone formation to bone resorption during the remodeling cycle (189). After bone resorption, IGF-1 is released

to promote osteoblastogenesis and bone formation by stimulating the expression of runt-related transcription factor 2 (Runx2) and mesenchymal stem cell (MSC) proliferation (168). Runx2 plays a major role in bone metabolism by regulating the differentiation of MSC into osteoprogenitors and osteoblasts (190).

Insulin is another anabolic hormone that promotes osteoblast differentiation and bone formation (191). It has been proposed that insulin increases osteoblastogenesis by suppressing Twist2, which is an inhibitor of Runx2 (192). In diabetic mice, insulin treatment has been found to improve bone quality and Runx2 function (193). Additionally, low insulin or insulin resistance is shown to suppress the Wnt/β-catenin pathway resulting in lower bone formation (194). There is also evidence of a feed-forward loop between insulin and bone wherein stimulation of osteoblast formation also increases OC production. Some OC is carboxylated and incorporated into the bone matrix, while the rest remains in the active uncarboxylated (or undercarboxylated) form. Active OC enters circulation and acts on adipose tissue to increase insulin sensitivity (125, 165), in addition to stimulating insulin production by the pancreatic β-cells (195). More research is needed, however, to fully elucidate the role of OC in glucose metabolism in populations without diabetes.

Leptin is positively associated with BMD and may have a direct anabolic action by increasing osteoblast proliferation and synthesis (196). However, leptin and BMD are also positively associated with fat mass which may partially explain the observed relationship. As described previously, leptin may also have a role GnRH secretion and the downstream production of estrogen. Low leptin is commonly reported in women with FHA (197) and leptin treatment is shown to increase estradiol along with LH concentration and LH pulse frequency (198). In a study by Welt and colleagues (198), three months of leptin treatment in women with FHA increased bone formation markers (BALP and OC) with no change in the resorptive marker NTX.

Finally, osteoblasts also have receptors for ghrelin and glucose-dependent insulinotropic peptide (GIP) which may promote osteoblast proliferation and activity (191). More hormones continue to be identified as potential contributors to bone homeostasis as understanding of skeletal health advances.

3.4.2 Nutrition Factors

Adequate nutrient intake is essential for bone growth and maintenance. Total energy intake, along with protein, carbohydrates, calcium, vitamin D, and vitamin K are especially important for bone health (2). There is substantial evidence that inadequate EA negatively affects bone health and increases the risk for low BMD in women (201, 202) and men (170), in addition to suppressing bone-regulating hormones such as estrogen, insulin, vitamin D, IGF-1, and leptin (203). If left untreated during adolescence, energy deficiency can impair bone accrual which may be irreversible. Barrack and colleagues (204) conducted a 3-year follow-up study on adolescent female runners (n=39) classified with normal and low bone mass. All runners had a significant increase in total body and site-specific BMC at follow-up, however, the rate of BMC gains in the low bone mass group was insufficient to "catch up" to the runners with normal bone mass. In fact, only about 10% of runners with low bone mass at baseline were able to increase BMC to normal levels by the 3-year follow-up. Although some of the runners who had low bone mass at baseline gained total and lean body mass, the slower accrual rate of BMC may be attributed to a high prevalence of menstrual irregularities and secondary amenorrhea reported by this group. In a study by Cialdella-Kam and colleagues (143), increasing EI by 360 $kcal \cdot d^{-1}$ over 6 months restored menstrual function in adult amenorrheic athletes but did not fully correct impairments to BMD for all athletes. Spinal z-score improved in one of the two athletes with low BMD and in the one athlete with spinal osteoporosis. However, hip z-score did not improve in the two athletes classified with low BMD at that site. Additionally, despite EA increasing to an optimal level (45.4 ± 14.7 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$), no change was found in serum markers of bone (re)modeling. Since structural changes in bone may take several months or years, a longer intervention may be required to restore normal BMD.

Hormone	Relationship to Bone Health	Consequence of LEA	
		Men	Women
Parathyroid Hormone	Regulates blood calcium by stimulating RANKL secretion and bone resorption to liberate calcium from hydroxyapatite crystals, as well as increasing calcium reabsorption in the kidney (176)	↔ (19)*	↔ (19, 106, 199)*
Vitamin D	Regulates calcium homeostasis by increasing intestinal calcium absorption and mobilization of calcium from the bone matrix (144)	*unknown*	*unknown*
Estrogen	Regulates bone resorption by inhibiting RANKL activity and increasing the expression of osteoprotegerin (OPG) (181, 182) Bone loss associated with estrogen deficiency in functional hypothalamic amenorrhea and postmenopausal women (179, 180)		↔ (19, 106)* (13)^ ↓ (184) ^
Triiodothyronine (T_3)	Promotes osteoblast activity and bone mineralization by binding to the thyroid hormone receptor, TRα, on osteoblasts and stimulating IGF-1 production (96, 186)	↔ (19)* (13)^ ↓ (20)*	↔ (19)* (13)^ ↓ (10, 11, 106, 121, 199)*
IGF-1	Promotes osteoblastogenesis and bone formation by stimulating the expression of runt-related transcription factor 2 (Runx2) and mesenchymal stem cell proliferation (168)	↔ (19)* (13)^ ↓ (170)*	↔ (19)* (13)^ ↓ (11, 106, 121)*
Insulin	Promotes osteoblastogenesis and bone formation by suppressing Twist2 (an inhibitor of Runx2) (192) Suppressed Wnt/β-catenin pathway and decreased bone formation associated with low insulin concentration and insulin resistance (194)	↔ (19)* (13)^ ↓ (107)*	↔ (106)* (13)^ ↓ (11, 19, 121)*
Leptin	Promotes bone formation directly by binding to receptors on osteoblasts and possibly indirectly through actions on IGF-1, PTH, and estrogen (196, 198)	↔ (19)* ↓ (107)*	↓ (11, 19, 106)* ↔ (200)^

Table 1. Bone regulatory hormones and response to low energy availability.
**acute and short-term controlled intervention trials; ^observational and cross-sectional studies*

The severity of bone health impairments induced by LEA is dependent on the degree and duration of energy restriction. In a study of sedentary women, the dose-response relationship between EA and bone (re)modeling markers was investigated during EA conditions equal to 10, 20, 30, and 40 kcal·kg FFM^{-1}·day^{-1} (12). Bone formation measured by PICP decreased linearly with EA, while bone resorption (urinary NTX) was only higher at EA of less than 20 kcal·kg FFM^{-1}·day^{-1}. In a crossover study by Papageorgiou and colleagues (19), changes in bone biomarkers were examined in a cohort of men and women exposed to 5 days of optimal (45 kcal·kg FFM^{-1}·day^{-1}) and low (15 kcal·kg FFM^{-1}·day^{-1}) EA. During both conditions, participants expended 15 kcal·kg FFM^{-1}·day^{-1} by running on a treadmill. In response to the LEA condition, PINP decreased by 13% and β-CTX increased by 19% in women. Men, on the other hand, showed no changes in PINP or β-CTX after the 5 days of energy restriction, conflicting with a previous study of male distance runners that found PINP decreased by 15% in response to three days of treadmill running with energy intake restricted to 50% of estimated needs (approximately 20 kcal·$kgBM^{-1}$) (170). These mixed findings suggest women may be more sensitive to the effects of LEA on bone (re)modeling and, therefore, at greater risk.

Carbohydrate availability may also play a role in bone health independent of total energy intake. Although the mechanism is not fully understood, it may partially be attributed to carbohydrate attenuation of proinflammatory cytokine IL-6, which has been shown to upregulate bone resorption by stimulating RANKL expression (149). In a study of elite athletes, markers of bone (re)modeling (PINP, CTX, OC) were examined in response to 3.5 weeks of a high-carbohydrate (HCHO) or energy-matched low-carbohydrate high-fat (LCHF) diet (205). Carbohydrate intake was 8.7 ± 0.4 g·$kgBM^{-1}$·d^{-1} in the HCHO group and 0.5 ± 0.1 g·$kgBM^{-1}$·d^{-1} in the LCHF group. Despite equal energy (220 kJ·$kgBM^{1}$·d^{-1}) and protein (2.1 ± 0.2 g·$kgBM^{-1}$·d^{-1}) intake between the two diets, fasting PINP and OC were lower in the athletes consuming a LCHF diet. Although the change in fasting CTX was not different between the two groups, there was a significant increase in the LCHF diet by 22% from baseline. In response to exercise, the LCHF diet showed

higher CTX immediately after a workout compared to HCHO. After an acute replenishment period of high carbohydrate intake, CTX returned to baseline in the LCHF diet, but markers of bone formation remained suppressed.

Carbohydrate intake during exercise has also been shown to attenuate bone resorption. Over an 8-day energy-matched training program, elite male runners were randomly assigned to consume a carbohydrate supplement (1 g maltodextrin per kgBM per hour of running) or an artificially sweetened placebo during all training runs (206). Carbohydrates provided 61% and 54% of total kcal in the carbohydrate and placebo groups, respectively, with no difference in total energy intake. On the day immediately after the 8-day training program ended, athletes completed an interval workout consisting of 10 x 800 m sprints while consuming a carbohydrate or placebo solution before, during, and immediately after the run. CTX was higher in the placebo group after an 80-minute recovery period compared to the carbohydrate group providing further evidence that carbohydrate availability attenuates exercise-induced bone resorption. However, the effect of carbohydrate on bone resorption may be limited to the immediate hours following exercise. Sale and colleagues (175) reported that when athletes consumed a carbohydrate solution (0.7 g CHO·kgBM^{-1}·h^{-1}) before, during, and immediately after exercise, the response of β-CTX and PINP was significantly lower compared to a placebo beverage immediately after running for 120 min at 70% VO_{2max}. However, there was no difference in bone (re)modeling markers between the carbohydrate and placebo conditions over the following 72 hours. Finally, Townsend and colleagues (207) examined the bone response to consuming a placebo or carbohydrate and protein (CHO + PRO) supplement (1.5 g CHO·kgBM^{-1}; 0.5 g PRO·kgBM^{-1}) immediately after or two hours post-exercise in experienced male runners. Immediate consumption of the CHO+PRO solution post-exercise attenuated the rise in β-CTX within the first hour of recovery and remained below baseline for the following 4 hours. When the CHO+PRO intake was delayed, β-CTX increased above baseline in the first two hours of recovery and then decreased at hours 3 and 4 post-exercise. Regarding bone formation, PINP was higher at 4 h

post-exercise in the immediate intake condition compared to placebo and delayed intake but there was no other difference in PINP response at any other timepoint. The attenuation of bone resorption in the CHO+PRO conditions was not long-lasting, as β-CTX returned to baseline 24 h later.

High dietary protein intake was previously thought to harm bone by increasing urinary calcium excretion (208) without any change in intestinal calcium absorption (209). An early study from 1920 found that adding milk to one subject's diet had little effect on calcium excretion in the urine or feces; however, adding lean beef increased calcium output (210). It was proposed that animal protein may have an acidic effect on the body by increasing the potential renal acid load (PRAL) due to the high sulfur-containing amino acids in meat. Since the body requires maintenance of a neutral pH, the increased acidity would need to be buffered by an alkaline substance. Based on early high protein studies that showed calcium was being lost without any increase in absorption, it was proposed that minerals such as calcium and magnesium would be extracted from bone to balance the pH (211). The idea that high protein intake, especially from animal sources, induced bone resorption became known as the acid-ash hypothesis (212). Currently, the nutrition recommendations for crewmembers on International Space Station (ISS) and National Aeronautics and Space Administration (NASA) missions are to limit animal protein intake to approximately 60% of total protein consumed each day (213). However, there is evidence that intestinal calcium absorption does, in fact, increase with higher protein intake at a rate that balances the increase in calcium output (214, 215), suggesting that protein intake does not negatively affect calcium homeostasis. A 2017 meta-analysis of randomized controlled trials and cohort studies found no effect of high protein intake on BMD, BMC, or fracture risk compared to low protein intake (216), refuting the acid-ash hypothesis. Moreover, dietary protein may even have a protective effect on bone by stimulating anabolic hormones such as IGF-1 (217).

Micronutrient intake is also necessary for normal bone growth and metabolism. The human skeleton reaches peak bone mass in the late 20s or early 30s, whereby bone mass reaches a plateau

followed by a steady decline (218). Calcium intake is essential for achieving peak bone mass during early growth and slowing the rate of age-related bone loss into adulthood (219). In the United States, the Recommended Dietary Allowance (RDA) for men aged 19-70 and women 19-50 is 1000 mg·day^{-1}, with a slightly higher RDA of 1,200 mg·day^{-1} for men over 70 and women over 50 years of age (220). According to NHANES data from 2017 to March 2020, average calcium intake in men met the RDA but was below the RDA for women of all age groups (221). As previously described, ionized calcium concentration is tightly regulated in the blood, and even slight decreases will stimulate PTH secretion and bone resorption. If calcium is not being sourced from the diet, the body will rely on stored calcium in the bones to maintain calcium homeostasis. Under normal conditions, approximately 100-300 mg of calcium is excreted in the urine (222), along with small losses in the gastrointestinal tract and sweat (223). Exercise will also disrupt calcium homeostasis and stimulate PTH secretion (224). Infusion of ionized calcium during exercise has been reported to attenuate the response of PTH and bone resorption (225). However, there are mixed findings on the effects of pre-exercise oral calcium intake on PTH and bone resorption markers. In a recent randomized crossover study, elite male rowers (n=16) underwent two exercise trials consisting of two repeated 90-minute rowing sessions under high and low calcium conditions using a dietary intervention (226). In both trials, participants were provided with energy-matched meals 120 minutes prior to the onset of exercise with either high (1000 mg calcium) or low (<10 mg calcium) calcium content. After each exercise session, there was a significant decrease reported in serum ionized calcium under the low calcium condition, in addition to elevated PTH and β-CTX-I. However, consumption of the high-calcium meals appeared to attenuate the exercise-induced decrease in ionized calcium and increase in PTH and bone resorption. These results suggest intake of high-calcium foods before repeated training bouts may help maintain serum calcium concentration and reduce the elevation of PTH and bone resorption in response to exercise. However, oral calcium supplementation may not have the same effect as calcium-rich foods on observed serum calcium and bone resorption. In a study of 51 men, oral administration of a calcium

citrate supplement (1000 mg elemental calcium) 30 min before exercise did not attenuate the increase in PTH or CTX in the 30 min after a 35-km cycling time trial compared to a placebo (227). However, participants in this study completed the exercise session under fasting conditions. It is possible that food intake may have a stronger effect on bone resorption post-exercise than calcium intake alone. Thus, calcium intake surrounding exercise may be more beneficial from whole foods as opposed to dietary supplements. More research is needed on the practical application of calcium supplementation surrounding exercise.

Calcium homeostasis is also regulated by vitamin D. The active form ($1,25(OH)_2D_3$) functions as a steroid hormone to regulate gene expression (228). Vitamin D has important roles in both bone formation and resorption by acting to increase intestinal calcium absorption, in addition to mobilizing calcium from the bones by stimulating osteoclast differentiation and activity. The RDA for vitamin D is 600 IU in men and women 2-70 years (220). Although there is some debate regarding the specific 25(OH)D cutoff values for vitamin D deficiency, the typical categories are severely deficient (<12.5 $nmol \cdot L^{-1}$), deficient (12.5-30 $nmol \cdot L^{-1}$), insufficient (30-50 $nmol \cdot L^{-1}$), and sufficient (50 $nmol \cdot L^{-1}$) (229). In the United States and Europe, the prevalence of vitamin D deficiency (serum 25(OH)D < 30 $nmol \cdot L^{-1}$) is estimated to be 5.9 and 13% among the general population, respectively, with vitamin D insufficiency (serum 25(OH)D < 50 $nmol \cdot L^{-1}$) reported as 24.0 and 40.4%, respectively (230). Rickets and osteomalacia are examples of a condition in children and adults, respectively, that are characterized by softening of the bones caused by vitamin D deficiency. Athletes likely have a higher vitamin D requirement than the general population given the higher rate of exercise-induced bone (re)modeling and calcium loss. Serum 25(OH)D concentration >75 $nmol \cdot L^{-1}$ is associated with higher calcium absorption and lower stress fracture risk compared to individuals with lower concentrations (231), suggesting maintenance of at least 75 $nmol \cdot L^{-1}$ serum 25(OH)D for optimal health and performance. The risk of vitamin D insufficiency varies based on several factors including sport, season, and athlete skin color (231). Exposure to solar ultraviolet B radiation

converts 7-dehydrocholesterol to previtamin D_3 and is the highest source of natural vitamin D available to humans (~3000 IU vitamin D_3 from 5-10 minutes of direct sun exposure to arms and legs) (117). Smaller amounts of vitamin D_2 and D_3 can also be attained through dietary sources such as fatty fish, shiitake mushrooms, egg yolks, and fortified foods. However, studies consistently report dietary vitamin D intake in athletes is below the RDA, regardless of sport (231). Thus, direct sunlight exposure is important for athletes with diets low in fatty fish and fortified foods. This may increase the risk of vitamin D insufficiency for certain athletes who train indoors or at high latitudes, shield their skin from the sun, or have skin with higher melanin pigment (228). It is unclear whether short-term supplementation in athletic populations has an effect on acute bone (re)modeling. In a study of generally healthy men with serum 25(OH)D < 75 nmol·L^{-1}, 12 weeks of vitamin D supplementation (20,000 IU weekly) did not affect markers of bone (re)modeling (CTX and OC) or BMD (total body or site-specific) (232). Furthermore, excess supplementation in athletes may negatively affect vitamin D status by increasing vitamin D catabolism. Owens and colleagues (233) investigated the effects of a weekly 35,000 IU or 70,000 IU bolus dose of vitamin D_3 administered over 12 weeks in elite athletes (n=40). At week 12, serum concentration of 25(OH)D was elevated in both groups compared to baseline, followed by a decrease after 6 weeks of supplementation withdrawal. However, inactive serum 24,25(OH)D concentration increased rapidly in the 70,000 IU supplement group above baseline at all time points including 6 weeks after supplementation withdrawal. Despite a small increase in 24,25(OH)D concentration reported in the 35,000 IU group, the peak concentration of the low dose was less than the higher dose supplementation (11.8 ± 1.9 nmol·L^{-1} compared to 17.3 ± 4.5 nmol·L^{-1}). These findings suggest that bolus high-dose vitamin D supplementation may cause an accelerated catabolic response by increasing the expression of 24-hydroxylase and the conversion of 25(OH)D to its inactive form. Thus, lower dosage supplementation administered at a higher frequency may have better outcomes for athletes in need of vitamin D supplementation.

In addition to its role in blood coagulation, vitamin K is another nutrient that is critical for normal bone metabolism. Vitamin K2 (menaquinone) is reduced to hydroquinone, which functions as a cofactor for the vitamin-K-dependent enzyme, γ-glutamyl carboxylase, in the γ-carboxylation of OC (126). Carboxylated OC regulates hydroxyapatite structure by binding with calcium during mineralization of bone matrix. There is also evidence that vitamin K has γ-carboxylation-independent roles in regulating bone (re)modeling by downregulating RANKL expression and osteoclast activity, in addition to promoting osteoblast function (234). Despite the importance of vitamin K in bone (re)modeling, there is mixed evidence regarding the association between vitamin K and BMD (235). A 2012 meta-analysis of vitamin K supplementation (phylloquinone [K1] or menaquinone [K2]) in women of all ages found that vitamin K was positively associated with BMD in the lumbar spine but had no effect on the femoral neck (236). Similar findings have been reported in female athletes. Braam and colleagues (237) reported vitamin K1 supplementation (10 mg daily) over two years did not increase lumbar spine or femoral neck BMD in elite female endurance athletes (n=37) compared to a placebo (n=42). In both groups, BMD at the femoral neck was lower than baseline suggesting no attenuation of bone resorption by vitamin K. However, adherence to supplement protocol was self-reported, which makes it challenging to evaluate compliance to the intervention. More research on vitamin K supplementation and bone health in active populations is warranted.

Inflammation and oxidative stress can negatively affect bone health by creating an imbalance in the bone (re)modeling cycle. Immune cells signal the release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukins (IL), in addition to reactive oxygen species (ROS) (238). Chronic inflammation impacts bone health through cytokine activation of osteoclast activity and downregulation of bone formation. Specifically, TNF-α, interleukin-1 (IL-1), and IL-6 upregulate the expression of RANKL promoting osteoclastogenesis and bone resorption (149). Food or supplement sources of antioxidants may have bone-protective effects by scavenging ROS and reducing oxidative stress (239). In particular,

omega-3 polyunsaturated fatty acid (PUFA) has antioxidant and anti-inflammatory properties which may decrease the rate of bone resorption. The three main types of omega-3 PUFA are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (240). ALA is the plant-based source of omega-3 PUFA and is converted in the body to EPA and DHA, which can also be directly attained from animal-based sources. In a study by Griel and colleagues (241), consumption of a high ALA diet from walnuts and flaxseed over six weeks reduced bone resorption (NTX) compared to consuming an average American diet that was higher in saturated fat and lower in PUFA. Additionally, a positive association between BMD and intake of ALA, EPA, and DHA has also been reported in normal and osteopenic Spanish women (242). Nonetheless, more randomized controlled trials are needed to fully understand the relationship between omega-3 PUFA and bone health.

3.4.3 Exercise

In the presence of optimal nutrition and metabolic conditions, physical activity has a positive effect on bone health across the lifespan. However, not all exercise modalities elicit an osteogenic response. Such a response depends on the magnitude, rate, frequency, and diversity of the loading pattern (243). Bone responds favorably to short bouts of dynamic loading applied at a relatively high impact and frequency (244). According to a position statement by Exercise and Sports Science Australia, participation in high-impact and intermittent sports is associated with greater bone mass compared to non-weight-bearing sports and untrained individuals (243). Although low-intensity activities such as walking may indirectly benefit bone through weight management (245, 246), it is unlikely to provide a sufficient osteogenic stimulus (247). The suggested exercise prescription for osteoporosis prevention suggests high-impact loading that applies a force greater than two times body mass (243). Cross-sectional studies show runners have higher BMD and bone strength compared to inactive controls (248, 249). However, lower total and site-specific (lumbar and femur) BMD has been reported in adolescent (250) and adult (251, 252) female endurance runners compared to athletes competing in other sports. Lower

BMD in this population may partially be attributed to a high prevalence of LEA and risk of DE/ED among female and endurance athletes (200). Additionally, running is a continuous and unidirectional exercise that may desensitize bone response due to overstimulation (244). Evidence from animal studies suggests intermittent loading with extended rest periods of at least 4 hours between sessions improves bone mechanosensitivity and formation (253). In a study of premenopausal women (n=26), changes in bone formation (OC and BALP) and bone resorption (TRAP5b and CTX) were assessed in response to a 2-week jumping intervention consisting of 10 jumps daily performed 5 times per week compared a control condition (254). Bone resorption measured by CTX was lower in the jumping group compared to baseline. Additionally, TRAP5b was lower in the jumping group compared to the control group, however, there were no changes in TRAP5b from baseline in either group. Interestingly, the jump intervention lowered BALP, a marker of bone formation. No changes were observed in OC within or between groups. To assess the optimal frequency of high-impact jumping to improve BMD, Bailey and colleagues (255) randomly assigned premenopausal women to 50 jumping exercises performed on 2, 4, or 7 days a week for 6 months. The jumping exercises consisted of 50 multidirectional, unilateral jumps. The same limb was trained for the entire duration of the study. In the women who performed the exercises daily, femoral neck BMD of the exercising limb increased by approximately 2% compared to the control limb and was higher than the lowest frequency intervention group. These findings were in agreement with an earlier study by Bassey and colleagues (256), which reported 50 daily jumps for 6 months increased BMD at the trochanter by 3.4% in premenopausal women.

Although LEA in any form can potentially compromise bone health, exercise during energy deficiency may attenuate some of the negative response. Papageorgiou and colleagues (106) conducted a crossover study in female runners to investigate changes in PINP and β-CTX after three days of optimal EA (45 kcal·kg FFM^{-1}·day^{-1}), LEA induced through dietary restriction (EI = 15 kcal·kg FFM^{-1}·day^{-1}), and LEA induced through a combination of dietary restriction and daily running ([EI = 45 kcal·kg FFM^{-1}·day^{-1}] + [EEE

= 30 kcal·kg FFM^{-1}·day^{-1}]). PINP was reduced after three days of LEA without exercise, but no change was reported after the combined LEA condition with running. No differences in bone resorption were found among the groups. These findings suggest the negative effects of LEA on bone formation may be counteracted or masked by the osteogenic effects of weight-bearing exercise, such as running.

It has been proposed that other high-impact exercise such as jumping may be a potential intervention to protect bone health during LEA given the relatively low energy cost and burden on the athlete (257). In a recent two-armed randomized crossover study, bone (re)modeling markers were assessed in nineteen recreationally active women who completed three days of balanced (45 kcal·kg FFM^{-1}·day^{-1}) and low (15 kcal·kg FFM^{-1}·day^{-1}) EA conditions (199). During the LEA condition, participants either completed two sets of 20 high-impact jumping exercises each day (n=10) or performed no exercise (n=9). In both LEA conditions, PINP was reduced after three days. The change in PINP, however, was not different between conditions suggesting bone formation was not affected by the jumping exercises under acute energy restriction. β-CTX only increased in the LEA conditioning without jumping and the change from baseline was greater than the change reported in the jumping condition. These findings indicate brief jumping exercises may attenuate the rise in bone resorption but not bone formation during short-term LEA. However, it is unlikely that an athlete or highly active person would refrain from physical activity completely or only perform brief jumping exercises in place of training. Therefore, intervention studies to investigate the effect of jumping exercises with concurrent sports training during short-term LEA are warranted.

3.4.4 Other Factors

Cigarette smoking has been identified as a modifiable risk factor for bone loss and the development of osteoporosis (258). However, little is known about the mechanism underlying smoking's effect on bone health. Based on observational data, it appears BMD is highest among individuals who have never smoked, but there is no evidence of a dose-response relationship between cigarette smoking

and bone health outcomes (259). The effect of smoking on bone density may be mediated through its effects on calcium and vitamin D absorption (260). A recent meta-analysis found that smokers had lower 25(OH)D concentrations compared to nonsmokers, regardless of vitamin D supplementation use (261). It is also possible that individuals who smoke cigarettes have other lifestyle factors that negatively impact bone health such as poor diet quality (262) and inadequate physical activity (263).

The relationship between alcohol consumption and bone health is somewhat complex. It is recognized that excessive alcohol intake of more than two or three drinks per day for women and men, respectively, is a risk factor for osteoporotic fractures (264). In a systematic review and meta-analysis of observational studies, osteoporosis risk was 1.34 times higher in individuals who consumed 1 to 2 alcoholic drinks per day compared to non-drinkers (265). Other studies, however, have reported positive bone health outcomes with low to moderate intake such as higher BMD and a slower rate of age-related bone loss (266). It is proposed that alcohol may slow bone loss by downregulating the activity of osteoclasts and osteoblasts, thereby, decreasing the rate of bone (re)modeling. More research is needed to fully understand the direct and indirect mechanisms by which alcohol interacts with bone health.

Caffeine may also negatively affect bone health by competitive inhibition of adenosine binding to the A_{2a} and A_{2b} adenosine receptors on osteoblasts and osteoclasts (267). Theoretically, antagonism of the A_{2a} and A_{2b} receptors would uncouple the bone (re)modeling cycle by simultaneously stimulating bone resorption and inhibiting bone formation. Caffeine may also downregulate bone formation by decreasing osteoblast vitamin D receptor expression (267). However, the long-term effects of caffeine on osteoporosis and fracture risk are unclear. In a recent systematic review and meta-analysis of observational studies, coffee intake was inversely associated with the risk of osteoporosis and hip fracture (268). However, total fracture risk was not associated with coffee consumption. A recent literature review by Berman and colleagues (267), on the other hand, suggested limiting caffeine intake to 400mg per day based on increased fracture risk reported above that amount in longitudinal observational studies. Based

on these conflicting findings, more research is needed to understand the possible risks of caffeine intake on bone health.

Non-Modifiable	Notes
Age	• Gradual decline in bone mass with age and accelerated loss after menopause in women
Sex	• Lower bone mass in women
Race	• Higher bone mass and lower prevalence of osteoporosis in Black individuals
Genetics	
Modifiable	**Recommendations for Bone Health**
Nutrition	• Adequate intakes of calcium and vitamin D, in addition to total energy, protein, and vitamin K • Limit alcohol intake (≤ 1 drink·d^{-1} women; ≤ 2 drink·d^{-1} men)
Physical Activity	• Engage in activities that put mechanical stress on bones such as weight-bearing sports, progressive resistance training, and plyometrics • Three to five sets of 10-20 high-impact exercises (e.g., plyometrics, jumping) performed 4-7 days per week at an intensity of at least 2x body weight
Body Weight	• Weight management to avoid low body mass index and obesity
Smoking	• Smoking cessation
Chronic Illness	
Certain Medications	

Table 2. Non-modifiable and modifiable factors related to bone health. (150, 180, 243, 264)

Certain medications have been shown to interfere with normal bone metabolism. Glucocorticoids are steroid medications with anti-inflammatory and immunosuppressive actions used to treat conditions such as rheumatoid arthritis, Chron's disease, multiple sclerosis, and asthma, among other conditions (269). Glucocorticoids promote bone resorption by suppressing OPG, allowing for the upregulation of RANKL and osteoclast proliferation. Secretion of estrogen is also decreased by glucocorticoids which may result in menstrual irregularities and amenorrhea. Finally, glucocorticoids reduce calcium absorption in

the duodenum and increase urinary calcium excretion leading to hypocalcemia and increased PTH secretion (270). Certain antiseizure medications (ASM) are associated with an increased risk of osteoporosis, lower BMD, and higher rates of bone (re)modeling which may accelerate age-related bone loss (271). Enzyme-inducing ASM such as carbamazepine, phenobarbital, phenytoin, and primidone disrupt vitamin D status by upregulating 24-hydroxylase which converts 25(OH)D to inactive 24,25-dihydroxyvitamin D (272).

4. Energy Availability and Body Composition

4.1 Seasonal Changes in Body Composition

Desired body composition varies based on the individual athlete, position, competition level, and sport. For high-contact sports, greater amounts of total and lean body mass may be advantageous for strength and power (273, 274). Endurance athletes, on the other hand, may strive to maintain leaner figures for speed and stamina (275). However, there is no "ideal" body shape for an athlete and the efforts made to alter body composition should be monitored and evaluated by a sports nutrition professional (276). Nonetheless, body composition is reported to change throughout the athletic season in both male and female athletes. An observational study of 57 elite male (n=39) and female (n=18) athletes competing in five different sports reported increases in FFM and decreases in FM between the preparatory and competitive phases of the season (277). The duration for each season varied between 5 to 10 months, and the average FFM increased in the male athletes by 1.3 ± 1.8 kg during the season. These findings are in agreement with another study which found male athletes increased body mass (0.7 ± 2.5 kg), FFM (1.1 ± 1.7 kg), and BMC (0.047 ± 0.097 $g \cdot cm^{-1}$), and a decrease in body fat percentage (0.6 ± 1.6%) during an athletic season (278). Walker and colleagues (279) observed similar results in 46 professional Australian soccer players who showed an increase in lean mass and decrease in FM from pre-season to mid-season. Bone parameters may also change during the athletic season. In a study of Division II women's lacrosse

players (n=20), total body BMC and z-scores were higher during the championship season compared to the off-season and pre-season (280). Similar findings were reported by Milanese and colleagues (281) in a study of 29 male professional soccer players in Italy, which found pelvic BMD increased by 2.2% from July (preseason) to the following May (end of season). However, lower limb BMC decreased during the season by 0.9% and there were no changes observed in pelvic or lower limb BMD. Chronic patterns in BMD throughout ten consecutive seasons were documented in an elite male soccer player beginning at the age of 30 years, with DXA measurements performed at 26 time points (282). BMD in the preferred leg (right) was 4.7% higher than the non-dominant leg (left). However, no correlation was found between total body or site-specific BMD and age or time of season. These findings indicate participation in elite-level soccer may help preserve bone mass late into the fourth decade of life. However, it is uncertain if a similar pattern exists in lower competition levels and recreational athletes.

Unintended changes in body composition can result from an imbalance between EI and EEE related to shifts in training volume. However, athletes may also be motivated to alter their body composition to improve athletic performance (283). However, without proper nutrition education athletes may find it challenging to achieve their desired outcomes. College athletes report struggling to gain and maintain body mass during the athletic season for several reasons related to energy intake such as lack of time to prepare and eat meals, in addition to fear of overeating and gaining body fat (284). A survey study by Devlin and colleagues (285) found that body composition and nutrient intake reported by elite male soccer players were not affected by their self-reported body composition goals. Athletes may also elect to take supplements despite the risk of contamination, adulteration, and poor regulation in the United States (286). In a survey of 115 male and 88 female Division I athletes, men were more likely to use supplements related to weight/muscle gain including protein powders, androstenedione, dehydroepiandrosterone (DHEA), creatine, hydromethylbutyrate (HMB), and weight gainers compared to female athletes (287). Weight gainer supplement use was reported by 10.3% of male athletes in this study,

suggesting a modest prevalence of male athletes with desires to increase total body mass. More research is needed on the methods used by athletes to alter body composition and whether self-reported goals align with observed outcomes.

4.2 Seasonal Changes in Energy Availability

In addition to body composition, fluctuations in EA may occur throughout the athletic season based on training schedule and periodization. However, there is mixed evidence on how EA changes in response to a competitive season. In a study of 88 athletes involved in five different non-weight and weight-sensitive sports, 12.5% were found to have LEA (< 30 kcal·kg FFM^{-1}·day^{-1}) during the preparatory phase of the season. However, by the end of the season, EA was higher across all five sports and no athletes reported LEA (288). In contrast, EA was reported higher in the pre- and post-season in Division I female soccer players, and lowest in mid-season (289). Although there was no difference in EA between the pre- and post-season measurements, EI was lower in the post-season indicating a concurrent decrease in EEE at that time. In a systematic review of endurance athletes, significant energy deficits were reported during the preparation and competition phases of training (290). In male athletes, the preparatory deficit was -304 kcal·d^{-1} and worsened to -2177 kcal·d^{-1} during the competitive phase. However, when stratified by sport, male runners reported the opposite trend with higher relative EI during the competitive phase compared to the preparatory phase (43.8 ± 3.2 vs 38.3 ± 8.6 kcal·$kgBM^{-1}$·d^{-1}). Female athletes reported deficits of -1145 kcal·d^{-1} and -1252 kcal·d^{-1} in the preparatory and competitive phases, respectively. In conclusion, the variations in EA over the course of an athletic season may be influenced by several factors including the sport, competition level, and sex.

4.3 Low Energy Availability and Body Composition

Reduced EA may negatively impact body composition and impair musculoskeletal training adaptations. In National-level female gymnasts and runners, Deutz and colleagues (291) reported a positive correlation between the largest daily energy deficits and DXA-derived body fat. Furthermore,

anabolic adaptations can be blunted in response to energy deficiency. Areta and colleagues (101) reported a 27% decrease in resting myofibrillar protein synthesis (MPS) in 15 young men (n=8) and women (n=7) after five days of intentional restriction to 30 kcal·kg FFM^{-1}·day^{-1}. MPS was restored to baseline after a single bout of resistance training, and protein intake was shown to further enhance MPS. However, conflicting findings were reported by Oxfeldt and colleagues (292) in a recent randomized trial of young women (n=30) who underwent a 10-day resistance and aerobic exercise training program with either optimal EA (50 kcal·kg FFM^{-1}·day^{-1}; n=15) or LEA (25 kcal·kg FFM^{-1}·day^{-1}; n=15). Despite adequate protein availability (2.2 g·kg LBM^{-1}·day^{-1}) and the incorporation of eight resistance training sessions during the intervention, MPS and LBM were reduced in the LEA group. Small reductions were also reported in RMR (-65 kcal) and T_3 (-0.29 nmol·L^{-1}) in the LEA group but not in the women with optimal EA. These findings suggest additional resistance training and protein availability may be insufficient to offset the negative effects of LEA on muscle adaptations.

5. Athletic Performance and Low Energy Availability

There is mixed evidence regarding the effects of LEA on sports performance with studies showing positive, negative, and neutral effects (293). As previously described, short-term LEA has been shown to impair bone and muscle growth adaptations (12, 292), and there has been interest in investigating other performance consequences. In a randomized trial of trained male endurance athletes (n=12), 14 days of EA reduced by 25%, 50%, and 75% progressively impacted cognitive and physical performance (20). A 25% reduction in EA (22.4 ± 6.3 kcal·kg FFM^{-1}·day^{-1}) was associated with higher cognitive restraint, reduced explosive power, altered lactate metabolism, and worsened well-being. Power output was decreased when EA was further reduced by 50% (17.3 ± 5.0 kcal·kg FFM^{-1}·day^{-1}). Finally, a reduction in T_3 concentration and lower lactate values post-exercise were observed during the most restrictive 75% reduction in EA (8.82 ± 3.33 kcal·kg FFM^{-1}·day^{-1}). Despite the alterations in T_3, no changes were found in

RMR during any of the EA conditions. Decreased strength (bench press) was also reported in amateur female fitness competitors (n=27) after a dieting period characterized by a 22.9 ± 13.8% reduction in EI over 19.8 ± 3.6 weeks (294). Moreover, it could take several months for muscle strength to recover. Rossow and colleagues (295) documented a 12-month case study of a natural male bodybuilder in the six months leading up to and following competition. The preparation phase was characterized by a concurrent reduction in EI and increase in aerobic exercise, along with reductions in 1RM squat, bench press, and deadlift. Squat and deadlift 1RM returned to baseline by four months post-competition, but bench press 1RM remained below baseline at six months post-competition.

Impairments in sport-specific training have also been reported by Woods and colleagues (296) in an observational study of ten male (n=5) and female (n=5) elite rowers over a four-week progressive training program. Rowing performance was assessed by 5k time trials at the start and end of the training period. Despite a 21 ± 7% increase in training load over the four weeks, there was no change in EI observed. Boat velocity and stroke rate volume were lower during the final time trial compared to baseline indicating a decrease in rowing performance. Additionally, scores from the Multiplecomponent Training Distress Scale questionnaire indicated an increase in fatigue and mood disturbances by the end of the training cycle. Similarly, Ackerman and colleagues (297) reported psychological issues were 2.4 times more likely to occur in female athletes with LEA (n=473) compared to athletes with adequate EA (n=527). LEA athletes reported higher rates of impaired judgment (2.09% vs 8.46%), lack of coordination (14.04% vs 20.51%), difficulty concentrating (7.59% vs 14.16%), irritability (21.63% vs 30.66%), and feelings of depression (10.25% vs 20.72%).

Several possible mechanisms could contribute to the effects of LEA on aerobic and endurance performance. First, a reduction in overall EI is typically also accompanied by a reduction in carbohydrate availability. Burke and colleagues (298) demonstrate that five to six days of a low-carb high-fat diet (< 50 gCHO, 80% total kcal from fat) with moderate EA (39 ± 4 kcal·kg FFM^{-1}·day^{-1}) increased fat oxidation rates

at the expense of metabolic economy in elite male racewalkers. Relative VO_2 during walking economy testing was 5 to 8% higher during the low carbohydrate condition. Prolonged periods of LEA with concurrent exercise training can also reduce muscle glycogen due to low carbohydrate availability. Although fat is also utilized as fuel during endurance events, low or depleted muscle glycogen will reduce the amount of substrate available for energy production (299). Another potential explanation is decreased iron availability by way of reduced micronutrient intake or increased hepcidin activity. Iron is essential for oxygen transport, and reduced metabolic economy has been shown in women with iron deficiency compared to women with normal iron status (300). With oxygen unavailable, energy production is shifted to less efficient anaerobic pathways, given one molecule of glucose only produces 2 molecules of adenosine triphosphate (ATP) during anaerobic glycolysis compared to 32 ATP molecules produced during oxidative phosphorylation (301). It is possible that the decrease in efficiency could further exacerbate the current energy deficiency resulting in a continuous LEA cycle.

On the other hand, some studies have reported improved aerobic outcomes despite the presence of LEA. In a survey of female ultramarathon runners (n=306), LEAF-Q scores were inversely associated with race-finishing times (50). Additionally, Stenqvist and colleagues (92) reported improved VO_{2max} in trained male cyclists after four weeks of high-intensity exercise with no compensated increase in EI. Despite a 3% decrease in absolute and relative RMR, there was a positive training response in VO_{2max} and functional threshold power. A case study of an Olympic-level female runner also demonstrated how EA can be manipulated appropriately to alter body composition and improve performance (302). Throughout the athlete's 9-year career, EA was manipulated at several time points to create a modest energy deficit (300 $kcal \cdot d^{-1}$) resulting in slowly progressive weight loss followed by weight recovery after competition. Despite the slight reduction in EA, the athlete screened as low risk using the IOC REDs CAT screening tool during most of the training cycle and competition. Fat mass and body weight were found to be negatively correlated with 1,500-m race finish time, suggesting the small energy deficit did not negatively impact

racing performance. Additional dose-dependent studies in larger cohorts are needed to fully understand when LEA becomes harmful to athlete health and sports performance.

6. Summary

Maintaining optimal EA seems straightforward, but there are situations where recreational and competitive athletes may be unable or unwilling to meet the recommended guidelines. Problematic LEA is a concern due to the health and sports performance consequences, especially related to long-term bone health. Fluctuations in EA also occur throughout the year based on training periodization and competition schedule. Studies have reported that even a few days of severely restricted EA can impair endocrine function and bone (re)modeling by accelerating the rate of bone resorption and impairing bone formation. Engaging in high-impact exercise may attenuate the bone resorptive response to LEA, but this has not been investigated in athletes during concurrent endurance training. Therefore, it is crucial to investigate additional interventions to protect bone health during short-term training periods with LEA.

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CHAPTER III

High-Impact Exercise Does Not Protect Against Bone Resorption in Female Runners During Low Energy Availability

ABSTRACT

This randomized, crossover study assessed the short-term effects of high-impact jumping exercises on markers of bone (re)modeling in female runners under controlled conditions of low energy availability (LEA) and exercise. Participants completed two, five-day experimental conditions of LEA (15 kcal·kg FFM^{-1}·day^{-1}) in the follicular phase of the menstrual cycle with a washout period of one month between conditions. During the five days of LEA, participants either ran on the treadmill daily without additional exercise (RUN) or ran on the treadmill in addition to completing 50 high-impact jumping exercises each day (RUN+J). Fasted markers of bone (re)modeling and bone-regulatory hormones were measured on the mornings immediately before and after each intervention period. Thirteen recreational female runners (age: 26.2 ± 3.8 years, VO_{2max}: 45.0 ± 6.9 mL·kg^{-1}·min^{-1}) began at least one experimental condition, and ten completed both five-day conditions. Data are presented as mean ± standard deviation. Bone resorption marker CTX-I was increased over time in both LEA conditions (main effect of time: *P*=0.004; RUN: 0.31 ± 0.20 - 0.36 ± 0.15 ng·mL^{-1}; RUN+J: 0.38 ± 0.21 - 0.42 ± 0.19 ng·mL^{-1}) with no difference between conditions (time by condition interaction: *P*=0.714). There were no changes in biomarkers related to bone formation (PINP and sclerostin) over time or between conditions. In conclusion, changes in bone (re)modeling markers were similar following five days of LEA and daily running with and without high-impact jumping exercises. These results suggest jumping exercises are not an effective countermeasure for accelerated rates of resting bone resorption during LEA in female runners who continue to engage in daily running.

Key Words: nutrition, female, endurance, bone remodeling markers

INTRODUCTION

Relative Energy Deficiency in Sport (REDs) is a syndrome that encompasses the detrimental effects of problematic low energy availability (LEA) on athlete health and sports performance (1). Athletes experience LEA when energy intake (EI) is insufficient to support total energy needs after accounting for exercise energy expenditure (EEE). Female athletes are often categorized as "high risk" for LEA if energy availability is less than 30 kcal per kg fat-free mass per day (kcal·kg FFM^{-1}·day^{-1}) (2), there is evidence of endocrine or metabolic perturbations (3), or the athlete screens positively using a validated screening tool (e.g., Low Energy Availability in Females Questionnaire (LEAF-Q) score > 8 (4)). The prevalence of LEA amongst runners is typically higher in women compared to men (5, 6), with an estimated 19 to 45% of recreational female runners considered at high risk for LEA (7, 8).

The recommended treatment for LEA is to correct the energy deficiency by increasing EI, decreasing EEE, or a combination of the two (9). However, there are certain periods during a training cycle where short-term LEA may be desirable to manipulate body composition and improve sports performance (10). It has been suggested that short-term LEA poses minimal risk to long-term health if the body can adapt and recover quickly. However, disturbances in markers of endocrine function and bone (re)modeling have been observed in as little as three to five days of LEA of less than 20 kcal·kg FFM^{-1}·day^{-1} (11–13). Thus, interventions to protect bone health during short-term LEA or recovery from REDs are warranted.

Biomarkers of bone (re)modeling are useful when assessing the short-term effects of LEA on bone health because it can take several months for changes in bone density and architecture to occur. N-terminal propeptide of type I procollagen (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX-I) are byproducts of the bone (re)modeling cycle. PINP and CTX-I are the recommended standard reference markers to evaluate bone formation and resorption, respectively (14). Under normal physiologic conditions, the rate of bone formation and resorption are tightly coupled, which allows for

the continuous renewal of tissue and strengthening of the bone. However, exposure to LEA can uncouple the (re)modeling process in women as evidenced by decreased PINP and increased CTX-I (15). Engaging in daily high-impact jumping exercises during short-term LEA without additional exercise has been shown to have a protective effect on bone health by attenuating the rise in bone resorption of recreationally active women (11). However, no study to date has investigated the short-term effects of jumping exercises on bone (re)modeling markers in women with LEA achieved through a combination of dietary restriction and exercise. Therefore, the purpose of this study was to evaluate the short-term effects of high-impact jumping exercises on bone (re)modeling markers in female runners during a controlled period of LEA compared to a period of LEA without jumping. A secondary aim was to compare the responses of bone-regulatory hormones between conditions.

METHODS

Overview of Study Design

This study employed a randomized crossover design to investigate the effect of high-impact jumping exercises on bone (re)modeling in recreational and trained female runners during short-term LEA. A crossover design was chosen to account for inter-subject variability in LEA and bone marker response (13, 16). A computer-based random number generator for research (randomizer.org, Version 4.0) (17) was used to randomize the participants into the condition order. Participants completed two, 5-day experimental conditions (D1-5) separated by at least one menstrual cycle. EA of 15 kcal·kg FFM^{-1}·d^{-1} on D1-5 was achieved through dietary restriction (EI = 30 kcal·kg FFM^{-1}·d^{-1}) and exercise (EEE = 15 kcal·kg FFM^{-1}·d^{-1}) consisting of daily treadmill running at 65-70% VO_{2max} with jumping exercises (RUN+J) or without (RUN). Experimental conditions began during the follicular phase of the menstrual cycle one to seven days after the onset of menses (Figure 1).

Study eligibility and fitness level were assessed during two preliminary screening visits, followed by three days of baseline diet and exercise tracking. Participants notified the research team at the onset of menstruation, and the intervention began within the following seven days based on the participant and laboratory availability. A fasting blood sample was collected on the morning before the first day of the intervention period (D0) or on the morning of the first day (D1) before any food consumption or exercise. Participants then underwent the RUN or RUN+J interventions on D1-5, followed by a fasted blood sample collected on the morning of D6. Compliance with the nutrition and exercise intervention was evaluated daily during the intervention period and included the collection of food packaging and uneaten items, daily measurement of body mass, and physical activity data collected by a GPS smartwatch. This study was approved by the Virginia Tech Institutional Research Board (IRB #22-168) and participants provided written informed consent before participating.

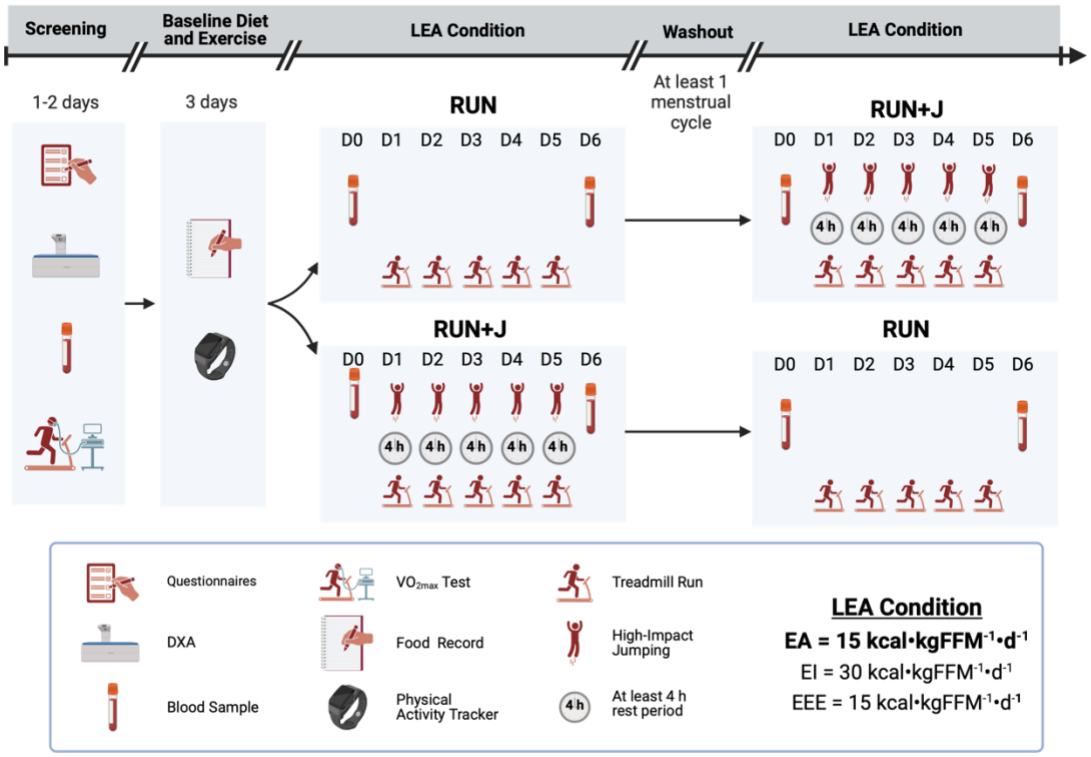

Figure 1. Overview of crossover study design. *Abbreviations: DXA, dual-energy x-ray absorptiometry; LEA, low energy availability; EI, energy intake; EEE, exercise energy expenditure. Figure created with BioRender.com*

Participants

Sixteen recreational female runners were recruited to participate in this study and fourteen were randomized into the first intervention period. Participants were recruited from the local area via flyers, social media, and digital communications. Eligible participants were naturally menstruating female runners (with self-reported normal menstrual cycles of 24 to 32 days) between 18-35 years of age with a body mass index (BMI) between 18.5-30 kg/m^2 and were non-smokers, not using hormonal contraceptives, not pregnant or lactating, and were free from bone injury within the previous 6 months. Individuals with low bone density (z-score < -2), low hemoglobin (< 12.0 $g \cdot dL^{-1}$), abnormal thyroid function (thyroid stimulating hormone < 0.4 $mIU \cdot L^{-1}$ or > 5.5 $mIU \cdot L^{-1}$) (18), and those who had recently recovered from an eating disorder within the last 12 months, or used medications that would affect study results (e.g., corticosteroids, anticonvulsants, gonadotropin-releasing hormone agonists) were excluded from study participation. Eligibility was confirmed using a standardized health history questionnaire, pregnancy test by urine, blood biochemistry, and body composition assessment by dual-energy x-ray absorptiometry (DXA) during the initial screening visit. All participants were considered low risk for existing LEA according to the Low Energy Availability in Females Questionnaire (LEAF-Q; score <8), which is a validated screening tool for LEA in female endurance athletes (4).

Screening

Participants reported to the laboratory for the initial screening visit in a post-absorptive state after a 10-hour overnight fast and were instructed to avoid exercise on the morning of their appointment. Figure 1 provides an overview of the screening procedures conducted. Standardized clothing was provided (t-shirt and shorts) and participants were instructed to remove all jewelry and void their bladders before the anthropometric and body composition measurements. Height was measured to the nearest 0.1 cm using a stadiometer (Welch Allyn Scale-Tronix 5002, Milwaukee, WI, USA) and body mass was measured

to the nearest 0.1 kg on a digital physician's scale (WB-110A NETP III, Tanita Corporation of America, Arlington Heights, Illinois, USA). Fat mass (FM), FFM, lean body mass (LBM), body fat percentage (BF%), and bone mineral density (BMD) were assessed using a fan-bean DXA (Lunar iDXA, enCORE Version 15, General Electric Healthcare, Madison, WI, USA). The DXA scanner was calibrated daily before the scanning sessions according to the manufacturer's instructions. Twelve of the thirteen DXA scans were performed and analyzed by the same licensed DXA technician (TS). Participants were in a supine position for all DXA scans, with hands in the mid-prone position and palms facing inward for the whole-body scan, and arms crossed over the chest and legs fully extended using the manufacturer's positioning aid for the lumbar spine and dual femur scans (19). A urine sample was collected to measure urine specific gravity (USG) using a handheld analog clinical refractometer (Fisherbrand, Thermo Fisher Scientific, USA) for analysis of hydration status. Euhydration was considered less than 1.020 $g \cdot mL^{-1}$ (20). A fasting blood sample was collected by venipuncture immediately following the DXA scans to assess concentrations of hemoglobin and thyroid stimulating hormone (TSH).

Maximal aerobic capacity (VO_{2max}) was determined using a standardized graded treadmill protocol (21) and indirect calorimetry (TrueOne 2400, Parvo Medics, Murray, UT, USA). The test began with a 5-min warm-up at 0% gradient and a speed predetermined by the participant. Treadmill gradient was increased to 2.5% for one minute, followed by a self-selected increase in "workload" of either 0.5 miles per hour (mph) or 2.5% gradient every minute thereafter until volitional exhaustion was reached. Rating of perceived exertion (RPE) was assessed during each stage using the Borg Scale (6-20). Oxygen uptake, respiratory exchange ratio (RER), and heart rate were assessed in 15-s increments. To be considered a valid measurement of VO_{2max}, participants had to achieve three of the four following criteria: 1) RER $\geq$ 1.10; 2) maximum heart rate within 10 beats of age-predicted maximum [208 – (0.7 x age)]; 3) evidence of a VO_2 plateau defined $\leq$ 1.5 $mL \cdot kg^{-1} \cdot min^{-1}$ change in VO_2 with increased workload; 4) final RPE $\geq$ 18.

Baseline Diet and Exercise

Habitual diet was assessed using food intake records collected on three consecutive days including two weekdays and one weekend day. Participants were provided with written and verbal instructions for recording the time, location, quantity, and brand names of foods, beverages, and dietary supplements consumed, in addition to a printed packet of standardized food diagrams for reference. Food records were analyzed by a registered dietitian (TS) using ESHA's Food Processor® Nutrient Analysis software (Version 11.9.14, ESHA Research, Salem, OR, USA). Typical exercise and physical activity were tracked on the same three days using a GPS smartwatch (Venu SQ, Garmin International, Olathe, KS, USA) which measured heart rate, step count, exercise duration, and estimated energy expenditure.

Experimental Conditions

Dietary Procedures

During the two experimental conditions, participants were provided controlled, weighed diets equal to 30 kcal·kg FFM^{-1} each day. Menus were designed by a registered dietitian using the nutrient analysis software, Nutrition Data System of Research (NDSR 2022, University of Minnesota, Minneapolis, MN, USA). Diets consisted of three meals and one pre-run snack using whole foods and commercial products and provided 55% of total energy from carbohydrates (CHO), 20% from protein, and 25% from fat. Two rotating menus were alternated daily (i.e., Menu A on D1, D3, and D5, and Menu B on D2 and D4) and contained animal-based sources of protein, including dairy (Appendix E). Alternative menus without meat products (including dairy) were available for vegetarian participants. Participants received meals each day for a 24-hour period in an insulated cooler to consume outside of laboratory visits. All food packaging and uneaten food items were returned and weighed to the nearest 0.5 g. Participants were instructed to consume meals at approximately similar times each day to avoid within-day fluctuations in energy balance (22) and to record the time of food consumption on a mealtime log.

Participants were instructed to consume the pre-run snack containing 30 g CHO one to two hours before running on the treadmill to standardize CHO availability during the exercise. Participants were instructed not to consume any other foods or beverages other than water and non-caloric beverages (e.g., black coffee, unsweetened tea). Consumption of beverages containing non-nutritive sweeteners (NNS) was permitted to increase adherence. A daily multivitamin (25 mcg of vitamin D3 as cholecalciferol, 160 mg of calcium as calcium carbonate, 18 mg of iron as ferrous fumarate; Nature Made, West Hills, CA, USA) was provided during the five days of energy restriction. Participants were instructed to take the multivitamin with a meal of their choice and record the time of consumption on the mealtime log. All prepared and returned food items were weighed to the nearest 0.5 g to estimate actual intake.

Running Procedures

On the first (D1) and last (D5) days of the experimental conditions, running economy (also known as "efficiency") was measured at the start of the prescribed exercise bout. Heart rate was measured during the test using a chest heart rate sensor (Polar H10, Polar Electro, Kempele, Finland). Participants ran at three moderately easy intensities for four minutes each at 0% gradient. Treadmill speed was set to 5.0, 5.5, and 6.0 mph or 5.5, 6 6, and 6.5 mph based on habitual running pace. The last two minutes of oxygen consumption and carbon dioxide production data were used to determine metabolic economy (mL oxygen consumed per kg body weight per minute relative to the set work performed) (23). Substrate utilization for each stage was calculated based on nonprotein RER values (24). Running economy was not assessed on D2-4.

On D1-5, participants completed supervised treadmill runs at 65-70% VO_{2max}. Running speed and duration were determined on D1 of the first experimental condition immediately following the economy test. VO_2 was continuously collected during a controlled titration run and speed was adjusted every four minutes until a steady-state VO_2 between 65-70% VO_{2max} was achieved in the third and fourth minutes.

The American College of Sports Medicine (ACSM) metabolic calculation (25) for running was used to initially predict running pace at 65% VO_{2max}: $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$) = 3.5 + (0.2 × S) + (0.9 × S × G), where S is speed in meters per minute ($m \cdot min^{-1}$) and G is gradient expressed as a decimal. Treadmill gradient was set to 1% during the supervised treadmill runs to reflect the slightly higher oxygen cost when running outdoors (26). Data from the titration run was used to determine the fixed running duration on D2-4. Using the stage determined to elicit as close to 65-70% VO_{2max} as possible, the last two minutes of absolute VO_2 ($L \cdot min^{-1}$) data were converted to Kcal using a thermal equivalent of oxygen for the nonprotein respiratory quotient (RQ) of 0.9 (5.078 $kcal \cdot L^{-1}$) (24). RQ of 0.9 was equal to the nonprotein food quotient of the provided control diet (67.5% CHO, 32.5% fat). Running duration on D1 and D5 was adjusted to include the energy expended during the running economy test (D1 and D5) and the titration run (D1). The same methodology was used where the average VO_2 from the last two minutes of each economy and titration stages were converted to Kcal (VO2 $L \cdot min^{-1}$ x 4.924 $kcal \cdot L^{-1}$ x 4 min) and summed together. Runs on D2-4, when there was no economy test performed, began with an optional warm-up run at 0% gradient at one of the three economy test speeds for five minutes. Daily run duration was adjusted to account for the energy expended during the economy test (D1 and D5), optional warm-up (D2-4), and jumping exercises (D1-5), as applicable, to elicit a daily EEE of 15 $kcal \cdot kg\ FFM^{-1}$. Participants were instructed to refrain from all physical activity outside of the supervised exercise sessions that were not related to activities of daily living (e.g., getting dressed, walking to the car).

Jumping Procedures

In the RUN+J condition, participants completed five sets of ten supervised depth jumps (50 total) from a height of 30.5 cm in the laboratory. A description of the jump movement is presented in Figure 2. Ground reaction force (GRF) was measured using NeuLog dual force plates (Eisco™ NeuLog™ Force Plate Logger Sensor NUL-225, Eisco Scientific, Victor, NY, USA) and target GRF was at least two times body mass

for each jump. A 60-second recovery period was taken between each set (27). Energy expenditure of the jumping exercise was determined on the first day of the RUN+J intervention using indirect calorimetry and assumed to remain consistent for the remainder of the intervention. Energy expended during the jumping exercises was accounted for in the daily EEE target and running duration was decreased as necessary to prevent EEE from exceeding 15 kcal·kg FFM^{-1}. Jumping exercises and the treadmill run were separated by at least four hours based on the amount of rest time required for optimal osteogenic response (28).

Figure 2. Depth Jump Procedure. *(A) Starting position was on top of a 30.5 cm box. (B) Participants stepped off the box and (C) landed evenly on both feet with knees bent to create momentum (D) for a fluid upward jumping movement and (E) final landing with knees slightly bent. This was repeated for five sets of 10 jumps with 60 sec rest between each set.*

Blood Biochemistry

Fasting blood samples were collected by venipuncture after an overnight, 10-hour fast. A 20 mL blood sample was collected on D0 and D6 to measure markers of bone (re)modeling (PINP, CTX-I, sclerostin) parathyroid hormone (PTH), reproductive hormones (progesterone and estradiol), thyroid hormones (free triiodothyronine [fT_3], free thyroxine [fT_4], and reverse T_3 [rT_3]), markers of iron status (serum iron, ferritin, total iron binding capacity [TIBC], iron saturation), insulin, insulin-like growth factor (IGF-1), cortisol, and leptin. Serum specimens were allowed to clot at room temperature for at least 15 minutes but no longer than 2 hours before centrifugation. Plasma specimens were kept on ice before centrifugation. Specimens were centrifuged at 3500 rpm for 13 minutes at 4°C (Centrifuge 5804R 15amp, Eppendorf, Enfield, CT, USA). CBC, TSH, PTH, ferritin, TIBC, iron, cortisol, and insulin were analyzed the same day at a commercial lab (LabCorp Burlington, Burlington, NC, USA). Aliquots of plasma and serum were stored at -80°C until analysis at the Virginia Tech Metabolism Core (Virginia Tech, Blacksburg, Virginia, USA). Single kit enzyme-linked immunosorbent assay (ELISA) was used to measure plasma PINP (MyBioSource, San Diego, California, USA, intra-assay coefficient of variation (CV) ≤ 5.07%) and rT_3 (Alpco, Salem, NH, CV not provided), and serum CTX-I (EuroImmun, Mountain Lakes, NJ, CV ≤ 5.29%), sclerostin (Thermo Fisher Scientific, Carlsbad, CA, CV ≤ 9.53%), progesterone (Abcam, Cambridge, MA, CV ≤ 11.4%), estradiol (Abcam, Cambridge, MA, CV ≤ 8.7%), fT_3 (Alpco, Salem, NH, CV not provided), fT_4 (Alpco, Salem, NH, CV not provided), IGF-1 (Alpco, Salem, NH, CV ≤ 5.83%), leptin (Alpco, Salem, NH, CV not provided).

Statistical Analysis

Statistical analyses were conducted in IBM® SPSS® Statistics software (Version 28.0.2.2 (14), IBM Corporation, Armonk, NY, USA) and graphs were generated in Graphpad Prism (version 10, GraphPad Software, San Diego, CA, USA). Shapiro-Wilk test was used to check the data for normality. Data not normally distributed was log-transformed before analysis. Paired t-test was used to compare differences

in total body mass and biochemistry at D0 of RUN and RUN+J. A linear mixed model was performed to assess outcomes for the impact of condition (RUN and RUN+J), time (D0 vs. D6), and condition by time interaction, with condition and time as fixed effects and participants as a random effect. Post hoc Bonferroni-adjusted paired t-tests or Wilcoxon-rank sum tests were conducted when the main effects were statistically significant. The magnitude of change was determined by Cohen's *d* effect size (small ≥ 0.20, medium ≥ 0.50, large ≥ 0.80) (29). Data are summarized as mean ± 1 standard deviation, and the level of significance was set at $P<0.05$.

RESULTS

Participant characteristics at baseline are shown in Table 1. Fourteen participants started at least one experimental condition, thirteen completed at least one condition, and ten participants completed both conditions. At baseline, seven participants were classified as Tier 1 athletes (Recreationally Active) and seven were Tier 2 (Trained/Developmental) according to the participant classification framework proposed by McKay and colleagues (30). Seven participants were allocated to start with the RUN condition and seven started in RUN+J (Figure 3). However, one participant that started in the RUN condition was excluded from data analysis due to noncompliance with the exercise protocol, resulting in 13 participants for the final analysis.

Characteristics	**Mean ± SD (range)**
Age (yr)	26.2 ± 3.8 (20.8 - 31.4)
Height (cm)	169.7 ± 5.3 (158.1 – 177.8)
Total Body Mass (kg)	66.8 ± 8.7 (48.8 - 80.1)
BMI (kg/m^2)	23.3 ± 2.7 (19.7 – 29.9)
Body Fat (%)	28.6 ± 5.2 (21.4 - 38.8)
Fat Mass (kg)	18.6 ± 5.5 (11.2 - 28.4)
Lean Mass (kg)	45.4 ± 4.2 (35.5 - 50.1)
Fat-Free Mass (kg)	48.1 ± 4.5 (37.6 - 53.0)
BMC (g)	2688.7 ± 315.4 (2096.0 - 3197.0)
Lumbar BMD (g/cm^2)	1.287 ± 0.187 (0.950 - 1.590)

Dual Femur BMD (g/cm^2)	1.096 ± 0.123 (0.970 - 1.350)
VO_{2max} (mL·kg^{-1}·min^{-1})	45.0 ± 6.9 (37.0 - 55.6)
LEAF-Q score	3.7 ± 2.1 (1-7)
Hemoglobin (g·dL^{-1})	13.1 ± 0.6 (12.1-14.2)
TSH (µIU·mL^{-1})	1.78 ± 0.22 (0.97-3.32)

***Table 1. Baseline characteristics (n=13)**. Abbreviations: BMI, body mass index; BMC, bone mineral content; BMD, bone mineral density; LEAF-Q, Low Energy Availability in Females Questionnaire; TSH, thyroid stimulating hormone*

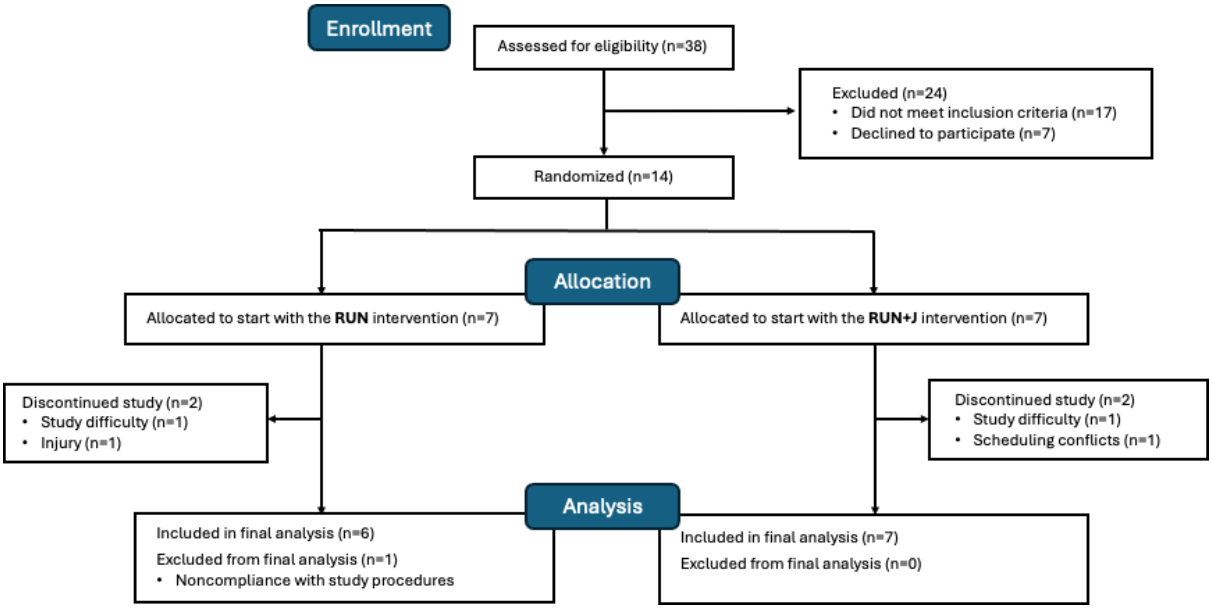

Figure 3. Enrollment flow diagram

Baseline dietary intake compared to the diets provided in the LEA conditions is presented in Table 2. As designed, EI and EA were lower in the LEA diets compared to baseline (both P < 0.001). The relative amount of CHO (P = 0.040), protein (P = 0.002), and fat (P < 0.001) were also lower during the LEA conditions. Habitual macronutrient distribution was 45.8 ± 7.7% total energy from CHO, 18.0 ± 3.7% from protein, and 35.8 ± 4.4% from fat compared to the LEA conditions of 55% CHO, 20% protein, and 25% fat. The provided calcium amount was not different during the intervention periods compared to baseline intake (P = 0.755). After correction for returned food waste, actual EI during RUN and RUN+J were 1388 ± 150 and 1383 ± 131 kcal, respectively. These intakes were not different from the planned EI of 1447 kcal (RUN, *P*=0.244; RUN+J, *P*=0.120).

Variable	Baseline	LEA Conditions	P-value
Energy Intake			
Absolute (kcal)	2108 ± 435	1447 ± 138	< 0.001
Relative (kcal·kgBM^{-1})	32.3 ± 8.5	21.8 ± 1.5	< 0.001
Energy Availability# (kcal·kg FFM^{-1}·day^{-1})	35.7 ± 9.6	15.0 ± 0.0	< 0.001
Carbohydrate			
Absolute (g)	245 ± 78	199 ± 19	0.052
Relative (g·kgBM^{-1})	3.8 ± 1.3	3.0 ± 0.2	0.040
Protein			
Absolute (g)	93 ± 17	72 ± 7	0.001
Relative (g·kgBM^{-1})	1.4 ± 0.3	1.1 ± 0.1	0.002
Fat			
Absolute (g)	84 ± 18	40 ± 4	< 0.001
Relative (% total kcal)	35.8 ± 4.4	25.0 ± 0.0	< 0.001
Calcium (mg)	838.8 ± 310.1	867.4 ± 79.7	0.755
Vitamin D (mcg)	3.7 ± 5.8	25.2 ± 0.2	< 0.001
Iron (mg)	14.4 ± 5.3	32.2 ± 1.3	< 0.001

Table 2. Baseline dietary intake compared to planned intakes during the LEA conditions. *Data reported as mean ± SD. Abbreviations: BM, body mass; FFM, fat-free mass*
#LEA calculated as (EI-EEE)·FFM^{-1}

D0 of the RUN and RUN+J conditions began 3 ± 2 days after the onset of menstruation and did not differ between conditions (*P*=0.460). There were also no differences in concentrations of estradiol (*P*=0.120), progesterone (*P*=0.541), or TBM (*P*=0.599) on D0 between conditions. Reductions in TBM during RUN and RUN+J were 1.3 ± 0.7 and 1.4 ± 0.7 kg, respectively, and did not differ between conditions (*P*=0.651).

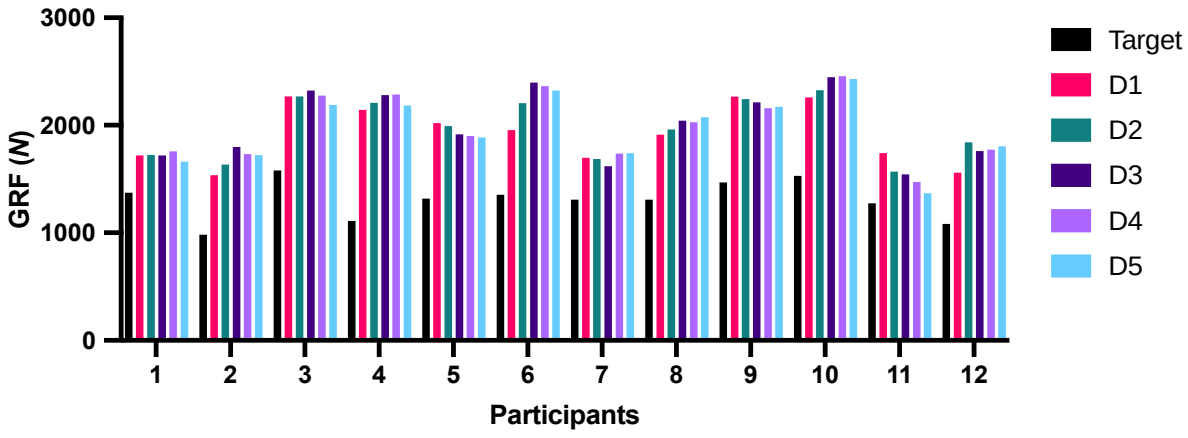

Figure 4. Daily ground reaction force (GRF) for depth jumps. *Individualized GRF target is represented by the black bars and the measured daily average GRF in the colored bars.*

Jumping Exercises

Jumping data are presented in Figure 4. Twelve participants completed the RUN+J intervention with a 100% completion rate for the daily jumping exercises. Average GRF for all five days was 1972 ± 288 newtons (*N*), which was equivalent to a GRF of 3.0 ± 0.4 times TBM. GRF for all participants exceeded the target minimum GRF of two times TBM on all five days.

Markers of Bone (re)Modeling

Bone (re)modeling markers are summarized in Table 3. There were no significant main effects of time, condition, or time by condition interaction for PINP. Baseline concentration of CTX-I at D0 was higher in the RUN+J condition compared to D0 of the RUN condition (*P*=0.009). Concentration of CTX-I was increased from D0 to D6 (main effect of time, *P*=0.004) with no interaction effect between conditions (*P*=0.714). There was a nonsignificant trend for decreased sclerostin over time (*P*=0.074) with no main effects of condition (*P*=0.911) or time by condition interaction (P=0.598).

	RUN		**RUN+J**				
	D0 (n=11)	**D6** (n=10)	**D0** (n=12)	**D6** (n=12)	**Time P-value**	**Condition P-value**	**Interaction P-value**
PINP (ng·mL^{-1})	75.6 ± 11.0	74.2 ± 11.9	74.1 ± 8.3	71.0 ± 11.9	0.426	0.457	0.246
CTX-I (ng·mL^{-1})	0.31 ± 0.20	0.36 ± 0.15	**0.38 ± 0.21***	0.42 ± 0.19	**0.004**	**<0.001**	0.714
Sclerostin (pg·mL^{-1})	161.3 ± 55.2	141.2 ± 28.1	149.9 ± 39.2	139.7 ± 30.9	0.074	0.911	0.598

Table 3. Concentrations of bone (re)modeling markers in RUN and RUN+J before (D0) and after (D6) each intervention period. *Data reported as mean ± SD.*
**significant from D0 in RUN*

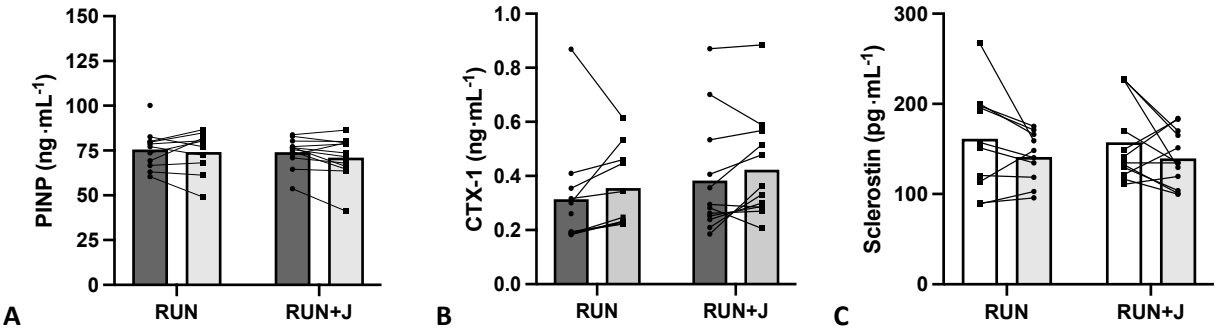

Figure 5. Individual changes in PINP, CTX-I, and sclerostin from D0 to D6 in RUN and RUN+J conditions. *Data presented as mean (bars) and individual changes (lines).*

LEA and Bone Regulatory Hormones

Endocrine markers of bone regulation and growth hormone concentrations at D0 and D6 in RUN and RUN+J are summarized in Table 4. Leptin was not normally distributed and was therefore log-transformed prior to analysis. Data summarized in Table 4 represents mean values before transformation.

All hormone concentrations at D0 were similar between conditions (*P*>0.05) except for fT_4 which was lower at D0 of RUN+J compared to RUN (*P*=0.036). Concentrations of fT_3, IGF-1, leptin, and insulin decreased from D0 to D6 (main effect of time), but no significant interaction effect was shown. There was a nonsignificant trend for a main interaction effect for fT_3 (P=0.161). Concentrations of fT_3 were 27% lower on D6 compared to D0 in RUN (*P*=0.022; *d*=0.87) but no change in fT_3 concentration was found following RUN+J (P=0.237, *d*=0.36) in the post hoc analysis.

	RUN		**RUN+J**				
Variable	**D0** (n=11)	**D6** (n=10)	**D0** (n=12)	**D6** (n=12)	**Time P-value**	**Condition P-value**	**Interaction P-value**
PTH ($pg \cdot mL^{-1}$)	31.6 ± 10.8	31.7 ± 11.1	31.9 ± 10.5	35.3 ± 10.7	0.484	0.585	0.309
Free T_3 ($pg \cdot mL^{-1}$)	3.1 ± 1.6	**2.6 ± 0.9[a]**	2.4 ± 1.0	2.1 ± 1.1	**0.010**	**0.041**	0.161
Free T_4 ($pg \cdot mL^{-1}$)	1.5 ± 0.3	1.4 ± 0.1	**1.4 ± 0.1[b]**	1.3 ± 0.1	0.066	**0.011**	0.637
rT_3 ($ng \cdot mL^{-1}$)	0.57 ± 0.05	0.59 ± 0.03	0.57 ± 0.04	0.59 ± 0.03	**0.014**	0.950	0.574
IGF-1 ($ng \cdot mL^{-1}$)	87.9 ± 5.9	**80.3 ± 6.4[a]**	91.3 ± 10.5	**79.8 ± 7.5[a]**	**<0.001**	0.225	0.308
Leptin ($ng \cdot mL^{-1}$)	8.5 ± 3.5	**4.5 ± 2.3[a]**	10.1 ± 4.9	**5.2 ± 4.5[a]**	**<0.001**	0.737	0.354
Insulin ($\mu IU \cdot mL^{-1}$)	5.6 ± 2.8	4.0 ± 2.4	5.6 ± 2.2	3.8 ± 1.6	**0.013**	0.808	0.942
Cortisol ($\mu g \cdot dL^{-1}$)	17.4 ± 5.1	18.4 ± 4.0	17.6 ± 3.3	17.4 ± 4.8	0.689	0.818	0.549
Estradiol ($pg \cdot mL^{-1}$)	68.1 ± 18.9	70.8 ± 22.1	73.7 ± 21.8	71.9 ± 21.6	0.889	0.359	0.267

Table 4. Hormone concentrations in RUN and RUN+J before (D0) and after (D6) each intervention period*. Data reported as mean ± SD. Abbreviations: PTH, parathyroid hormone; T_3, triiodothyronine; T_4, thyroxine; IGF-1, insulin-like growth factor-1*
[a]significant from D0 in the same condition
[b]significant from D0 in RUN

Iron Profile

Serum iron and iron saturation were not normally distributed and therefore log-transformed prior to analysis. Data summarized in Table 5 represents mean values before transformation. D0 concentrations for all iron markers were similar between RUN and RUN+J (*P*>0.05). Concentrations of ferritin were higher at D6 compared to D0, with no changes in serum iron, TIBC, or iron saturation. Concentrations of ferritin

increased by 36% under the RUN condition (*P*=0.012, *d*=0.990) but the change was not statistically significant following RUN+J (+8%, *P*=0.0.236, *d*=0.362).

	RUN		RUN+J				
Variable *(normal values#)*	**D0** (n=11)	**D6** (n=10)	**D0** (n=12)	**D6** (n=12)	**Time P-value**	**Condition P-value**	**Interaction P-value**
Ferritin (ng·mL^{-1}) *(15-150)*	29.8 ± 27.8	**43.2 ± 35.0[a]**	38.8 ± 24.4	42.9 ± 24.4	**0.012**	0.839	0.207
TIBC (µg·dL^{-1}) *(250-450)*	352.0 ± 42.3	354.2 ± 40.2	358.1 ± 47.4	350.4 ± 36.7	0.446	0.413	0.616
Serum Iron (µg·dL^{-1}) *(27-159)*	67.0 ± 36.7	88.5 ± 99.5	90.9 ± 48.4	63.4 ± 22.9	0.444	0.495	0.167
Iron Saturation (%) *(15-55)*	19.4 ± 10.9	19.2 ± 10.4	25.3 ± 12.4	18.4 ± 7.0	0.230	0.297	0.264

Table 5. Markers of iron status in RUN and RUN+J before (D0) and after (D6) each intervention period.
Data reported as mean ± SD. Abbreviations: TIBC, total iron binding capacity
[a]=significantly different from D0 of the same condition
#Normal values provided by LabCorp at time of analysis.

Running Economy

Six participants selected to complete the economy test at 5.0, 5.5, and 6.0 mph and seven participants selected 5.5, 6.0, and 6.5 mph. Selected paces were equivalent to 61.3 ± 5.9, 67.6 ± 7.4, and 73.6 ± 8.4% relative VO_{2max} at stages 1, 2, and 3, respectively. Absolute VO_2 increased by approximately 10% (+0.18 L·min^{-1}) between each stage in both conditions at D1 and D5. No main effects of time, condition, or time x condition interaction were shown for VO_2 at any stage (Table 6). RER decreased by 7% between the first two stages and by less than 2% between stages 2 and 3 in both conditions at D1 and D5. RER was lower at D5 compared to D1 at all three stages with no significant main effects of condition (*P*>0.05 all stages) or interaction (*P*>0.05 all stages). Substrate utilization shifted in favor of fat oxidation at all three stages at D5 compared to D1 in both conditions. CHO oxidation (g·min^{-1}) decreased at stages 1, 2, and 3 by 44%, 30%, and 25%, respectively, while fat oxidation (g·min^{-1}) increased by 47%, 79%, and 111%, respectively.

	RUN		RUN+J			
	D1 (n=11)	**D5** (n=10)	**D1** (n=12)	**D5** (n=12)	**Time (P)**	**Interaction (P)**
Stage 1						
RER	0.87 ± 0.04	0.81 ± 0.03	0.87 ± 0.03	0.80 ± 0.03	**<0.001**	0.766
CHO ($g \cdot min^{-1}$)	1.19 ± 0.32	0.68 ± 0.21	1.18 ± 0.32	0.65 ± 0.26	**<0.001**	0.872
Fat ($g \cdot min^{-1}$)	0.41 ± 0.16	0.60 ± 0.11	0.42 ± 0.12	0.62 ± 0.13	**<0.001**	0.861
VO_2 ($L \cdot min^{-1}$)	1.79 ± 0.25	1.76 ± 0.23	1.80 ± 0.29	1.77 ± 0.32	0.385	0.982
Stage 2						
RER	0.93 ± 0.02	0.87 ± 0.02	0.93 ± 0.03	0.86 ± 0.04	**<0.001**	0.937
CHO ($g \cdot min^{-1}$)	1.80 ± 0.34	1.29 ± 0.30	1.81 ± 0.35	1.24 ± 0.46	**<0.001**	0.709
Fat ($g \cdot min^{-1}$)	0.25 ± 0.09	0.45 ± 0.09	0.27 ± 0.11	0.47 ± 0.16	**<0.001**	0.864
VO_2 ($L \cdot min^{-1}$)	1.97 ± 0.28	1.96 ± 0.25	2.00 ± 0.32	1.96 ± 0.33	0.501	0.789
Stage 3						
RER	0.95 ± 0.03	0.88 ± 0.04	0.94 ± 0.03	0.88 ± 0.05	**<0.001**	0.775
CHO ($g \cdot min^{-1}$)	2.17 ± 0.47	1.57 ± 0.45	2.11 ± 0.52	1.54 ± 0.58	**<0.001**	0.831
Fat ($g \cdot min^{-1}$)	0.19 ± 0.11	0.42 ± 0.14	0.22 ± 0.14	0.45 ± 0.19	**<0.001**	0.888
VO_2 ($L \cdot min^{-1}$)	2.15 ± 0.29	2.13 ± 0.26	2.16 ± 0.33	2.15 ± 0.32	0.617	0.862

Table 6. Comparison of substrate utilization and oxygen consumption during a three-stage metabolic economy test from D1 to D5. *Data reported as mean ± SD. Abbreviations: RER, respiratory exchange ratio; CHO, carbohydrate; VO_2, oxygen consumption.*

DISCUSSION

It has been speculated that the addition of high-impact exercise during short-term periods of LEA could be a potential countermeasure for the negative impact of LEA on bone health. Previous studies have examined bone metabolic response to LEA without exercise, LEA with running, and LEA with high-impact jumping. This is the first study to investigate the combined effects of high-impact exercise and running on bone (re)modeling markers in female recreational runners under controlled conditions of LEA. Using a crossover design, we found no additional benefit of the jumping exercises on PINP, CTX-I, or sclerostin following five days of LEA and running compared to LEA with running alone.

Neither LEA condition altered PINP, suggesting the suppression of bone formation typically observed during LEA (15) is either mitigated or masked by weight-bearing exercise. These results are in line with a similar study of active women that reported no change in PINP following three days of LEA (15 $kcal \cdot kg\ FFM^{-1} \cdot day^{-1}$) with daily running (13). However, observed changes in PINP may vary based on the form and/or duration of exercise. For example, completing 20 high-impact jumping exercises twice a day

was not shown to prevent reductions in PINP following three days of LEA (15 kcal·kg FFM^{-1}·day^{-1}) in active women (11). In the present study, participants ran continuously on the treadmill each day for 65 to 82 min (70.8 ± 8.9 min) covering approximately 10 km per session, which is a common training distance for recreational runners (31).

Contrary to our hypothesis, jumping exercises did not prevent the increase in bone resorption during LEA. Our finding of increased CTX-I following both LEA conditions is in agreement with previous research, which reported that five days of LEA (15 kcal·kg FFM^{-1}·day^{-1}) with daily running increased bone resorption (β-CTX AUC) by 19% in active women (15). Ihle and Loucks (12) also reported increased rates of bone resorption (urinary amino-terminal cross-linked telopeptide of type I collagen; NTX) in sedentary women following five days of severely restricted LEA (10 kcal·kg FFM^{-1}·day^{-1}) and uphill walking. Conversely, other studies have reported no change in β-CTX following three days of LEA (15 kcal·kg FFM^{-1}·day^{-1}) with running (13) or jumping exercises (11) in similar populations. The discrepancy in these results may be partially attributed to the differences in study duration. Although CTX responds acutely to stimuli such as food intake and exercise (32), it may take several days to detect changes in resting concentrations of active individuals. This could partially explain why CTX-I was shown to increase in the current study but not in similar three-day investigations.

Neither of the LEA conditions had a significant effect on sclerostin concentrations. Sclerostin is a protein secreted by osteocytes that regulates the synthesis of osteoblasts by inhibiting the Wnt/β-catenin pathway (33), thereby reducing bone formation. Sclerostin has been shown to increase in response to LEA (15 kcal·kg FFM^{-1}·d^{-1}) without exercise (34) but not following the same level of LEA with moderate-intensity running (15). These results suggest LEA without impact exercise may be at higher risk for suppressed bone formation due to the increase in sclerostin compared to LEA with exercise.

Reduced concentrations of fT_3, IGF-1, leptin, and insulin, and increased rT3 concentration following the LEA conditions reflect similar changes reported in short-term LEA experimental trials (13, 15, 35, 36).

These data indicate participants were adherent to the dietary protocol and in a state of energy deficiency. The additional jumping exercises did not affect the response of IGF-1, leptin, insulin, or rT_3 to the LEA exposure. However, concentrations of fT_3 were only significantly decreased following the RUN condition. Previous studies have reported similar findings of nonsignificant changes in total T_3 in exercising women following three days of LEA (15 kcal·kg FFM^{-1}·d^{-1}) with either running (13) or jumping exercises (11) but the variability in T_3 response to LEA with impact exercise is not well understood.

Estradiol regulates bone resorption by promoting the expression of osteoprotegerin, a decoy receptor for receptor activator of nuclear factor-kappa β ligand (RANKL), and downregulating osteoclast activity (37). Bone loss associated with estrogen deficiency has been shown in young amenorrheic endurance athletes (38) and postmenopausal elite runners not on hormone replacement therapy (39). In the present study, no changes in resting estradiol concentration were shown following either condition, which is in agreement with previous studies at the same level of EA (11, 13, 15). In contrast, 24-h mean estradiol concentration was shown to decrease by 15% after five days of LEA equal to 10 kcal·kg FFM^{-1}·d^{-1} (40). It is possible that estradiol concentrations are not influenced until EA is below 15 kcal·kg FFM^{-1}·d^{-1}, which may partially explain why the present study and previous investigations have not detected changes in estradiol concentration.

The increase in ferritin independent of the other iron markers indicates an acute stress response to LEA. Resting concentration of hepcidin, the master regulatory hormone of iron metabolism, has been shown to increase in male distance runners following three days of LEA (20 kcal·kg FFM^{-1}·d^{-1}) with daily running for 75 min at 70% VO_{2max} (41). Hepcidin regulates iron availability by inactivating ferroportin, the iron export channel on macrophages, hepatocytes, and enterocytes (42). Downregulating ferroportin sequesters iron resulting in elevated concentrations of ferritin. Future investigations of LEA in female athletes that include hepcidin measurements are warranted.

Suppressed resting energy expenditure is a common surrogate marker for LEA (43) that is reflective of a metabolic adaptation to conserve energy. However, data are lacking on whether adaptations in EEE also occur during LEA. In the present study, RER was lower on D5 compared to D1 at all stages of the running economy test, suggesting a shift in substrate utilization in favor of fat oxidation. Despite the increased rate of fat oxidation, there were no changes in VO_2 at any stage on D5 compared to D1. These findings suggest an adaptive exercise response to short-term LEA by improving fat oxidative capacity during submaximal running without increasing the energetic cost. However, our analysis was limited to the last two minutes in each stage of the economy test and serial measurements of respiratory gases were not collected during the long-duration treadmill runs. Future studies should investigate changes in substrate utilization and metabolic adaptations during endurance exercise to better understand how LEA affects EEE.

This study was limited by the small sample size and unbalanced number of participants who completed the RUN (n=10) and RUN+J (n=12) conditions. Additionally, jumping exercises during the RUN+J condition were unidirectional and only targeted impact on the lower limbs. A greater osteogenic response may have been stimulated by exercises targeting the trunk or upper body because running already provides a high-impact stimulus to the lower limbs. Measurements of PINP and CTX-I reflect the synthesis and degradation of type 1 collagen, which is not specific only to bone tissue (44). It is not possible to know if the changes in PINP and CTX-I are solely reflective of bone (re)modeling or the site-specific region where the (re)modeling occurred. It is possible short duration LEA studies, such as the present one, do not find changes in PINP because bone resorption proceeds bone formation in the remodeling cycle. Follow-up blood samples were only collected on the morning of D6 and may have missed delayed changes that would influence the interpretation of our findings. Furthermore, these findings are not generalizable to athletes of different racial or ethnic backgrounds (45), elite athletes, older female athletes who are at risk for age-related bone loss, or men.

In conclusion, our results indicate that the combination of high-impact jumping exercises with daily training does not provide additional bone-protective benefits in female runners during LEA compared to running alone. Further research is needed on the relationship between acute changes in bone (re)modeling markers and long-term outcomes in bone health to better understand the risk associated with short-term energy restriction.

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CHAPTER IV

Seasonal Stability of Body Composition and Dietary Intake in Division I Male Soccer Players

ABSTRACT

This longitudinal, observational study assessed body composition and energy availability (EA) of Division I male soccer players at the start and end of the Spring non-championship season using dual-energy x-ray absorptiometry (DXA), three-day diet records to assess energy intake (EI), and exercise logs to estimate exercise energy expenditure (EEE). EA was calculated by subtracting EEE from EI and dividing by fat free mass (FFM). Measurements of aerobic fitness, strength, wellness, and self-reported body composition goals were also assessed. Twenty-three athletes (19.7 $\pm$ 1.1 years, 177.6 $\pm$ 6.3 cm, 76.0 $\pm$ 6.1 kg) completed the initial testing visit and 15 completed the follow-up visit at the end of the season. At the start of the season, athletes reported consuming 2748 $\pm$ 683 kcal·day^{-1} with an EA of 33.9 $\pm$ 10.7 kcal·kg FFM^{-1}·day^{-1}. No changes in body composition, EA, or macronutrient intakes were found at the end of the season compared to the start, despite over half of athletes reporting they wanted to increase total and/or lean body mass during the season. There were no differences in body composition or fitness outcomes between athletes who decreased EA (n=6) during the season compared to those who increased EA (n=6). These results indicate body composition and EA of Division I male soccer players remain relatively stable during the non-championship season, and additional nutrition support may be necessary to help athletes gain total and lean body mass.

Key Words: nutrition, energy intake, athlete, soccer, DXA

INTRODUCTION

Soccer is an endurance-based field sport characterized by continuous movement with brief high-intensity efforts (1). Along with technical skills, soccer athletes are expected to maintain a high level of fitness that is influenced by training, nutrition, and lifestyle factors (e.g., sleep, stress management) (2, 3). Body composition may also play a role, with increased total body mass (TBM) and body fat negatively affecting sports performance in male soccer players (4). However, body composition is reported to change throughout an athletic season in response to training, in addition to intentional efforts to enhance athletic performance or achieve a certain body image (5, 6).

It is recommended that attempts to change body composition should occur during a non-competitive phase of training (7). For National Collegiate Athletic Association (NCAA) male soccer players, the preferred time to alter body composition is during the off-season or possibly the Spring season. In the United States, the NCAA soccer season is divided into a championship season in the Fall and a non-championship season in the Spring. During the Fall season, athletes compete in at least 20 matches and are permitted to train 20 hours per week (up to 4 $hr \cdot day^{-1}$) with a mandatory day off (8). In the Spring, however, teams are only permitted to play exhibition matches on five days of the season, and two days off are required each week. Contact between coaches and players is also limited to eight hours per week during the initial three to four weeks of the Spring season and 15 hours per week during the remaining 45 days. Additionally, team training goals are typically focused on gaining muscle strength and hypertrophy, as opposed to conditioning and field play in the Fall. Silvester and colleagues (9) reported increases in TBM and lean tissue pre- to postseason in the Fall but it is unknown if similar changes occur during the Spring.

In addition to training, changes in body composition are affected by dietary intake. An imbalance between energy intake (EI) and daily energy requirements can result in a state of reduced or low energy availability (LEA). Energy availability (EA) describes the amount of energy that can be used to support acts

of daily living and normal bodily function after accounting for exercise energy expenditure (EEE). EA is calculated by subtracting EEE from dietary EI and is commonly reported relative to fat-free mass (FFM) as kcal·kg FFM^{-1}·day^{-1} (EA = [EI – EEE] / FFM) (4). Optimal EA (~45 kcal·kg FFM^{-1}·day^{-1}) is necessary for weight maintenance; however, athletes may intentionally restrict EI or increase EEE to achieve LEA for TBM and/or fat loss (10). There is no agreed-upon threshold value for problematic LEA in men, however, a recent randomized trial suggested health and performance impairments occur somewhere between 9 and 25 kcal·kg FFM^{-1}·day^{-1} in male endurance athletes (11). Although short periods of reduced EA may not cause harm to an athlete (12), chronic or severe LEA may become problematic and impair sports performance (10). Problematic LEA is associated with health consequences such as mood and hormone disturbances, decreased bone mineral density (BMD), and a weakened immune system. Athletes with problematic LEA may also experience impaired training response and difficulty achieving body composition goals. Additionally, LEA is not always intentional. Some athletes may lack the proper nutrition knowledge, resources, or environment necessary to achieve optimal EA. More research is needed on how EA changes in response to a competitive season and its relationship to changes in body composition.

Therefore, the primary purposes of this study were to profile body composition and EA of Division I collegiate male soccer players at the start of a Spring athletic season and evaluate longitudinal changes in body composition and sports performance in relation to EA. The secondary purpose was to determine the methods utilized by athletes to intentionally alter body composition.

MATERIALS AND METHODS

Study Design

This longitudinal observational study assessed dietary intake, body composition, strength, and aerobic fitness of Division I male soccer players at the beginning (January) and end (April) of the 2023

Spring season (Figure 1). Self-reported wellness outcomes, along with goals and methods utilized to alter body composition, were also assessed.

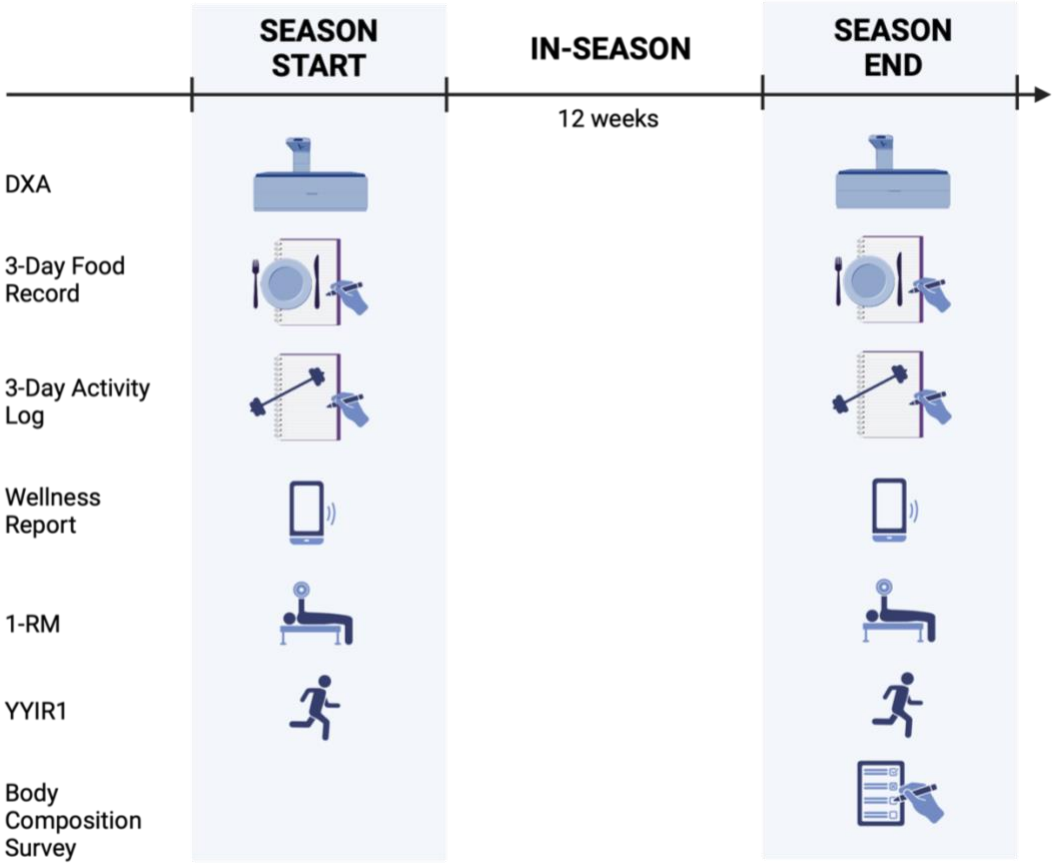

Figure 1. Overview of study design. *Participants underwent testing visits at the start and end of the Spring season separated by 12 weeks. DXA, dual-energy x-ray absorptiometry; 1-RM, 1-repetition maximum bench; YYIR1, Yo-Yo Intermittent Recovery Test Level 1. Created with BioRender.com*

Participants

Thirty-six active roster Division I male soccer players were approached for this observational study. Eligible participants were free from injury, eligible for unrestricted team participation, and over the age of 18 years. Details about the study and risks involved were provided before obtaining written informed consent. This study was approved by the Virginia Tech Institutional Review Board (IRB #22-901) in agreement with the Radford University Athletics Department.

Anthropometry and Body Composition

Height, TBM, and body composition were measured with participants wearing minimal clothing (athletic shorts, t-shirt, and socks) with all jewelry removed. Height was measured to the nearest 0.1 cm using a stadiometer and TBM to the nearest 0.1 kg on a digital physician's scale (Welch Allyn Scale-Tronix 5002, Milwaukee, WI). Body composition and bone parameters were assessed using a fan-beam dual-energy X-ray absorptiometry (DXA) scanner (Lunar iDXA, enCORE Version 15, General Electric Healthcare, Madison, WI). All scans were performed and analyzed by the same licensed technician to ensure consistency. Daily calibrations of the DXA scanner were performed according to the manufacturer's instructions. Participants laid supine with hands placed in the mid-prone position for the whole-body scan and with arms crossed over chest and legs extended fully for the lumbar spine and dual femur scans per manufacturer instructions. All participants were in the post-absorptive state after a 10-hour overnight fast and refrained from exercise on the morning of their appointment. Urine specific gravity (USG) was measured using a handheld analog clinical refractometer (Fisherbrand, Thermo Fisher Scientific, USA) for determination of hydration status. Suboptimal hydration status was defined as a USG value greater than 1.020 $g \cdot mL^{-1}$ (13).

Dietary Intake

Dietary intake was assessed from diet records collected on three consecutive days (Sunday to Tuesday) during the first and last weeks of the season. Written and verbal instructions were provided for recording the time, location, quantity, and brand names of foods, beverages, and dietary supplements consumed. A printed packet of standardized food diagrams was provided as a reference. All completed diet records were reviewed by a registered dietitian (TS) and missing information was clarified as necessary. Diet records were analyzed using ESHA's Food Processor® Nutrient Analysis software (Version 11.9.14, ESHA Research, Salem, OR), which has a database of over 146,000 foods.

Exercise Energy Expenditure

During the three-day monitoring period at the beginning and end of the season, participants also recorded all exercise sessions. Participants were instructed to record the time, activity, duration, and intensity ranging from light to hard. Details regarding team field training and weightlifting sessions were obtained from the team's strength and conditioning coach. Exercise logs were reviewed by a certified exercise specialist (TS) and clarified with participants as necessary. EEE was estimated using activity codes and metabolic equivalents (METs) in the ESHA Food Processor® software, which contains 933 activities with corresponding METs.

Energy Availability

Estimations of EA were calculated as EI minus EEE divided by FFM (4). The three-day observation period of diet and exercise at the start of the season included one day of rest and two days of training. The observation period at the end of the season included one day of rest, one of training, and one match played away from home. EA cutoff of < 25 kcal·kg FFM^{-1}·day^{-1} was used to identify athletes at risk for LEA based on the upper range suggested by Jurov et al. (11). Athletes were categorized into three groups (increased, decreased, maintained) at the end of the season based on ±1 kcal·kg FFM^{-1}·day^{-1} reported change in EA compared to the start.

Wellness Surveys

As part of standard team requirements, participants completed an electronic wellness survey (GPS DataViz, Carlsbad, CA) each morning with eight questions related to sleep, mood, stress, fatigue, soreness, and urine color (Table 1). Each question was scored out of 12.5 points with a total daily wellness score out of 100 calculated from the sum of all eight questions.

Category	Question	Possible Responses (score)
Sleep Quality	Please rate your sleep quality from last night.	Very restful (12.5) Good (10) Difficulty falling asleep (7.5) Restless (5) Insomnia (2.5)
Sleep Duration	How much sleep did you get last night?	More than 8 hr of sleep (12.5) Between 6 and 8 hrs (9.4) Between 4 and 6 hrs (6.3) Less than 4 hrs (3.1)
Mood	How would you rate your mood today?	Very positive mood (12.5) Generally good mood (10) Less interested in others (7.5) Snappy with teammates, family and friends (5) Highly annoyed, irritable, down (2.5)
Stress	How would you rate your stress level?	Very relaxed (12.5) Relaxed (10) Normal (7.5) Feeling stressed (5) Highly stressed (2.5)
Academic Stress	How would you rate your current academic stress?	Very low (12.5) Low (10) Normal (7.5) High (5) Very high (2.5)
Fatigue	How would you rate your current fatigue level?	Very fresh (12.5) Fresh (10) Normal (7.5) More tired than normal (5) Always tired (2.5)
Soreness	How would you rate your current muscle soreness?	Feeling great (12.5) Feeling good (10) Normal (7.5) Increase in soreness/tightness (5) Very sore (2.5)
Urine Color	What is your urine color this morning?	Clear (12.5) Lighter yellow (10.4) Yellow (8.3) Dark yellow (6.3) Amber (4.2) Brown (2.1)

Table 1. Daily Wellness Report Questions. *Daily wellness scores were calculated from the sum of all question responses. Daily wellness scores were out of 100. Questions republished with permission from GPS DataViz (Carlsbad, CA).*

Endurance Capacity

Aerobic capacity and ability to recover from intense exercise were evaluated using the Yo-Yo Intermittent Recovery Test Level 1 (YYIR1) (14). This test was developed in elite soccer players to evaluate endurance capacity (15) and has been shown to correlate with maximum oxygen uptake (VO_{2max}) (16). The team's strength and conditioning coach (LM) administered the test at both time points for all players on the team. The YYIR1 test consisted of two 20m shuttle runs, with 10 seconds of active recovery after each 40m. Intervals were repeated with increased speed until the participant reached exhaustion. The starting speed was 10.0 $km \cdot h^{-1}$ and increased to 12 $km \cdot h^{-1}$ and then 13 $km \cdot h^{-1}$, followed by increased increments of 0.5 $km \cdot h^{-1}$ thereafter. Pace was controlled using an audio recording. Participants were scored based on the last successfully completed level or total distance covered.

Strength Assessment

One-repetition maximum (1RM) for bench press was completed at the start and end of the season. The team strength and conditioning coach or intern supervised each 1RM attempt after athletes completed a basic warmup progression. For the lift attempt, athletes were in a supine position with feet planted firmly on the floor. Load was progressively increased after each successful lift until failure was reached. To qualify as a successful repetition, the athlete had to touch the barbell to his chest followed by complete arm extension without assistance. The strength-to-weight and strength-to-LBM ratios were calculated using TBM and LBM measured at that point in the season.

Nutrition and Body Composition Survey

During the final laboratory visit, participants completed a survey related to self-reported goals and actions taken to alter body composition during the season. The survey was developed based on unstructured conversations between the part-time dietitian and the athletes in previous athletic seasons.

Questions targeted intentional dietary changes (if applicable), sources of nutrition information, and supplement usage. The full survey can be found in Appendix I.

Statistical Analysis

Statistical analyses were conducted using IBM SPSS statistics software (Version 28.0.2.2 (14), IBM Corporation, Armonk, NY). Descriptive summaries are presented as mean ± standard deviation. The Shapiro-Wilk test for normality was used to test all variables for normality. Paired t-tests were performed to assess changes in TBM, body composition, bone density, and EA between the start and end of the season. Independent sample t-tests were performed to compare changes in dietary intake, body composition, and performance measurements between athletes who increased vs. decreased EA across the season. Comparisons were not made for the athletes that maintained EA given the small sample size. Significance was set at $P < 0.05$.

RESULTS

Out of the 36 eligible athletes approached, 26 enrolled in the study, 23 completed the initial testing visit, and 15 completed both visits, including one goal keeper at both time points (Figure 2). Three participants withdrew before the initial testing visit and eight withdrew before the final testing visit due to scheduling conflicts, time constraints, or ineligibility/withdrawal from team participation.

	Start of Season (n=23)
Age (yr)	19.7 ± 1.1
Height (cm)	177.6 ± 6.3
Body mass (kg)	76.0 ± 6.1
Fat mass (kg)	11.9 ± 2.6
Fat-free mass (kg)	64.1 ± 5.1
Body fat (%)	16.4 ± 3.0
BMC (g)	3437.1 ± 315.2
Total BMD (g/cm^2)	1.442 ± 0.095
Lumbar BMD (g/cm^2)	1.377 ± 0.123

Dual femur BMD (g/cm^2)	1.420 ± 0.130

Table 2. Baseline Characteristics of Collegiate Male Soccer Players at the Start of Spring Season*. Data presented as mean ±SD. BMC, bone mineral content; BMD, bone mineral density*

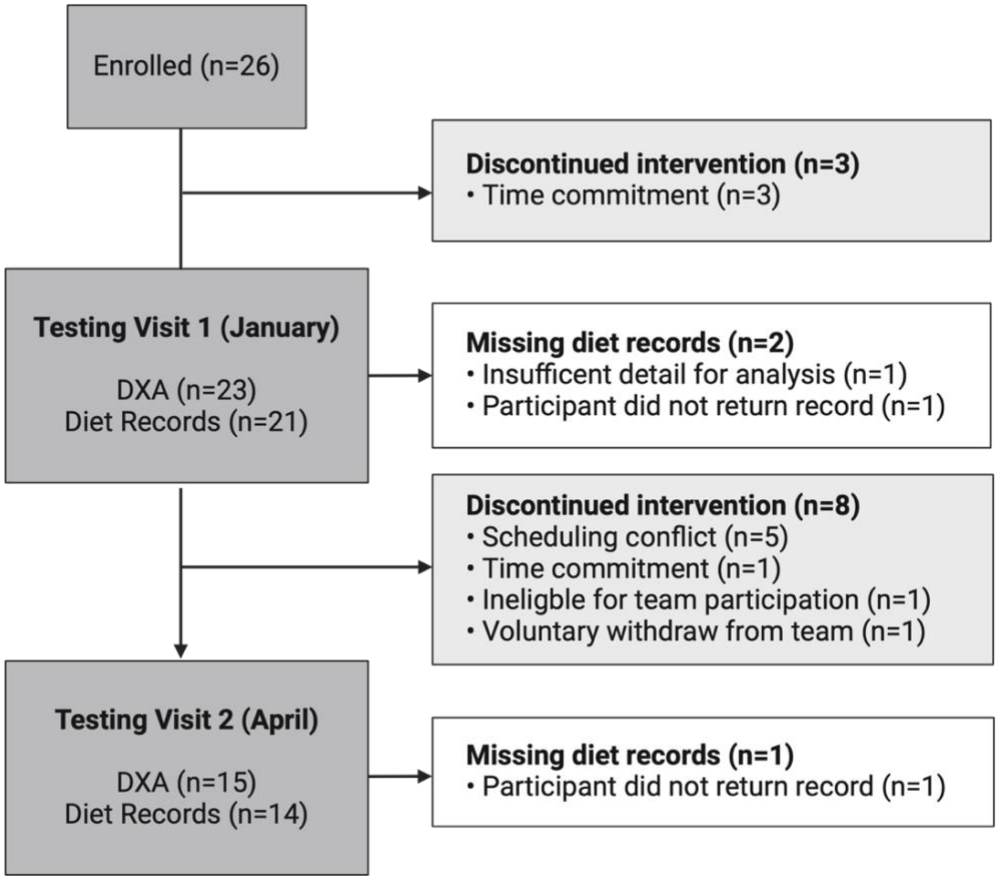

Figure 2. Flow of Athlete Participation. *DXA, dual-energy x-ray absorptiometry. Created with BioRender.com*

Baseline Characteristics

At the start of the season, DXA scans were conducted for 23 participants and 21 participants completed diet and exercise records (Figure 2). Anthropometric data are summarized in Table 2. The average EA reported over the three-day observation period was 33.9 ± 10.7 kcal·kg FFM^{-1}·day^{-1}, with an average EI of 2748 ± 683 kcal and EEE of 589 ± 394 kcal. Nineteen percent (n=4) of athletes reported EA

< 25 kcal·kg FFM^{-1}·day^{-1}, and only one of these athletes returned for the follow-up visit at the end of the season. Relative CHO and protein intakes were 4.3 ± 1.3 and 1.9 ± 0.6 kcal·kg^{-1}, respectively.

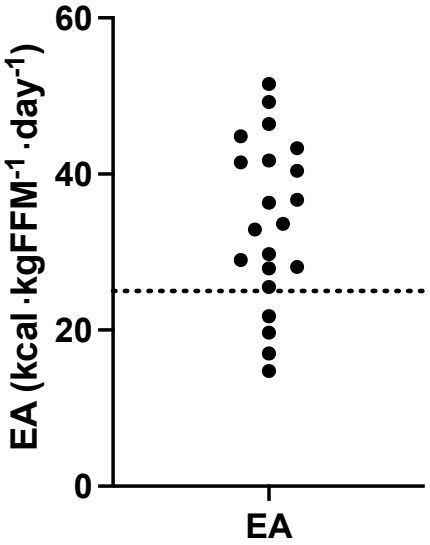

Figure 3. Energy availability at baseline (n=21). *Data presented as individual values (solid circles). Dotted line represents LEA cutoff of 25 kcal·kg FFM^{-1}·day^{-1}. Abbreviations: EA, energy availability; FFM, fat-free mass*

Longitudinal Data Across the Season

Energy Availability

Fourteen participants completed diet records at both the start and end of the season (Table 3). There were no differences in total energy, CHO, protein, or fat intakes at the end of the season compared to the start. As a group, EA was similar at both time points (P=0.259). Six athletes reported increases in EA, six reported decreases, and two reported no change. The one athlete with LEA at the start of the season who returned for the follow-up visit, reported a twofold increase in EA from 21.7 to 42.2 kcal·kg FFM^{-1}·day^{-1}. LEA at the end of the season was reported by only one athlete who showed a decrease in EA from 36.3 to 16.1 kcal·kg FFM^{-1}·day^{-1}. The change in relative CHO and protein intake was different between the athletes with increased and decreased EA, with greater positive change in the athletes that increased EA (Table 4).

Measure	Start of Season	End of Season	P-value
Energy Intake (kcal)	2845.9 ± 733.5	3002.9 ± 1057.5	0.509
Relative Energy Intake (kcal·kg^{-1})	37.5 ± 10.4	39.3 ± 14.7	0.564
EA (kcal·kg FFM^{-1}·day^{-1})	35.9 ± 9.2	39.9 ± 15.9	0.295
CHO (g)	326.0 ± 98.3	320.3 ± 120.7	0.855
Relative CHO (g·kg^{-1})	4.3 ± 1.4	4.2 ± 1.7	0.770
Protein (g)	149.0 ± 37.1	158.6 ± 56.5	0.496
Relative Protein (g·kg^{-1})	2.0 ± 0.6	2.1 ± 0.8	0.573
Fat (% total kcal)	33.6 ± 7.3	36.3 ± 4.4	0.173

Table 3. Dietary Intake at the Start and End of Spring Season for all athletes (n=14). *Data presented as mean ±SD. EA, energy availability; CHO, carbohydrate*

	Increased EA (n=6)			Decreased EA (n=6)		
	Start of Season	End of Season	Difference	Start of Season	End of Season	Difference
Dietary Intake						
EI (kcal)	2593 ± 936	3519 ± 1439	925 ± 567	3201 ± 503	2578 ± 499	-624 ± 424***
Relative EI (kcal·kg^{-1})	35.4 ± 13.6	47.2 ± 19.5	11.7 ± 8.0	41.6 ± 7.4	33.3 ± 6.9	-8.3 ± 5.1***
EA (kcal·kg FFM^{-1})	34.0 ± 12.9	50.2 ± 18.9	16.2 ± 10.7	38.8 ± 5.5	32.1 ± 8.7	-6.8 ± 7.4**
CHO (g)	296.6 ± 119.0	379.6 ± 153.2	82.9 ± 81.8	362.7 ± 78.8	271.8 ± 81.4	-90.9 ± 91.0**
Relative CHO (g·kg^{-1})	4.1 ± 1.7	5.1 ± 2.0	1.0 ± 1.2	4.7 ± 1.1	3.5 ± 1.1	-1.2 ± 1.1**
Protein (g)	131.9 ± 46.7	173.0 ± 84.0	41.1 ± 55.2	167.9 ± 18.1	141.1 ± 23.2	-26.8 ± 29.7*
Relative Protein (g·kg^{-1})	1.8 ± 0.7	2.3 ± 1.2	0.5 ± 0.8	2.2 ± 0.3	1.8 ± 0.3	-0.4 ± 0.3*
Fat (% total kcal)	34.2 ± 8.0	37.7 ± 3.1	3.4 ± 8.0	34.0 ± 4.5	36.1 ± 5.9	2.0 ± 4.0
Body Composition						
Body mass (kg)	73.7 ± 5.5	74.4 ± 3.4	0.8 ± 2.6	77.1 ± 3.2	77.7 ± 4.5	0.6 ± 2.0
Fat mass (kg)	11.7 ± 3.2	12.2 ± 3.2	0.3 ± 1.8	12.5 ± 2.8	12.1 ± 2.3	-0.4 ± 1.3
Lean body mass (kg)	59.0 ± 4.4	59.4 ± 4.4	0.4 ± 1.7	61.2 ± 3.1	62.1 ± 3.6	0.9 ± 1.7
Body fat (%)	16.1 ± 3.8	16.5 ± 4.6	0.4 ± 2.0	16.9 ± 3.5	16.3 ± 2.7	-0.6 ± 1.5
BMC (g)	3318.2 ± 398.8	3328.6 ± 378.4	10.4 ± 39.2	3446.3 ± 116.7	3480.5 ± 127.2	34.2 ± 22.5
Total BMD (g/cm^2)	1.424 ± 0.100	1.431 ± 0.101	0.007 ± 0.013	1.408 ± 0.028	1.420 ± 0.031	0.012 ± 0.018
Lumbar BMD (g/cm^2)	1.314 ± 0.096	1.330 ± 0.106	0.015 ± 0.023	1.381 ± 0.080	1.392 ± 0.085	0.011 ± 0.015
Mean femur BMD (g/cm^2)	1.395 ± 0.131	1.400 ± 0.129	0.005 ± 0.011	1.355 ± 0.039	1.372 ± 0.042	0.017 ± 0.011

Table 4. Comparison of Dietary Intake and Body Composition in Athletes with Increased vs Decreased EA*. Data presented as mean ±SD. EI, energy intake; EA, energy availability; CHO, carbohydrate. Significantly different from Increased EA: *** P<0.001, ** P<0.01, * P<0.05*

Body Composition and Bone Parameters

As summarized in Table 5, there were no changes in TBM or body composition between the start and end of the season. For bone parameters, there was an increase in total body bone mineral content (BMC) and BMD of the total body, lumbar spine, and dual femur at the end compared to the start of the season. No athletes presented with low BMD (z-score < -1.0) at any site at either time point. Changes in TBM, FFM, fat mass, percentage body fat, total BMC, lumbar BMD, and mean femur BMD were not different between the athletes with increased vs. decreased EA at the end of the season (Table 4).

Measure	Start of Season	End of Season	P-value
Total Body			
Body mass (kg)	76.6 ± 5.0 (67.3 - 84.6)	77.4 ± 4.9 (69.2 – 85.9)	0.166
Fat mass (kg)	12.2 ± 2.7 (7.9 – 17.2)	12.3 ± 2.7 (8.9 – 16.3)	0.735
Fat-free mass (kg)	64.4 ± 4.1 (54.7 – 69.8)	65.1 ± 4.3 (54.2 – 70.6)	0.143
Lean mass (kg)	61.0 ± 3.9 (51.9 – 66.0)	61.6 ± 4.1 (51.4 – 66.7)	0.155
Body fat (%)	16.6 ± 3.3 (12.1 – 23.4)	16.7 ± 3.3 (12.1 – 22.6)	0.913
BMC (g)	3433.2 ± 283.3 (2739.0 – 3875.0)	3454.0 ± 276.8 (2765.0 – 3880.0)	0.028
Total BMD (g/cm^2)	1.437 ± 0.087 (1.31 – 1.62)	1.447 ± 0.086 (1.32 – 1.63)	0.028
Regional			
Lumbar BMD (g/cm^2)	1.35 ± 0.11 (1.15 – 1.50)	1.37 ± 0.11 (1.18 – 1.51)	0.013
Mean femur BMD (g/cm^2)	1.39 ± 0.10 (1.24 – 1.53)	1.40 ± 0.10 (1.24 – 1.54)	0.010
Arms			
Total Mass (kg)	9.5 ± 1.0 (8.0 – 11.8)	9.7 ± 1.1 (8.5 – 11.9)	0.239
Fat mass (kg)	1.3 ± 0.3 (0.9 – 1.9)	1.3 ± 0.3 (1.0 – 1.9)	0.755
Lean mass (kg)	7.8 ± 0.9 (6.3 – 9.6)	7.9 ± 0.9 (6.5 – 9.7)	0.267
BMC (g)	456.9 ± 49.3 (386.0 – 555.0)	462.2 ± 53.3 (391.0 – 561.0)	0.239
Legs			
Total Mass (kg)	27.4 ± 1.7 (23.7 – 30.1)	27.6 ± 1.5 (24.5 – 29.6)	0.474
Fat mass (kg)	4.4 ± 1.0 (2.9 – 6.8)	4.3 ± 0.9 (2.8 – 5.7)	0.572
Lean mass (kg)	21.6 ± 1.5 (18.5 – 23.5)	21.8 ± 1.4 (18.3 – 23.8)	0.170
BMC (g)	1392.0 ± 120.0 (1109.0 – 1586.0)	1396.8 ± 119.4 (1114.0 – 1576.0)	0.253
Trunk			
Total Mass (kg)	34.8 ± 2.5 (30.9 – 39.0)	35.2 ± 2.6 (30.9 – 39.4)	0.119
Fat mass (kg)	5.6 ± 1.6 (2.9 – 8.0)	5.8 ± 1.7 (3.7 – 8.4)	0.417
Lean mass (kg)	28.1 ± 1.7 (24.2 – 31.3)	28.4 ± 2.0 (23.5 – 30.9)	0.287
BMC (g)	1026.5 ± 111.3 (770.0 – 1210.0)	1031.8 ± 102.6 (788.0 – 1222.0)	0.447

Table 5. Dual-energy x-ray absorptiometry measurements at the Start and End of Spring Season (n=15). *Data presented as mean ±SD (range). BMC, bone mineral content; BMD, bone mineral density*

Performance Outcomes

Thirteen participants completed the performance tests at both time points (Table 6). Average aerobic fitness scores, strength-to-weight ratio, and strength-to-LBM ratio increased by 10%, 11%, and 12%, respectively. Seasonal changes in aerobic fitness and strength ratios were not different between the athletes that increased vs. decreased EA at the end of the season.

Measure	Start of Season	End of Season	P-value
Aerobic YYIR1 score	54.0 ± 7.3	59.5 ± 4.5	0.002
Strength-to-weight ratio	1.07 ± 0.21	1.20 ± 0.20	<0.001
Strength-to-LBM ratio	1.35 ± 0.25	1.51 ± 0.25	<0.001

Table 6. Performance measurements at the Start and End of the Spring Season for Division I male soccer players (n=13). *Calculations: Strength-to-weight ratio = 1RM bench press (kg) / TBM (kg); Strength-to-LBM ratio = 1RM (kg) / LBM (kg). Data presented as mean ±SD. YYIR1, Yo-Yo Intermittent Recovery Test Level 1; LBM, lean body mass; 1RM, one repetition maximum; TBM, total body mass.*

Wellness Survey

No change was observed in the three-day wellness scores (n=15) between the start and end of the season (70.4 vs 73.5, *P* = 0.247). Wellness scores were not different between the athletes that increased vs. decreased EA during the season. However, the one athlete with EA < 25 kcal·kg FFM^{-1}·day^{-1} at the end of the season had a 17.4% decrease in average wellness score (83.5 vs 69.0).

Body Composition Surveys

Body Composition Surveys were completed by 15 participants. Most athletes reported the desire to build muscle (66.7%) and/or increase TBM (53.3%) during the Spring season. Five participants reported a desire to lose body fat, four to maintain current weight, and one to lose weight. Of the eight who reported a desire to gain TBM, five met their goal and gained 2.3 ± 1.8 kg with 1.8 ± 1.9 kg as FFM and 0.5 ± 0.6 kg as fat mass. Six of the ten who reported the desire to build muscle had increased FFM at the end of the season (1.9 ± 1.4 kg FFM). Only one of the five participants who desired body fat loss had decreased

fat mass at the end of the season. Two of four participants with a desire to maintain TBM were within 0.5 kg of their starting measurement at the end of the season, while the other two participants gained 2.1 ± 1.1 kg, of which 1.6 ± 0.6 kg was FFM.

Methods	n (%)
Change the types of food eaten	11 (73.3%)
Change the amount of food eaten	9 (60%)
Participants in extra workouts in addition to supervised team training	9 (60%)
Started following a new diet or way of eating	5 (33.3%)
Used nutrition supplements	5 (33.3%)
Asked for advice from the team Strength and Conditioning Coach	5 (33.3%)
Sought advice from the internet	4 (26.7%)
Asked advice from the team Registered Dietitian	3 (20%)
Asked advice from a teammate	2 (13.3%)
Sought advice from a published book or resource	1 (6.7%)
No changes made	1 (6.7%)

Table 7. Methods employed to achieve body composition goals (n=15).

Tables 7 and 8 summarize the approaches taken during the season to achieve body composition goals. The top three methods were changing the types of foods consumed, the amount of food consumed, and engaging in extra workouts outside of team training. One-third of participants also reported following a new dietary pattern and/or using nutrition supplements. Five participants reported following a higher protein diet, one eliminated dairy products, and one began intermittent fasting. Increased intake of protein-rich foods, fruits and vegetables, portion sizes, and overall EI were the top dietary changes (Table 8). The use of sports nutrition supplements was also highly prevalent in this population, with 80% (n=12) reporting the use of one or more supplements (Table 9). Protein powders/shakes were the most consumed supplements followed by pre-workout and magnesium.

In the free response section of the survey, athletes reported improving diet quality by switching to more whole grains and eating more “real” and “clean” foods. Additionally, two participants reported

eating less frequently by reducing snacking and both of these participants had increased TBM and FFM at the end of the season compared to the start.

	Increased % (n)	Decreased % (n)	No Change % (n)
Overall calories eaten	60% (9)	6.7% (1)	33.3% (5)
Portion sizes	60% (9)	13.3% (2)	26.7% (4)
Number of meals each day	26.7% (4)	6.7% (1)	66.7% (10)
Number of snacks each day	20% (3)	26.7% (4)	53.3% (8)
Protein foods	73.3% (11)	0% (0)	26.7% (4)
Fruits and vegetables	60% (9)	6.7% (1)	33.3% (5)
Dietary supplements	26.7% (4)	0% (0)	73.3% (11)

Table 8. Self-reported dietary changes of Division I male soccer players during the athletic season (n=15).

Supplement Category	**% (n)**	**Supplement details (n)**
Protein powder/shakes	60% (9)	
Pre-workout	33.3% (5)	
Individual vitamin(s) or mineral(s)	26.7% (4)	Magnesium (3), Vitamin D3 (1), CoQ-10 (1), Zinc (1)
Multi-vitamin	13.3% (2)	
Fish oil or omega-3	13.3% (2)	
Meal replacement shakes	13.3% (2)	
Weight gainers	0% (0)	
Testosterone boosters	0% (0)	
Appetite suppressants	0% (0)	
Other	13.3% (2)	Ashwagandha (2), Creatine (1), CBD (1), Powdered Greens (1)
None	3 (20%)	

Table 9. Self-reported supplement use of Division I male soccer players (n=15).

DISCUSSION

This study assessed EA and body composition of Division I male soccer players at the start of a non-championship Spring season, and examined longitudinal changes in EA, body composition, and performance outcomes. We found that EA, TBM, LBM, and fat mass did not change across the Spring season for the group, and there were no differences in body composition or fitness outcomes between the athletes who decreased EA during the season compared to those who increased EA. Total BMC, total and site-specific BMD, and relative strength improved throughout the season regardless of EA status.

This study is the first to examine EA status of Division I male soccer players at the beginning and end of the non-championship segment of an athletic season. Prevalence of LEA (< 30 kcal·kg FFM^{-1}·day^{-1}) in collegiate female soccer players has been reported to range between 26 and 66.7% at various points throughout the season (296, 330). In the current study, LEA (< 25 kcal·kg FFM^{-1}·day^{-1}) was identified in 19.0% and 7.1% of athletes at the start and end of the season, respectively. These results suggest collegiate male soccer players may be at a lower risk for LEA compared to their female counterparts. It is important to note that three of the four athletes who reported EA < 25 kcal·kg FFM^{-1}·day^{-1} at the start of the study withdrew before the end of the season. It is possible that the athletes with reduced EA may have had lower motivations to complete the additional study requirements.

For an individual athlete, it is difficult to predict how EA will fluctuate throughout training. Theoretically, it could be easier for an athlete to increase EA as the season progresses when training volume decreases to focus more on match play and recovery. However, EA may also decrease, particularly in collegiate athletes, later in the academic semester when time demands are presumably higher for coursework and team activities. In a study of elite athletes (27% female) from five sports, EA was increased during the competition phase of the athletic season compared to preseason (19). However, in the current study, EA was similar at the end of the season compared to the start. Interestingly, half of the participants reported increased EA at the end of the season, while the other half reported decreased EA. The

participants who reported increased EA at the end of the season tended to have lower baseline TBM, EA, and intakes of total energy, CHO, and protein compared to the participants who reported a decrease in EA. It is possible that these athletes may have focused more on increasing their dietary intake given the greater room for improvement. In Division I female athletes, Reed and colleagues (17) found that EA decreased from preseason to midseason but returned to preseason values during the postseason. However, the fluctuation in EA across the athletic season may be different in the Fall compared to the Spring. The preseason in the Fall is characterized by a high level of training volume and intensity, usually involving two-a-day sessions, which then decreases once the regular season begins. Spring season, on the other hand, typically does not have the same high-intensity preparatory phase.

Daily energy intake reported in the current study was 2748 $\pm$ 683 kcal (36.6 $\pm$ 9.7 kcal·kg^{-1}) and 3003 $\pm$ 1058 kcal (39.3 $\pm$ 14.7 kcal·kg^{-1}) at the beginning (n=21) and end (n=14) of the season, respectively. These intakes are similar to the findings of a recent systematic review (20), which reported daily EI of 2813 $\pm$ 498 kcal (37.7 $\pm$ 6.2 kcal·kg^{-1}) in male soccer players. However, this intake is slightly below the Union of European Football Associations (UEFA) expert group recommendation of 2900-2500 kcal·day^{-1} (2). Soccer players utilize CHO as the primary fuel source during matches, which is generated from a combination of anaerobic glycolysis and CHO oxidation. Relative CHO intake of the current study was at the lower end of the UEFA recommended range for in-season training (3-8 g·kg^{-1}) and below the current sports nutrition guidelines for high-intensity endurance programs (6-10 g·kg^{-1}) (7). These findings are similar to CHO intakes reported in collegiate (21), professional (22), and senior soccer players (23). Some athletes may be hesitant to increase their CHO intake for fear it may cause weight gain or reduce performance, despite contrary evidence indicating CHO intake benefits soccer performance (24, 25). Protein and fat intakes, on the other hand, were at the upper end of the UEFA and ACSM recommended ranges.

Soccer players in the current study displayed similar TBM, but higher average fat mass (11.9 vs 10.6 kg) and percent body fat (16.4 vs 13.9%) at baseline compared to a previous study of Division I soccer

players also measured via DXA (4). However, the athletes in the previously mentioned study were recruited from a team that consistently ranked in the top 25 nationally, and it has been suggested that higher-level soccer players have lower body fat (26). Comparatively, athletes in this current study competed at the Division I level but the team was not nationally ranked.

Despite over half of participants reporting a desire to gain TBM and/or lean tissue, there were no changes in body composition measurements at the end of the season compared to the start. These results are in agreement with a previous study conducted in NCAA Division III male soccer players, which found no changes in TBM or percent body fat over an 11-week Fall season (27). Body composition also appears to be relatively stable in professional soccer players between the preseason and postseason (28, 29). In contrast, a study of Division I male soccer players by Silvestre and colleagues (4) reported increased TBM and LBM across the Fall season. It is possible the resources available to student-athletes at different Division I programs may be partially responsible for the discrepancy in results. Participants in the current study did not have access to either a full-time sports dietitian or routine sports nutrition education. Previous studies have documented a positive correlation between nutrition knowledge and body composition (30) and sports performance (31). Providing additional nutrition support during the athletic season may assist athletes in achieving body composition and performance goals.

Aerobic and strength performance were increased at the end of the season compared to the start, regardless of EA status. Due to the relatively small prevalence of LEA, dietary intake was likely sufficient to support training adaptations. Future studies should investigate the effect of EA on soccer-specific performance testing.

As a group, there were no changes in wellness scores observed between the start and end of the season. Eleven out of the fifteen participants reported higher wellness scores at the end of the season and the change in wellness score was not influenced by the individual change in EA. More research is needed on the perceived wellness of collegiate soccer players in the Fall season compared to Spring.

Athletes compete in considerably more matches in the Fall and travel more frequently. The additional travel in the Fall may contribute to perceived stress and impaired recovery (32).

In this current study, athlete perceptions of their dietary intakes did not match the three-day dietary assessments. At the end of Spring season, 73.3% (n=11) of athletes reported having increased protein-rich foods and 60% (n=9) reported higher EI, however, there was no difference in protein or EI between the start and end of the season. Current strategies to promote healthy weight gain include the incorporation of a modest energy surplus and fairly rigorous strength training (33). One intuitive strategy that athletes may use to increase energy intake is to include energy-dense snacks throughout the day. Interestingly, two of the athletes in this study who had increased TBM and lean tissue at the end of the season reported the opposite. Instead, they reduced snacking frequency and increased EI during mealtimes. It's possible that adding in more snacks could have the opposite of the intended effect by promoting prolonged satiety and reducing EI during meals. Therefore, an individualized approach is recommended based on athlete preference.

More athletes in this study sought nutrition advice from the strength and condition coach and the internet than the part-time, team dietitian. Froil and colleagues (34) reported similar observations in a large cohort of Division I athletes, wherein the top sources of information were family members, fellow athletes, and the athletic trainer. Evidence suggests working with a sports dietitian may improve athletic performance (35, 36), recovery (35), body composition (36), and diet quality (37) in collegiate athletes. More research is needed to understand why sports dietitians may be underutilized or not seen as a trusted source of nutrition information by some athletes.

There are limitations of this study that must be considered when interpreting the results. First, participants were recruited from a single soccer program and may not be representative of other Division I programs, especially those with more nutrition resources available (e.g., full-time dietitian, fueling stations, dedicated athlete dining halls). There may also be differences in player motivations and coaching

philosophies across Division I programs that would influence the changes observed throughout the season. Another limitation is the reliance on self-reported data. Athletes tend to underreport energy intake (38) which can lead to miscalculations in EA. This study attempted to reduce risk of underreporting by providing written and verbal instructions on how to quantify dietary intake, in addition to immediate post-collection review by a registered dietitian to clarify any ambiguity. However, the use of multiple dietary assessment methods (e.g., weighed food records with 24-h recalls) may improve accuracy in future studies (38).

In summary, results of the current study found that dietary intake and body composition of Division I male soccer players remained consistent over a non-championship Spring season. Approximately 40% of athletes did not meet their self-reported goals of increased TBM and/or lean tissue during the season, which may have resulted from inadequate nutrition knowledge or awareness of dietary intake. Bone measures and performance outcomes improved during the season regardless of EA status, which may be attributed to the small prevalence of LEA observed in this sample.

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CHAPTER V

Conclusions and Future Directions

High-Impact Exercise and Low Energy Availability

Short bouts of daily high-impact exercise in combination with daily running were not found to prevent the increase in resting bone resorption in female runners under a controlled condition of LEA. No changes in bone formation were shown under either condition of LEA with or without jumping. It is unclear why free T_3 did not decrease during the LEA with jumping condition and whether the apparent sparing effect of jumping on the expected fall in T_3 is beneficial during LEA. Based on the findings from this study and a similar five-day investigation of LEA with exercise in active women (1), it is uncertain whether short-term periods of LEA (three to five days) with high-impact running pose a significant risk to the balance between bone formation and resorption. Although the rate of bone resorption did increase in the present study, there was no suppression of bone formation found. Based on the existing literature, it is more likely the negative consequence of LEA on bone tissue is mediated through suppressed estradiol concentration, which does not appear to occur with EA of 15 kcal·kg FFM^{-1}·day^{-1}.

Seasonal Profiles of EA and Body Composition

Several studies have reported seasonal changes in EA and body composition (2–4), with the most noticeable changes occurring between pre-season and competition. Our results contradict these findings, showing no change in EA or body composition from pre- to post-season. However, the current study was conducted during the Spring season, which is the non-championship segment of the National Collegiate Athletic Association (NCAA) athletic season. The Spring season is characterized by lower training volume and fewer matches. Contact between coaches and players is also reduced during the Spring season, and athletes must be self-motivated to complete additional training independently. Future studies should

examine EA and body composition profiles of collegiate soccer players at institutions that represent various levels of competition and resource availability.

Implications for Future Research

Participants in Study 1 were classified as Recreationally Active (Tier 1) and Developmental Athletes (Tier 2) according to the framework proposed by McKay and colleagues (5). This sample is reflective of the general population and does not represent athletes competing at the state, national, or international level. Therefore, results from this study cannot be generalized to high-caliber athletes in Tiers 3-5. Highly Trained and Elite athletes may already engage in multiple daily training sessions depending on the time of season, and adding in additional exercise may not be feasible. Allowing for longer rest periods between training sessions may be more beneficial for these athletes. Often field/skills training is completed immediately before or after strength training sessions. The lack of recovery between the two sessions may reduce the osteogenic response to exercise. Future studies should explore the timing of multiple-day training sessions on bone (re)modeling markers in Highly Trained and Elite athletes.

Qualitative studies on LEA etiology are also warranted to provide better care for specific athlete populations. LEA etiology cannot be generalized for all athletes given the unique environments and circumstances each athlete experiences. The challenges faced by a college student-athlete may be uniquely different from a professional, masters, or amateur athlete. These types of studies will allow for the development of more targeted interventions. In Study 2, participants had an increase in absolute and relative strength, regardless of whether EA increased or decreased throughout the season, suggesting the exercise stimulus (i.e., training program) was sufficient for strength gains. However, many athletes struggled to gain total and lean mass indicating EA may not have been high enough to create an anabolic environment for tissue growth and repair. Applied practice would benefit from future studies that interview athletes and characterize the practical barriers to achieving optimal EA.

Subjective Insights

While conducting the studies within this dissertation, several conversations arose regarding the lack of standardized EA calculations and the need for possible adjustment. The current approach for calculating EA involves estimating energy intake (EI), exercise energy expenditure (EEE), and fat-free mass (FFM). The technical issues with estimating these components were previously described in Chapter II. What requires further discussion is the estimation of EEE. In the 2018 IOC consensus statement on REDs (6), it was recommended to calculate EEE as the energy expended above rest during a bout of exercise. However, EEE does not factor in the energy cost of recovery (e.g., excess post-exercise oxygen consumption, EPOC) or acts of daily living. EPOC accounts for an additional energy expenditure of approximately 25-70 kcal above rest after high-intensity interval training and moderate-intensity steady-state exercise, with the precise energy cost of EPOP dependent on the intensity of the activity and the individual athlete (7). Leisure time activity and acts of daily living are also overlooked by the current EA calculation. The daily activity level of participants in Study 1 ranged from predominantly sedentary (e.g., desk jobs) to moderately active (e.g., standing on feet most of the day) based on their occupations. A majority of our participants were also somehow involved with a college campus (i.e., students, faculty, or staff). Some of our participants biked or walked to campus each day, while others walked frequently between buildings for classes or meetings. However, because this type of activity is not a planned *exercise*, it is not taken into account when calculating EA. Based on the GPS watch data, activity energy expenditure (AEE; all energy expenditure above rest) was 313 $\pm$ 293 kcal (-1.1 to 952 kcal) higher than our targeted EEE. When we recalculated the level of EA for Study 1 using AEE instead of EEE, the EA value was 7.5 $\pm$ 5.8 kcal·kg FFM^{-1}·d^{-1} (-3.6 to 14.2 kcal·kg FFM^{-1}·d^{-1}), approximately half of the planned 15 kcal·kg FFM^{-1}·d^{-1}. Further discussions are warranted for the estimation of EEE when EA calculations are used in clinical practice.

Another consideration regarding LEA research is the discrepancy between how LEA is studied in the lab and experienced in the field. A review of the major translational errors has been summarized by Heikura and colleagues (8), however, one of the other considerations not mentioned in their review is the modality by which LEA is induced. Several studies now have shown bone (re)modeling markers and certain hormones respond differently during LEA conditions with and without exercise (1, 9). These results suggest LEA repercussions are less severe when participants engage in some form of exercise. This is important for informing clinical practice because results from LEA studies should only be compared when similar methodologies are used. For example, low T_3 has consistently been shown to decrease in response to LEA and has been proposed as an objective biomarker to screen for Relative Energy Deficiency in Sport and the Male Athlete Triad (8, 10, 11). However, results from Study 1 showed free T_3 was only reduced from baseline during the run-only LEA condition and not affected during LEA with running and jumping. Hutson and colleagues (9) reported similar results, showing reductions in total T_3 after three days of LEA without exercise but no change in T_3 after three days of LEA with daily jumping. In both studies, LEA was controlled in all conditions to 15 kcal·kg FFM^{-1}·d^{-1}. This provides further evidence questioning the use of a single cutoff value for LEA and suggests the LEA conditions must also be considered when evaluating objective biomarkers.

Finally, stronger efforts in sports nutrition research are needed to recruit participants from diverse racial and ethnic backgrounds. In Study 1, two participants identified as African American/Black, one as Asian, and one as Two or More Races. However, three of these participants dropped out at various points of the study and only one completed both interventions. The geographic location of where this study was conducted may have partially contributed to the low representation of diverse participants. According to the United States Census Bureau (12), Montgomery County, where this study was conducted, has a population of 98,915, of which 86.1% identify as 'White alone'. Similarly, in Study 2, ten of the 23 enrolled athletes identified as Black (n=5), Hispanic (n=3), Asian (n=1), or Two or More Races (n=1). Of the

eight participants who withdrew from study participation, five were from diverse ethnic/racial backgrounds. Future studies that explore the barriers to study participation experienced by diverse athletes are necessary to move the field forward.

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APPENDICES

Appendix A: Study 1 Virginia Tech Institutional Review Board Research Protocol

Division of Scholarly Integrity and Research Compliance
Institutional Review Board
North End Center, Suite 4120 (MC 0497)
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732
irb@vt.edu
http://www.research.vt.edu/sirc/hrpp

VIRGINIA TECH

MEMORANDUM

DATE: February 14, 2024

TO: Enette Larson-Meyer, Elaina Lynn Marinik, Firoozeh Tarkesh, Anna Morozov, Janet T Rinehart, Trisha Marie Sterringer, Lauryn Faith Mericle

FROM: Virginia Tech Institutional Review Board (FWA00000572)

PROTOCOL TITLE: The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners

IRB NUMBER: **22-168**

Effective February 14, 2024, the Virginia Tech Institution Review Board (IRB) approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

https://secure.research.vt.edu/external/irb/responsibilities.htm

(Please review responsibilities before beginning your research.)

PROTOCOL INFORMATION:

Approved As:	**Expedited, under 45 CFR 46.110 category(ies) 9**
Protocol Approval Date:	**March 13, 2024**
Protocol Expiration Date:	**March 12, 2025**
Continuing Review Due Date*:	**February 19, 2025**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

ASSOCIATED FUNDING:

The table on the following page indicates whether grant proposals are related to this protocol, and which of the listed proposals, if any, have been compared to this protocol, if required.

PROTOCOL TITLE:

Include the full protocol title.

The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners

PROTOCOL NUMBER:

Include the number assigned in Protocol Management (verify this has been added before submitting protocol to HRPP).

22-168

PRINCIPAL INVESTIGATOR:

Full Name and Degrees: Dawnine Enette Larson-Meyer, PhD
Department: Human Nutrition, Foods, and Exercise
Telephone Number: 540-231-1025
Email Address: enette@vt.edu

FUNDING:

Sponsor(s): no sponsor
Funded already or in the proposal phase?: Proposal phase
Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: N/A

VERSION NUMBER/DATE:

Include the version number and date of this protocol. Versions should start at 1.0.

Version 6.0

REVISION HISTORY:

Use this table to keep track of changes. Add more rows as needed.

Revision #	Version Date	Brief Summary of Changes (i.e., the different sections)	Consent Change?
1	3/1/2022	Clarified time commitment for subject participation and payment structure in the protocol and consent form (Sections 1.0, 15.4, 17.4). Added more details regarding future undergraduate study personnel in section 26.	No
2	3/16/2022	Increased compensation to $100 split into two payments of $25 and $75. (Section 15.4)	Yes

		Clarified exclusion criteria: no contraceptive use, no recent (within 12 mo) or current eating disorder (Section 12.2) Expanded on protocol justification (Section 3.3) Clarified running time of protocol (Section 8.1)	
3	3/29/2022	Added pregnancy test after the 3-week washout period on day 1 of the second experimental condition (Sections 8.1, 8.2, 12.3, 17.4)	Yes
4	5/20/2022	Protocol for impact loading exercise sessions modified slightly to include recovery period (Section 8.2) Additional assays (leptin, cortisol, ferritin, 25(OH)D, Nesfatin-1) (Section 4.1 and Table 1 in Section 8) Added vitamin D questionnaire at baseline (Section 8.2) Change to weight safety endpoint (Section 4.2 and justification in section 7.2) Change timepoints for running economy Added menstrual cycle question to phone screening form	Yes
5	6/24/2022	Baseline DXA moved from visit 2 to visit 1 (Section 8.2) Added a mealtime log during the 5-day experimental phases (Section 8.2) Protocol for treadmill run decreased to 65%VO2max (previously 70%VO2max) (Section 8.2)	Yes
6	9/6/2022	Inclusion criteria adjusted to include participants ages 18-35 (previously 18-30) and removed running requirement of 30 miles/week and VO2max criteria (Section 12) Additional iron status markers (TIBC and serum iron) (Section 4.1, Table 1 Section 8) Exclusion criteria: criteria for low BMD adjusted to z-score <-2 (Section 12)	Yes
7	7/10/2023	Inclusion criteria adjusted to include participants with a BMI 18.5-30 (Section 12)	Yes
8	9/8/23	Increase sample size to 20	No

Table of Contents

1.0Study Summary

Study Title	The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners
Study Design	Randomized Cross-Over Intervention Study
Primary Objective	Determine the effect of short-term, high-impact loading on biomarkers of bone remodeling in trained, female long-distance runners in the presence of low energy availability.
Secondary Objective(s)	Exploratory Aim: Examine the relationship between undercarboxylated osteocalcin and impact loading on glucose metabolism during acute low energy availability.
Study Population	Eumenorrheic, female long-distance runners who do not have existing low energy availability.
Sample Size	20
Research Intervention(s)/ Investigational Agent(s)	Research volunteers will undergo two experimental conditions of low energy availability in a randomized order: Low energy availability with daily running only (RUN) and low energy availability with daily running and impact loading (RUN+IL). Each 5-day experimental condition will be initiated in the follicular phase and separated by a washout period of one menstrual cycle (approximately 24 days).
Study Duration for Individual Participants	19 days of intervention and study visits including one screening visit, one baseline testing visit, 3 days of tracking normal diet and exercise, and two experimental conditions of 7 days separated by a 3-week washout period of normal diet and exercise
Acronyms and Definitions	ACSM, American College of Sports Medicine; BMD, bone mineral density; CBC, complete blood count; CGM, continuous glucose monitoring; CTX, C-terminal telopeptide of type 1 collagen; DXA, Dual-Energy X-ray Absorptiometry; HIP Laboratory, Human Integrated Physiology Laboratory; LEA, low energy availability; NEM Laboratory, Nutrition and Exercise Metabolism Laboratory; P1NP, N-terminal propeptide of type 1 procollagen; Trap5b, tartrate-resistant acid phosphatase; TSH, thyroid-stimulating hormone; unOC, undercarboxylated osteocalcin; VO2 max, maximal oxygen uptake during exercise, also known as aerobic capacity;

2.0 Objectives

2.1 Describe the purpose, specific aims, or objectives of this study:

1) Determine the effect of short-term, high-impact loading on biomarkers of bone remodeling in energy-restricted, female long-distance runners.
2) Examine the relationship between undercarboxylated osteocalcin and impact loading on glucose metabolism during acute low energy availability.

2.2 State the hypotheses to be tested:

The addition of 50 high-intensity impact loading jumping exercises per day to usual run training will result in less suppression of bone formation following 5 days of endurance running in an energy-restricted condition compared to daily running in an energy-restricted state without high-impact loading exercises.

3.0Background

3.1 Summarize the relevant prior research on this topic and gaps in current knowledge within the field of study:

Maintaining adequate energy intake is essential for athletic performance and overall general health. Yet, many athletes experience low energy availability (LEA) by failing to consume enough calories to meet their energy demands. Energy availability is the amount of energy remaining from dietary energy intake (EI) to support general health and bodily functions after accounting for exercise energy expenditure (EEE), and is commonly expressed relative to fat-free mass as kilocalories per kilogram of fat-free mass (FFM) per day (kcal/kgFFM/d). According to the Life History Theory, the human body will adapt to conserve energy under conditions of biological stress, such as energy-restriction and LEA, by downregulating biological processes that are less essential for immediate survival (Shirley, Long et al. 2022). The performance and health consequences of LEA are characterized by the syndrome known as Relative Energy Deficiency in Sport (RED-S) (Mountjoy, Burke et al. 2018). Specific to this proposed study, athletes suffering from RED-S and chronic LEA may experience several inter-related health consequences involving endocrine function, reproduction, and bone metabolism. Previous studies indicate LEA is associated with increased risk for bone stress injuries and low bone mineral density (BMD) (Tenforde, Carlson et al. 2016, Tenforde, Carlson et al. 2018, Gibbsm Battuv et ak, 2014), in addition to suppression of certain bone-regulating hormones including estrogen, insulin, vitamin D, triiodothyronine (T3), and insulin-like growth factor (IGF-1) (McCall, Ackerman 2019).

Previous Studies on LEA and Biomarkers of Bone Metabolism. Biomarkers of bone formation and resorption are often used as surrogate markers for assessing bone metabolism in the short term given that it can take months or even years for changes in bone microarchitecture and BMD to manifest. In a study of 8 male distance runners, serum N-terminal pro-peptide of type 1 collagen (P1NP), a marker of bone formation, and IGF-1 decreased by 15% and 17%, respectively, after 3 consecutive days of treadmill running with energy intake restricted to 50% of estimated needs (Zanker and Swaine 2000). In a more recent cross-over design study (Papageorgiou, Elliott-Sale et al. 2017), changes bone biomarkers were measured in 11 women and 11 men in response to 5 days of daily running under optimal EA conditions (45 kcal/kgFFM/d) and 5 days of daily running under low EA conditions (15 kcal/kgFFM/d). In both conditions, participants ran on a treadmill until an energy expenditure of 15 kcal/kgFFM was achieved. Diets provided 60 kcal/kgFFM/d and 30 kcal/kgFFM/d for the optimal and low EA conditions, respectively. In the female participants, P1NP was significantly lower after the low EA condition

compared to optimal EA. Additionally, ß-carboxyl-terminal cross-linked telopeptide of type 1 procollagen (ß-CTX), a marker of bone resorption, increased 19% in response to the low EA condition and this increase was significantly different from the change in ß-CTX observed in the optimal EA condition. No significant change in P1NP or ß-CTX were observed in men. This suggests women may be more sensitive to the effects of LEA on bone metabolism and, therefore, be at greater risk. In another cross-over design study of 10 female runners, changes in P1NP and ß-CTX were measured after completion of three, 3-day experimental conditions: optimal EA (45 kcal/kgFFM/d), LEA achieved through dietary restriction only (15 kcal/kgFFM/d), and LEA achieved through a combination of dietary restriction and daily running (15 kcal/kgFFM/d) (Papageorgiou, Martin et al. 2018). In the combined diet and exercise LEA condition, participants received diets providing 30 kcal/kgFFM/d and expended 15 kcal/kgFFM/d during a treadmill run. This study found P1NP was significantly reduced after 3 days of LEA achieved through diet only, but not after the combined LEA condition with running. These findings suggest the negative effects of LEA on bone metabolism may be counteracted or masked by the osteogenic effects of weight-bearing exercise, such as running. There were no significant changes observed in ß-CTX in response to either LEA condition. Based on these short-term studies, it appears suppression of bone formation occurs before measurable increases in bone resorption. Additionally, the severity of bone impairments may depend on the degree of energy restriction given the dose-response relationship observed between LEA and select bone turnover markers in exercising women (Ihle and Loucks 2004).

Benefit of High-Impact Loading on Bone Metabolism. High-impact loading exercises such as jumping, bounding, and plyometric training place a high level of mechanical strain on bone and can elicit osteogenic adaptations (Hutson, O'Donnell et al. 2021). There have been a limited number of studies showing mixed results on the short-term effects of high-impact loading on bone biomarkers (Rantalainen, Heinonen et al. 2009, Rogers, Dawson et al. 2011). Additionally, very few studies have included energy intake assessments and most studies have been conducted in non-athletes. In one study of 26 female non-athletes, markers of bone formation (osteocalcin and bone specific alkaline phosphatase (BAP)) and bone resorption (tartrate-resistant acid phosphatase (TRAP5b) and CTX) were measured in response to either a control condition or jumping intervention (Kishimoto, Lynch et al. 2012). Volunteers in the jump group performed 10 jumping exercise per day at a frequency of 5 times a week for 2 weeks. Bone resorption measured by CTX was lower in the jump group compared to baseline. Additionally, TRAP5b was significantly lower in the jump group compared to the control group, however, there was no significant changes in TRAP5b from baseline in either group. Interestingly, the jump intervention significantly lowered BAP, a marker of bone formation. No changes were observed in osteocalcin within or between groups. This study was limited based on lack of energy intake assessment and the potential for inter-subject variability in bone response to jumping exercises. Despite the theoretical basis for high-impact exercise as a countermeasure for bone resorption during LEA, there have been no controlled trials to date that have investigated this theory in energy-restricted athletes.

Undercarboxylated Osteocalcin (unOC) and Glucose Metabolism. This study seeks to contribute to understanding the potential association between osteocalcin in glucose metabolism in humans. Undercaboxylated osteocalcin (unOC) is the active form of osteocalcin and is released in response to osteoclast activity during bone resorption (Moser and van der Eerden 2019). The role of unOC to regulate glucose metabolism in humans is unclear (Lin, Brennan-Speranza et al. 2018). In a cross-sectional study, unOC was found to be associated with insulin secretion and sensitivity in lean male patients (BMI <25 kg/m^2) (Fernández-Real, Izquierdo et al. 2009). Subjects in this study were generally healthy and non-athletes. In a meta-analysis of osteocalcin and glucose metabolism, a weak negative correlation ($r=-0.09$, $p<0.5$) was found between unOC and fasting plasma glucose in women (Liu, Guo et al. 2015). However, the association between unOC and glucose metabolism was found to be stronger in men than in women. There is limited data on the effects of LEA on glucose metabolism in athletes without diabetes. One study of 7 male long-distance runners found muscle glycogen was reduced by approximately 30% after 3 days of endurance training under energy-restricted conditions (EA = 18.9 ± 1.9 kcal/kgFFM/d) (Kojima, Ishibashi et al. 2020). No studies to date have investigated the relationship between undercarboxylated osteocalcin and glucose metabolism in energy-restricted athletes.

3.2 Describe any relevant preliminary data:

N/A

3.3 Based on the existing literature, provide the scientific or scholarly rationale for and significance of your research and how will it add to existing knowledge:

Maintaining adequate energy intake is essential for athletic performance and general health. Yet, many athletes experience low energy availability (LEA) by failing to consume enough calories to meet their energy demands. Recent studies estimate that the prevalence of LEA in athletes ranges from 22% to 58% with a high prevalence in women and endurance sports (Logue, Madigan et al. 2020). Chronic LEA is associated with several inter-related health consequences involving endocrine function, reproduction, and bone metabolism. Impaired bone health is one of the most concerning consequences of chronic energy restriction. Left untreated, prolonged energy deficiency may impair bone accrual during adolescence and bone formation in adulthood, leading to an elevated risk of fracture and osteoporosis later in life (Papageorgiou, Dolan et al. 2018).

The recommended treatment for LEA is to increase energy availability to optimal levels (45 kcal/kgFFM/d) by increasing EI, decreasing EEE, or a combination of the two (Kuikman, Mountjoy et al. 2021). However, inadequate energy is not always intentional as in the cases of disordered eating/eating disorders and "cutting weight". Some athletes may experience LEA unintentionally due to factors such as inadequate knowledge of fueling recommendations, decreased appetite, lack of time, or low food security (Wasserfurth, Palmowski et al. 2020). Therefore, not all athletes may be willing or able to achieve optimal energy availability. Given the significant risk to long-term bone health, strategies to counteract the effects of LEA on bone metabolism are necessary.

Compared to inactive controls, runners on average have higher BMD and bone strength (Scofield, Hecht et al. 2012). However, lower total and site-specific BMD has been reported in female endurance runners compared to sprinters and athletes competing in higher impact sports (Tenforde, Carlson et al. 2018, Mudd, Fornetti et al. 2007). Lower BMD in this population may partially be attributed to a high prevalence of LEA and risk of disordered eating (DE) and clinical eating disorders (EDs) among female and endurance athletes (Melin, Tornberg et al. 2015). Mechanical loading through weight-bearing exercises, such as high-impact loading activities and resistance training, provides an osteogenic stimulus and non-pharmacological approach to improving bone health (Hart, Nimphius et al. 2017, Beck, Daly et al. 2017). In a cross-sectional study of male distance runners, BMD was found to be significantly higher in runners who reported routine engagement in resistance training compared to runners who did not weight train and untrained controls (Duplanty, Levitt et al. 2018).

Despite the advantages of resistance training on bone health, recommending additional resistance training to athletes with LEA may further exacerbate energy deficiency if EI is not increased. Thus, physical activity interventions to counteract LEA must be designed to achieve maximal osteogenic responses with the minimal possible energy cost. Adding additional exercise without EI compensation may worsen the state of energy deficiency can cause further damage to the athlete's health. Even if an athlete does increase EI, adherence to additional routine resistance training is another potential issue. A survey of

667 competitive distance runners found only 60% of respondents engaged in routine resistance training, with middle-distance (800-3,000 m) runners reporting higher participation in strength and conditioning activities compared to long-distance (5k to half-marathon) runners (Blagrove, Brown et al. 2020). Middle-distance runners were 2.7 and 6.7 times more likely to engage in resistance training compared to long-distance and ultra-distance runners, respectively. An alternative to approach to resistance training that would also apply mechanical loading to the bone is high-impact loading exercises like jumping and plyometric training. Brief jumping exercises have the potential to cause a significant osteogenic bone response with very little energy expenditure, given that a relatively low volume of 10-50 impacts/day at a frequency of 4-7 days/week is required to produce osteogenic effects in premenopausal women (Kishimoto, Lynch et al. 2012, Bailey and Brook-Wavell et al. 2010). The protocol in this proposed study will use jumping exercises at a volume of 5 sets of 10 jumps each day (50 jumps/day) with a 60 second rest between each set. Jumps will be at an intensity of 2x body weight and performed on 5 consecutive days. This approach has several benefits for runners with LEA, with the first being a relatively low energy cost of the jumping intervention which will prevent worsening the LEA state. The second benefit is the short time commitment required for high-impact loading exercises. Given the high running volume of most long-distance runners, engagement in additional cross-training such as resistance exercises may be a challenge. Thus, athletes may find it easier to adherence to the jumping exercises proposed in this study compared to other exercise interventions that require more time and effort. Based on the elevated risk of LEA and low BMD in female endurance runners, this population would benefit from this proposed study.

Findings from this proposed study will be of interest to sports dietitians and athletic trainers based on its potential to improve the clinical management of bone loss in female athletes with LEA through the use of brief, high-impact loading exercises. Future trials based on findings of the proposed study will likely explore the long-term efficacy of high-impact loading during prolonged LEA in active individuals across the spectrum of physical activity levels.

4.0Study Endpoints

4.1 *Describe the primary and secondary **study** endpoints. See links below for discussion of study endpoints and how they may differ from study objectives. These are most common in clinical trials but are sometimes applicable to other types of biomedical research, as well as social, behavioral, or educational research. See link below for a discussion.*

https://docs.google.com/document/d/1Wocz7K7a0hCQJPPO_khh5l1SQQjhGDDGHzcOPRHR5Tw/edit?usp=sharing

Primary Endpoints

Change in Biomarkers of Bone Remodeling in Blood including N-terminal propeptide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, and C-terminal telopeptide of type 1 collagen.

Change in Hormone and Metabolic Markers in Blood including parathyroid hormone, estrogen, insulin-like growth factor 1, hepcidin, insulin, cortisol, leptin, Nesfatin-1, and thyroid hormones

24-h Glucose

Secondary Endpoints

Change in Running Economy
Change in Body Weight
Change in ferritin, TIBC, iron, vitamin D

*4.2 Describe any primary or secondary **safety** endpoints. These should be included for all studies that are greater than minimal risk. (Minimal risk: The probability and magnitude of harm or discomfort anticipated in the research that are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.)*:

Safety Endpoints
Excess weight loss (>5 lbs. during the 5-day experimental conditions)
Excessive change in absolute value for 24-hour glucose concentration
Excessive muscle soreness and/or pain
Fainting or light-headedness
Muscle or joint injury
General fatigue

5.0Study Design and Statistical Analysis Plan

5.1 Describe the basic study design/approach (e.g., qualitative study using five focus groups of first year students to describe assimilation into the university community; randomized controlled trial of a behavioral change intervention to increase dietary intake of whole grains; pre- post-test evaluation of new pedagogical techniques to improve adult literacy):

This study is a randomized, cross-over intervention study that will evaluate the effect of brief, high-impact loading exercises on biomarkers of bone metabolism in energy-restricted, eumenorrheic female runners. Volunteers will complete two, 5-day experimental conditions in a randomized order separated by one menstrual cycle (approximately 3 weeks). Experimental conditions will include a dietary intervention of energy intake equal to 30 kcal/kgFFM/d using controlled diets and an exercise intervention of daily treadmill running with or without an additional 50 impact loading exercises.

5.2 Describe corresponding data analysis plan/approach (e.g., content analysis of focus group transcripts; descriptive analysis followed by linear regression modeling; nonparametric analysis of pre- and post-test measures):

Data will be analyzed using IBM SPSS statistics software. Paired t-test will be used to detect differences in bone biomarkers and hormones within and between experimental conditions (Aim 1). Associations between undercarboxylated osteocalcin concentration and glucose metabolism (i.e., interstitial glucose concentration and serum insulin) will be analyzed using regression analysis (Aim 2). Data will be summarized as mean $\pm$ 1 standard deviation. The significance level will be set a priori at $p<0.05$.

6.0Setting

6.1 *Describe the sites or locations where your research team will conduct the research. Consider each of the items listed below:*

- *Identify where your research team will identify and recruit potential subjects.*
- *Identify where the team will perform the research procedures.*
- *Describe the composition and involvement of any community advisory board(s).*
- *For research conducted in other locations, describe:*
  - *Site-specific regulations or customs affecting the research at those locations.*
  - *Local scientific and ethical review structure at those locations. Examples include work in other cultures or ethnic groups (within or outside of the U.S.) and work with churches. The HRPP will provide additional guidance for international research.*

The research will be conducted at Virginia Tech. Our research team will identify and recruit potential research participants and also perform the research procedures in the Human Integrative Physiology (HIP) Laboratory in the Garvin Innovation Building, 233 Wallace Hall, and the Nutrition and Exercise Metabolism (NEM) Laboratory in the research building located on 2270 Kraft Drive in the CRC.

7.0Study Intervention(s)/Investigational Agent(s)

7.1 Describe the study interventions (including behavioral interventions) and/or investigational agents (e.g., drugs or devices) to be used in this study. Consider each of the items listed below:

- *Drug/Device Handling: If the research involves drugs or devices, describe your plans to store, handle, and administer the drugs or devices so that they will be used only on subjects, and only by authorized investigators.*
- *Describe whether any of the following will be used: microwaves, X-rays, DEXA scans, general anesthesia, or sedation*
- *If control of the drugs or devices used in this protocol will be accomplished by following an established, approved organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.), please reference the SOP in this section.*

The study intervention will consist of controlled meals providing 30 kcal/kgFFM/d and exercise intervention (running with and without impact loading exercises) that will result in energy expenditure of 15 kcal/kgFFM. Menus will be designed by a registered dietitian (T.Sterringer) and provide 55% of total calories from carbohydrates, 20% from protein, and 25% from fat (example diet uploaded). A daily multi-vitamin will be provided during the nutrition intervention to ensure micronutrient needs are met during the acute energy restriction.

The Research does not involve administration of drugs. The research does involve use of Dual-energy x-ray absorptiometry (DXA/DEXA) scans that will be performed at 1 timepoint during the study (baseline). DXA scans will be performed by an ISCD Certified Bone Densitometry Technologist (T. Sterringer).

7.2 List the name of all drugs (including any vitamins, supplements, herbs, or nicotine) to be used in the study. Indicate whether they have FDA approval, and list any limitations for their use:

A standard over the counter, multi-vitamin and mineral supplement (Nature Made) will be provided during the 5-days of controlled experimental diet to ensure micronutrient needs are met in the presence of energy restriction. Nature Made multivitamin supplements are verified by the United States Pharmacopeia (USP), a nonprofit organization that offers third-party verification of product quality and labeling accuracy.

7.3 List all devices, how they will be used, their purpose in the study, and if they will be used in a manner consistent with their approved uses. If they will be used in ways that are not yet FDA approved, indicate whether they need an IDE or a determination that they are exempt from the IDE Determination. If a determination of significant risk or non-significant risk is needed for any of the devices, include the researcher's recommendation for each of those devices:

The medical devices/equipment used in this study include the DXA and the CGM sensors.

The DXA will be used to assess total body composition at baseline. Both devices are FDA approved and the research will involve employment of these devices for approved uses. Scans will be performed only by members of the research staff who are trained and certified bone densitometry technologists (CBDT) through the International Society of Clinical Densitometry.

The CGM will be used only for research and not diagnostic purposes for the intended FDA approved intent of monitoring blood glucose concentration over several days. Prescriptions are not required to obtain CGM devices from Abbott Laboratories (FreeStyle Libre) if they are to be used for research purposes. Dr. Larson-Meyer has experience using CGM for research purposes as part of an ongoing research project (IRB #21-561).

7.4 If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

- *Identify the holder of the IND/IDE/abbreviated IDE.*
- *Explain procedures followed to comply with sponsor requirements for FDA regulated research for the following:*

	Applicable to:		
FDA Regulation	**IND Studies**	**IDE studies**	**Abbreviated IDE studies**
21 CFR 11	**X**	**X**	
21 CFR 54	**X**	**X**	
21 CFR 210	**X**		
21 CFR 211	**X**		
21 CFR 312	**X**		
21 CFR 812		**X**	**X**
21 CFR 820		**X**	

N/A

8.0Procedures Involved

8.1 Describe and explain the study design:

This study will employ a cross-over design in which 12 eumenorrheic women between the ages of 18 and 35 will complete two experimental conditions in a randomized order using a computer program with a random number generator. The cross-over study design will help control for inter-subject variability in response to LEA (Guebels, Kam et al. 2014, Reed, De Souza et al. 2015).

As outlined in the table, eligible participants will undergo two, 5-day experimental conditions separated by one menstrual cycle (approximately 24 days) consisting of an energy-restricted diet and endurance training regimen. Experimental conditions will include a dietary intervention of energy intake equal to 30 kcal/kgFFM/d using controlled diets and an exercise intervention of daily treadmill running with one condition of run only (RUN) and one condition with running and 50 high-intensity, impact loading "jumping" exercises (RUN+IL) After providing informed consent, participants will be randomly assigned to the RUN or RUN+IL condition. Outcome data will include assessment of serum biomarkers of bone metabolism and circulating hormones important for bone metabolism. Compliance to the nutrition and exercise regimen will be evaluated at regular intervals and include collection of packaging and uneaten food at the end of each 5-day condition, measurements of body weight before and after each experimental phase to assess change, and physical activity data collected by accelerometer and digital technology provided by the research team (Garmin smart watch and My PT Hub app).

Dietary Intervention. During the two experimental conditions, participants will be provided controlled, weighed diets equal to 30 kcal/kgFFM/d. Energy intake will be manipulated individually based on the FFM of participants measured via DXA. Diets will be prepared by a registered dietitian (TS) in the research kitchen in Wallace Hall and standardized between conditions to include three meals and one snack. Menus will consist of similar whole foods and commercial products that provide approximately 55% of total calories from carbohydrates, 20% protein, and 25% fat. Diets will be modified based on participant allergies or preferences, within reason. Participants will be instructed to consume the meals and snack at approximately the same time each day to avoid within-day fluctuations in energy balance (Fahrenholtz, Sjödin et al. 2018). They will also be instructed not to consume any other foods or beverages other than water and non-calorie beverages (e.g., black coffee, unsweetened tea). A daily multi-vitamin will be provided to participants during the experimental conditions to provide adequate micronutrient intake during the energy-restricted state. The key investigators are both registered dietitians with experience assessing energy balance. The PI and has extensive experience conducting controlled feeding trials.

Exercise Sessions. Participants will undergo supervised exercise sessions consisting of treadmill running with and without high-impact exercises on 5 consecutive days in the NEM laboratory on two occasions separated by one menstrual cycle (approximately 24 days). On the first day of each experimental condition, exercise energy expenditure (EEE) will be measured using indirect calorimetry (ParvoMedics) during a controlled "titration" run to help determine running speed at 65% VO2max. A heart rate monitor will be used simultaneously to measure heart rate. Total EEE for the exercise session is approximately 15 kcal/kgFFM. From this, the running protocol (duration) needed to expend or "burn" a total of 15 kcal/kgFFM will then be determined and used throughout the study. In the RUN condition, participants will run on the treadmill run at that pace for the amount of time needed to expend 15 kcal/kgFFM each day of the five-day intervention. Running time on the treadmill will vary based on participant body weight, percent body fat, VO2max, and running efficiency. Treadmill duration will be determined based on an expenditure of 15 kcal/kgFFM while running at an intensity of 65% VO2max. We estimate total running time for participants to fall within a range of approximately 50-65 minutes. For example, a runner with the following characteristics: body weight = 62kg, body fat % = 30%, VO2max = 55 ml/kg/min, would need to run for 58 minutes to meet an energy expenditure of 15 kcal/kgFFM. Runners with a higher running efficiency and VO2max will have slightly shorter running durations compared to runners who are less efficient. In the RUN+IL condition, participants will start by performing 5 sets of 10 high-impact loading jumping movements for a total of 50 impacts/session at intensities greater than 2x bodyweight with 60 seconds of rest between sets to stimulate an osteogenic response according to the guidelines for osteoporosis prevention recommended by the Exercise and Sport Science Australia (ESSA) (Beck, Daly et al. 2017). Intensity of the jumping exercises will be assessed using ground reaction force measured using dual force plates. Participant body weight will be obtained before each exercise session to ensure GRF of jumping exercises is at the desired intensity. The digital scale and monitor will be separate, and body weight will not be shared with the participant. Indirect calorimetry will also be used simultaneously on day one of the intervention (only) to assess the energy expended during the jumping exercises. Participants will then run on the treadmill at 65% VO2max until combined energy expenditure of impact loading and treadmill running reaches the target 15 kcal/kgFFM. Participants will also wear an accelerometer and smart watch devices during the experimental conditions to measure activity level. All activity tracking devices will be provided by the research team. Participants will be instructed to refrain from all physical activity outside of the supervised exercise sessions that are not related to activities of daily living (e.g., getting dressed, walking to the car).

Table 1. Overview of Data Collection

Overview of Data Collection								
			RUN and RUN+IL Experimental Conditions					
	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Body Mass (weight)	x	x	x	x	x	x	x	x
Body Composition by DXA	x							
BMD (total body, dual femur, lumbar spine) by DXA	x							
Urine collection for hydration status	x							
Pregnancy test	x	x*						
Low Energy Availability in Females Questionnaire	x							
Vitamin D Questionnaire	x							
Blood via venipuncture								
CBC	x							
TSH	x							

Progesterone		x						
Vitamin D (total and free 25(OH)D)		x						
N-terminal propetide of type 1 procollagen		x						x
Undercarboxylated osteocalcin		x						x
Sclerostin		x						x
C-terminal telopeptide of type 1 collagen		x						x
Parathyroid hormone		x						x
Estradiol		x						x
Insulin-like growth factor-1		x						x
Hepcidin		x						x
Markers of iron status (Ferritin, iron, total iron-binding capacity [TIBC])		x						x
Insulin		x						x
Thyroid hormones (Free triiodothyronine (T3), free thyroxine and rT3)		x						x
Leptin		x						x
Cortisol		x						x
Nesfatin-1		x						x
Health outcomes								
Continuous glucose monitoring			x	x	x	x	x	
Compliance and Fitness Testing								
Habitual dietary intake (food records for 3 days)	x							
Habitual exercise tracking (smart watch for 3 days)	x							
Running Economy			x				x	
Aerobic Capacity (VO2max)	x							
Treadmill running at 65% VO2max			x	x	x	x	x	
Physical Activity tracking (smart watch)			x	x	x	x	x	
Mealtime Log			x	x	x	x	x	

Note: The 5-day intervention, RUN or RUN+IL, occurs on days 2-6

pregnancy test performed at only 2 timepoints: baseline and day 1 of the **second experimental condition after the 3-week washout period*

8.2 Provide a description of:

- *All research procedures being performed*

- *If the study has more than one procedure, session, and/or subject population, describe each procedure, session, and/or study population separately. For complex studies, you are encouraged to include a figure or chart.*

Phone Screen

Those who respond to the investigatior's advertisements will be scheduled to complete a brief telephone (or HIPAA-compliant Zoom) screening to confirm basic eligibility criteria. Participants will be made fully aware of the eligibility criteria, time commitment, possible risks and their right to withdraw from the study at any time. A phone screening script and phone screening data collection form will be used for phone screening conducted by a registered dietitian nutritionist.

Baseline Screening (approximately 1 hour)

Informed Consent: Participants will be provided an informed consent form following the phone screening and in advance of coming to the laboratory.

Health History: Subjects will be asked to complete a standard health/medical history questionnaire, which will be used to screen for health issues (e.g., coronary or congenital heart disease) or other reasons (medications which influence study results) that would preclude participation (see uploaded Health History Screening Questionnaire). This questionnaire has been used by Dr. Larson-Meyer for clinical studies for the past 17 years.

Low Energy Availability in Females Questionnaire (LEAF-Q): Subjects will be asked to complete the LEAF-Q, which will be used to screen for risk of existing LEA, medication use, menstrual function, and injury history. The LEAF-Q has been validated as a screening tool for LEA in female endurance athletes (Melin, Tornberg et al. 2014). Individuals who score as "high risk" for LEA will be provided referral information to a local sports dietitian.

Body Weight Height and Composition: Body weight and height will be measured on a digital physician's scale. Percent body fat and fat-free mass will be measured in all subjects via DXA scan.

Bone Mineral Density: Total body, dual-femur, and lumbar spine BMD will be measured in all subjects via DXA scan. Scans will be conducted by T. Sterringer who is an ISCD Certified Bone Density Technologist.

Urine sample and pregnancy test: All participants will be required to provide a small cup of urine immediately before the DXA at baseline. The urine will be evaluated for hydration status via specific density using a refractometer. A pregnancy test will be performed on the urine sample for all participants.

Baseline Laboratory Testing Session (approximately 1 hour).

All measurements will be performed in the morning after a 12-hour fast (no caffeine) with the participants instructed not to engage in heavy exercise for 36 hours prior to testing and to adequately hydrate the evening prior.

Blood Draw: Blood will be drawn by venipuncture after a 12-hr fast (no caffeine) for the following: CBC, TSH, and progesterone. In total, blood will be draw five times during the study by venipuncture from a vein in the arm. These include baseline, and the days immediately before and after each experimental

condition as explained below. Blood will be drawn with the participant seated quietly in a phlebotomy chair. Approximately 10 ml will be obtained during the baseline visit.

Aerobic capacity (i.e., VO2max): A graded exercise test will be performed on a treadmill to assess aerobic fitness via indirect calorimetry (Parvo Medics TrueOne 2400). Heart rate will be measured during the test by a heart rate strap and sensor. The test will begin with a 5-minute warm-up at 0% grade and at a speed predetermined by the participant. Following the warm-up phase, the workload will be increased each minute by increasing treadmill speed or grade by 0.5 mph or 2.5%, respectively until the participants can no longer continue or volitional exhaustion is reached. The entire exercise testing protocol will last 12-20 minutes.

Habitual Diet and Exercise (3 days)

Habitual Diet and Exercise: Following laboratory testing, participants will be instructed on completing food records for 3 days (2 weekday, 1 weekend). Food records will be analyzed using Nutrition Data System for Research (NDSR; University of Minnesota), a dietary analysis software program. Participants will also wear a smart watch over this same time period that will be provided by the research team. The smart watch will use an app to measure heart rate and estimate energy expended during structured exercise or physical activity; this will be used to help estimate the participants total energy expenditure. It will be necessary to have the GPS function turned on during the collection of physical activity data but only data related to time, distance or intensity and no GPS coordinates will be downloaded or recorded.

Vitamin D questionnaire: Participants will complete a questionnaire to assess vitamin D status over the previous month (DE Larson-Meyer). The vitamin D questionnaire includes a 52-item food frequency questionnaire, 6-item supplement section, and 7 questions related to sunlight exposure.

Intervention

After baseline screening and testing, eligible participants will be scheduled to complete two experimental conditions in a randomized order beginning on the second or third day of menstruation, depending on the participant's schedule and available appointments. Each experimental condition will require participants to come into the Nutrition and Exercise Metabolism (NEM) laboratory on 7 consecutive days. Table 1 outlines (section 8.1) provides an overview of data collection for each scheduled laboratory visit. On day 1, fasted blood draws will be collected in the morning after a 12-hour overnight fast and body weight will be measured on a digital physician's scale. Participants will complete the LEA experimental trials on days 2-6. A follow-up blood sample will be collected on the morning of day 7 after an overnight fast and body weight will be measured on a digital physician's scale.

Pregnancy Test: a pregnancy test will be performed after the 3-week washout period on day 1 of the second experimental condition.

Blood Draw: Blood (approximately 20-25 ml) will be drawn the days before and after each LEA experimental trial by venipuncture after a 12-hr fast (no caffeine) for the following: Progesterone, total and free 25(OH)D, N-terminal propetide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, C-terminal telopeptide of type 1 collagen, parathyroid hormone, estradiol, insulin-like growth factor, hepcidin, ferritin, TIBC, iron, cortisol, leptin, nesfatin-1, insulin, free T3, free T4, rT3. In total, blood will be draw five times during the study by venipuncture from a vein in the arm with a total of 110 ml obtained for the duration of the study. Blood will be drawn with the participant seated quietly in a phlebotomy chair.

Dietary Intervention: During the two experimental conditions, participants will be provided controlled, weighed diets of 30 kcal/kgFFM/d. Total energy of diets will be individualized based on the participant's FFM measured via DXA. Diets will be prepared by a registered dietitian (T. Sterringer) in the research kitchen in Wallace Hall and standardized between conditions to include three meals and one snack. Menus will consist of the same whole foods and commercial products that provide diets consisting of approximately 55% carbohydrates, 20% protein, and 25% fat. For example, a woman weighing 54.5 kg (120 lbs.) with a body fat percentage of 22% (FFM = 42.5 kg) would be provided with a diet containing 1275 kcal (55% total calories = 175g carbohydrates, 20% = 64g protein, 25% = 35g fat) based on 30 kcal/kgFFM (example menu uploaded). Participants will be instructed to consume the meals and snack at approximately the same time each day to avoid within-day fluctuations in energy balance (Fahrenholtz, Sjödin et al. 2018). They will also be instructed not to consume any other foods or beverages other than water and non-calorie beverages (e.g., black coffee, unsweetened tea). A daily multi-vitamin will be provided to participants during the experimental conditions to ensure adequate micronutrient intake during the energy-restricted state. Participants will be asked to log the times they consume each meal and snack in a mealtime log for the 5-day experimental phases. The primary investigator (E. Larson-Meyer) is a registered dietitian and has extensive experience with assessment of energy balance with controlled feeding trials.

Exercise Sessions: Participants will undergo supervised exercise sessions consisting of treadmill running with and without high-impact exercises on 5 consecutive days in the NEM laboratory on two occasions separated by one menstrual cycle (approximately 24 days). On the first day of each experimental condition, exercise energy expenditure (EEE) during the sessions will be measured using indirect calorimetry (ParvoMedics) and heart rate to ensure total EEE is approximately 15 kcal/kgFFM. In the RUN condition, participants will complete a 5 min warm-up (at 5.5, 6 or 6.5 mph) and then run on a treadmill at 65% VO2max for the duration determined at baseline (see protocol in section 8.1) to elicit an EEE of 15 kcal/kgFFM. In the RUN+IL condition, participants will perform impact loading movements (jumping) and a running protocol. Participants will come to the lab in the morning to perform 5 sets of 10 impact loading movements for a total of 50 impacts/session at intensities greater than 2x bodyweight. Each jumping set will be separated by 60 seconds of rest in alignment with guidelines for osteoporosis prevention recommended by the Exercise and Sport Science Australia (ESSA) (Beck, Daly et al. 2017). Ground reaction force of impacts will be measured using dual force plates. Approximately 4-6 hours later, participants will return to the lab to complete a 5-min run and warm-up on the treadmill at 65% VO2max until a combined energy expenditure of the morning impact loading exercise and afternoon/evening treadmill running reaches 15 kcal/kgFFM. A recovery period of at least 4 hours is recommended between impact loading sessions for optimal osteogenic bone response (Hart, Nimphius et al. 2017). Participants will wear accelerometers and smart watch devices during the experimental conditions to measure activity level. Participants will be instructed to refrain from all physical activity outside of the supervised exercise sessions that are not related to activities of daily living (e.g., getting dressed, walking to the car).

Body Weight: Body weight will be obtained before each supervised exercise session begins using a digital physician's scale to monitor significant changes in body weight during the intervention. Participant body weight will also be needed for monitoring the impact of the jumping exercises using ground reaction force in the RUN+IL condition. The scale and monitor will be separate and daily body weight will not be shared with participant.

Ground Reaction Force (GRF): Ground reaction force of jumping exercises will be performed using dual force plates. Participants will perform 5 sets of 10 vertical jumps with 60 seconds of rest between each set. Jumping exercises will be tailored until intensity reaches a threshold of at least 2x body weight of participant. These jumping exercises will be used in the RUN+IL condition.

Running Economy: Running economy will be assessed on the first and last day of each experimental condition at the start of each supervised treadmill run. Heart rate will be measured during the test by a heart rate strap and sensor (Polar). The test will begin with participant running for 4-minutes at three moderately easy speeds of 5.5, 6 6, and 6.5 mph. The last two minutes of oxygen consumption and carbon dioxide production data will be used to determine metabolic economy (ml oxygen consumed per kg body weight per minute relative to the set work performed). This test will last 12 minutes. Following cumulation of the standard economy test, the workload will be increased to the 65% VO2max. Oxygen consumption will be measured for the first 4 to 10 minutes of the steady state run.

Continuous Glucose Monitor (CGM): Interstitial glucose concentration will be measured during the two, 5-day experimental conditions using a CGM device which is typically worn on the back of the upper arm. This requires that a small amount of interstitial fluid (0.5 microliters) be sampled every 15 minutes (96 times per day) throughout the course of the 5-day experimental conditions. CGM will be placed on the day before each LEA condition and removed on the day after each condition.

8.3 Describe:

- *Procedures or safeguards intended to reduce the probability and magnitude of risks. (For example: Reducing the risk of injury in a virtual reality study either by having the subjects sit during the study or by providing an obstacle-free space for walking.)*
- *Be sure to describe all drugs and devices used in the research, when they will be administered or used, and their purpose.*
- *Methods used to collect data about subjects. Please upload all data collection forms to Protocol Management. Some common examples are:*
  - *Screening questionnaires*
  - *Survey(s), including online surveys*
  - *Demographic questionnaire(s)*
  - *Interview guide(s), e.g., questions or pool of questions for semi-structured interviews*
  - *Focus group guide(s)*
  - *Other documents used to collect data*

The following safeguards will be employed to reduce the probability and magnitude of risks associated with study participation. The specific risks are highlighted in Section 17.

Energy Restriction: Potential risks associated with energy restriction include loss of lean body mass (LBM), suboptimal macronutrient and micronutrient intake, hypoglycemia, and mood disturbances. These risks will be minimized by providing a daily multivitamin to participants for the duration of the 5-days of energy restriction. Menus will also be designed to provide 20% of total calories from protein to help preserve LBM. To reduce risk of hypoglycemia, diets will provide 55% of total calories from carbohydrates and participants will be encouraged to consume the provide snack containing 30g carbohydrate approximately 30 minutes before the exercise sessions. Additionally, menus will be designed to prioritize foods with fiber and low energy density that promote fullness and satiety (e.g., leafy greens, fresh non-starchy vegetables, whole grains and other high-fiber foods) to reduce physical and

psychological burden of energy restriction. Participants with existing menstrual disturbances (determined by progesterone and self-report), LEA (determined by LEAF-Q score), and/or low BMD (determined by DXA) will be excluded from the study to prevent worsening existing conditions.

High-Intensity Exercise: Potential risks associated with endurance running and high-impact loading exercises include musculoskeletal injuries, changes in blood pressure, gastrointestinal discomfort, fainting, and dizziness. These risks will be minimized by having participants assessed prior to each exercise session by a trained research member to evaluate readiness for physical activity. Additionally, participants will complete all exercise sessions in the lab under the supervision of a certified strength and conditioning specialist with CPR certification (T. Sterringer).

CGM: Potential risks associated with the CGM include discomfort during the insertion, pain, inflammation, redness/rash, swelling, minor bleeding and minor infection at the site.
These risks will be minimized by having a trained member of the research staff perform the procedure under aseptic conditions. Participants might also experience the aforementioned symptoms as a result of contact between the adhesive pad of the sensor and the skin. In rare cases, an infection can spread to other parts of the body. Allergic reactions can develop in response the adhesive used to keep the CGM in place. If these symptoms occur, participants have the ability to remove the CGM at will. Symptoms typically resolve within a short time (approximately one week).

Questionnaires and Study Logs: All study questionnaires (except the food records) will be collected with the participant sitting in a private setting in the laboratory. Questionnaires will be placed in each participant's study file date entered for data analysis.

Blood draws: Blood will be collected using universal precautions by a trained technician. Blood will be drawn by venipuncture from a vein in the arm with the participant resting in a phlebotomy chair. Blood will be drawn at five times during the study (baseline, RUN day 1, RUN day 7, RUN+IL day 1, RUN+IL day 7) with a total of 110 ml of blood collected over the course of the 5-6 weeks.

DXA scan: Participants will be exposed to a very low dose of ionizing radiation as part of the DXA scan at baseline only. DXA procedure will be performed by trained staff. Participants will be informed of the risk of radiation exposure prior to study enrollment. Female participants will complete a pregnancy test by urine immediately before the DXA.

VO2max/Aerobic capacity: Trained research personnel will be present during the test to correctly place the mouthpiece, monitor all variables during the test and support the participant at the end of the test.

8.4 What data will you collect during the study and how you will obtain them? Please include descriptions of electronic data collection, database matching, and app-based data collection:

Anthropometric and basic demographic (age) data will be recorded on data sheets and manually entered into a database (excel format) on a secure computer. Select data (DXA results, VO2max, ground reaction force) may be transferred electronically directly from the DXA, metabolic cart, or force plates into excel spread sheets, if possible. Blood results will be entered directly from laboratory sheets provided by a commercial laboratory or the Metabolic Core at Virginia Tech. Heart rate, energy expended and exercises performed will be downloaded onto the laboratory computer from the Smart Watch. Glucose concentration in interstitial fluid samples by time will be downloaded directly from the CGM sensor onto the lab computer.

8.5 *Who will transcribe or code audio and/or video recordings?*:

N/A

8.6 *Include a description of any deception to be used in the study. Include justification for the use of deception (why the deception is necessary), describe the debriefing process, and describe how the study meets <u>all</u> the following criteria for alteration of consent (deception is considered an alteration of informed consent):*

- *The research involves no more than minimal risk to the subjects*
- *The alteration will not adversely affect the rights and welfare of the subjects*
- *The research could not practicably be carried out without the alteration/deception*
- *(Optional but encouraged in most cases) Subjects will be provided with additional pertinent information after participation (i.e., debriefing for studies involving deception)*

N/A

8.7 *If the study involves long-term follow-up (once all research related procedures are complete), describe what data will be collected during the follow up period and when it will occur*:

N/A

9.0Data and Specimen Long Term Storage and Use

9.1 *If you will store data or specimens for future use, describe where you will store the data or specimens, how long they will be stored, and how and by whom the data or specimens will be accessed*:

All data will be stored in a locked cabinet in Dr. Larson-Meyer's laboratory which will also be locked to only authorized personnel. The computer data will be stored in the locked lab on a computer that is password protected. All de-identified data will be kept indefinitely.

9.2 *For specimens, list the data to be stored or associated with each specimen*:

Blood and urine samples will be stored in a -80-degree freezer in the HIP laboratory currently located in the Garvin Building. Samples will be labeled with the participants' study code (see section 9.4 below), the visit number and the date and time of the

collection. No identifying information will be written on specimen samples. The freezer is located in locked room/laboratory.

Blood analyzed by the Metabolic Core at Virginia Tech, housed in the Integrated Life Sciences Building, may also be temporarily stored in a freezer in this laboratory immediately before, during or after analysis.

9.3 Describe the procedures to release data or specimens outside of the research team, including the process to request a release, approvals required for release, who can obtain data or specimens, and what data will be provided with specimens:

Some de-identified blood and urine samples will be sent to a commercial laboratory for analysis based on cost savings. There are currently no plans to release data outside of the research team.

9.4 Describe the identifiers to be included with stored data or specimens, as well as any key or code that could be used to make them identifiable. Describe where the code will be stored, who will have access to it, and when it will be destroyed:

Study Codes using a combination of letters and numbers will be used to de-identify subjects from their personal information. No obvious identifiers will be stored with the data; the data spreadsheet, however will include each participants' age and starting weight as part of the de-identified data. Original de-identified data collection sheets will be stored in a locked file cabinet as part of study records; scans of some de-identified information may be kept in a password-protected electronic file that is accessible only to research personnel. During the active phase of the study, a master document (key) that will contain the participants name, assigned study code and randomization order will be kept in a password-secured file that will be accessible only to the PI and authorized study personnel (doctoral student in charge of the study, T. Sterringer). The key will be destroyed 6 to 12 months after collection of data from the last participant. De-identified data may be kept indefinitely. Blood and urine samples will be destroyed after 5 years.

9.5 Please select the identifiers you will obtain (whether directly from participants or from another source), including but not limited to:

☒	*Name*
☒	*Geographical subdivisions smaller than a state, including street address, city, county, precinct, zip code, and equivalent geocodes (note, the initial three digits of a zip code are not considered identifiable)*
☒	*Elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death, and single year of age over 89 and all elements of dates (including year) indicative of such age (note, such ages and elements may be aggregated into a single category of age 90+)*
☒	*Phone numbers*

☐	*Fax numbers*
☒	*Electronic mail addresses (e-mail)*
☐	*Social Security numbers*
☐	*Medical record numbers*
☐	*Health plan beneficiary numbers*
☐	*Account numbers*
☐	*Certificate/license numbers*
☐	*Vehicle identifiers and serial numbers, including license plate numbers*
☐	*Device identifiers and serial numbers*
☐	*Web Universal Resource Locators (URLs)*
☐	*Internet protocol (IP) address numbers*
☐	*Biometric identifiers, including finger and voice prints (audio recording)*
☐	*Full face photographic images and any comparable images (including video recording)*
☐	*Student record number or identification number*
☐	*User name for online or computer accounts*
☐	*Any other unique identifying number, characteristic, or code (note this does not mean the unique code assigned by the investigator to code the data)***:** Click here to explain.

10.0 Sharing of Results with Subjects

10.1 Describe whether you will share results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) with subjects or others (e.g., the subject's primary care physician). If so, describe how you will share the results and include this information as part of the consent document. Upload materials you will use to explain the results to subjects:

At the conclusion of the study or when the participants' involvement in the study ends, interested participants will be provided with individual results related to their body composition, BMD, fitness, and pertinent blood markers. These data will be summarized on a TBD summary document that the participant can pick up at the lab or have (upon request) mailed to them at a provided address. This form will be submitted as an Addendum before it is provided to the first participant. Participants will only be provided results during the study if it is determined that the participant has a result for any measured outcomes that is out of the normal range; in this case the participant would be provided information about the value and asked to see their personal health care provider. The participants will also be notified when a summary of the study findings is published if an active email address is on file.

11.0 Study Timelines

11.1 Describe:

- *The duration of an individual subject's participation in the study (for example, 1 hour, 2-4 weeks, 3-5 years).*
- *The amount of time expected to enroll all study subjects (weeks, months, years, etc.)*

- *The amount of time expected for the investigators to complete this study including primary data analyses.*

The duration of an individual's participation in this study will be approximately 6 to 10 weeks, which will include a Zoom or telephone call for screening, a screening visit in the laboratory, a baseline visit, three days of habitual diet and exercise tracking, two experimental conditions, and a washout period of 1 menstrual cycle (approximately 3 weeks). The actual time and frequency of the subject's visits will depend on their schedule and that of the study staff. Participants will begin the study on a rolling basis, but the entire study will take place across approximately 6 months based on enrollment of 1-2 participants per week. The investigators will complete primary data analyses within the following year but all analyses of study data may not occur for up to ten years following study completion.

12.0 Inclusion and Exclusion Criteria

12.1 Describe how you will screen individuals for eligibility. When will screening occur and what procedures will you use? Upload any screening scripts or surveys to Protocol Management:

Those who respond to the investigation's advertisements will be asked to complete a brief telephone (or Zoom) screening to confirm basic eligibility criteria. Participants will be made fully aware of the eligibility criteria, time commitment, possible risks and their right to withdraw from the study at any time. A phone screening form will be used for this purpose conducted by a research team member who is also a registered dietitian nutritionist (RDN). The Low Energy Availability in Females Questionnaire will be used to screen for risk of existing LEA, medication use, menstrual function, and injury history.

12.2 Describe the eligibility criteria that define who will be included and who will be excluded from enrollment for each procedure of your study. Include any geographic criteria (e.g., Virginia Tech undergraduate students, a national sample of adults with engineering degrees, minors aged 8-12 in the New River Valley, university faculty in Virginia and Paris, France):

Eligible participants will be well-trained, eumenorrheic female runners between the ages of 18-35 years that are weight-stable with a BMI between 18.5-30 kg/m^2. Participants with LEAF-Q scores ≥8, existing menstrual disturbances measured by progesterone and self-report, or low BMD (z-score <-2) will be excluded from the study to prevent worsening existing conditions and recommended to follow up with their primary care physician and/or a registered dietitian nutritionist. During the phone screening, participants will be asked if they have ever been diagnosed with an eating disorder and the circumstances surrounding the diagnosis. If the participant has just recently recovered (within the last 12 months) or is still in recovery, she will be excluded from the study. The LEAF-Q was not developed to assess disordered eating behavior, however, a study in ultra-marathon female runners found a significant association between high LEAF-Q scores and disordered eating (Folscher, Grant et al. 2015). Athletes who score 8 or higher on the LEAF-Q will be excluded from the study and referred to a local sports dietitian. Participants using contraceptives (oral contraceptives, injections, IUD, etc.) will be excluded from this study. Contraceptive use may mask menstrual irregularities and oral contraceptives may influence glucose metabolism (Lopez, Schultz 2007), a secondary outcome of this study. Adequate training status for the study protocol will be assessed based on running volume, frequency, and VO2max. Eligible participants must be able to run on 5 days/week for at least 60 minutes to meet the training requirements of the study protocol. Additional exclusion criteria include history of fracture in previous 6

months, medication use that could potentially affect bone metabolism (e.g., corticosteroids, anticonvulsants, heparin, gonadotropin-releasing hormone agonists), pregnancy, lactation, abnormal TSH, and routine engagement in mechanical loading exercises. Participants must be willing to consume the diets provided, however, diets will be modified based on patient allergies or preferences, within reason. Participants will be excluded if they have dietary restrictions or preferences that would prevent them from consuming meals that fit within the experimental conditions. For example, we cannot modify diets to meet low-carbohydrate preferences given the experimental diets must contain 55% of total calories from carbohydrates. Similarly, participants who are unable to consume high-fiber diets will be excluded.

12.3 Indicate specifically whether you will include or exclude each of the following special populations: (You may not include members of these populations as subjects in your research unless you indicate them in the description of your subject population.)

- *Minors, as defined by state law where the study is performed (infants, children, teenagers)*
- *Pregnant women (can be included in minimal risk studies by mentioning in section 13.1)*
- *Prisoners (including all incarcerated individuals)*
- *Adults not capable to consent on their own behalf*

None of the above will participate.
Pregnant women are excluded because intentional energy restriction is not appropriate during pregnancy. A pregnancy test will be performed at baseline prior to the DXA scan and after the 3-week washout period on Day 1 of the second experimental condition.

13.0 Vulnerable Populations

13.1 If the research involves individuals who are vulnerable to coercion or undue influence, please describe additional safeguards you will include to protect their rights and welfare. Consider the applicable items listed below:

- *If the research involves Virginia Tech students, indicate whether these are students of any of the investigators. If so, describe whether the activities will take place during class time as part of the curriculum and the steps you will take to reduce the possibility that students feel obliged to participate in order to improve their course grade. The HRPP can provide further guidance as needed. Describe whether you will request access to student records (e.g., SAT, GPA, GRE scores).*
- *If the research involves employees of Virginia Tech or the research sponsor, describe steps you will take to ensure that the employees are freely participating and describe how their data will be protected from inspection by their supervisors.*
- *If the research involves Virginia Tech NCAA athletes, you must obtain approval from the athletic department.*
- *For research involving Montgomery County Public Schools, you must obtain county approval (after obtaining contingent Virginia Tech approval). Other locales have different requirements; please check on these and describe here. Approval is typically granted by the superintendent, principal, and classroom teacher (in that order). Approval by an individual teacher is insufficient. School approval, in the form of a letter or a memorandum should be uploaded as a supporting document.*

- *If the research involves pregnant women, review "CHECKLIST: Pregnant Women (HRP-412)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves prisoners, review "CHECKLIST: Prisoners (HRP-415)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research (minors), review the "CHECKLIST: Minors (HRP-416)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves cognitively impaired adults, review "CHECKLIST: Cognitively Impaired Adults (HRP-417)" to ensure that you have provided sufficient information in this protocol.*

This research study has the potential to include students and employees of Virginia Tech. However, during the consenting process, the participants will be made aware that only members of the research study team will have access to their data and that this data will utilize a coding system making their data unidentifiable. This data will be locked away and they will be made fully aware of their right to withdraw from the study at any time. If Virginia Tech athletes are interested in the study, they will only be allowed to participate during their off-season and after first obtaining approval from the athletics department.

14.0 Number of Subjects

14.1 Indicate the total number of subjects to be enrolled and how this number was determined (e.g., sample size calculation [show], number of available subjects in a finite pool, number of tests funding award would allow):

A power analysis determined that 12 women will be required to detect a clinically significant change of 10μg/L in PINP with 80% power at $P < 0.05$ based on results from a similar study of LEA during dietary restriction (Papageorgiou, Martin et al. 2018). We aim to enroll 20 participants to account for possible dropout.

14.2 If this is a multi-site study, indicate the number of subjects to be enrolled at this site and the total to be enrolled from all sites:

N/A

14.3 If applicable, indicate the number of potential subjects you expect to screen for enrollment, and the number of subjects you will need to complete the research procedures:

We anticipate that we may need to screen between 50-60 participants to recruit and complete the required 12 participants.

14.4 If the study has more than one procedure, indicate the total number of subjects to undergo each procedure separately:

All enrolled participants will undergo the two experimental procedures.

15.0 Recruitment Methods

15.1 Describe when, where, and how you will recruit potential subjects:

Participants will be recruited through flyers placed at strategic locations (gyms, fitness, and recreational centers, etc.) at colleges and universities in the New River Valley (including Virginia Tech), local running stores, running groups in New River Valley, and through targeted listservs (VT News), emails, and social media posts.

15.2 Describe the source of subjects (for example, clinic patients with specific conditions, students in the library, community members at a gathering, or members of a local gym):

We will recruit from the general population of athletes and active individuals. This will include recruiting members of local running groups and residents of New River Valley. We welcome diverse participants from all racial, ethnic, educational, financial, and social backgrounds. Given the nature of this laboratory-based intervention, subject recruitment will be limited to individuals residing in the New River Valley and surrounding areas. Increased recruiting efforts will be targeted at Radford City County given the increased diversity of this county compared to the other 4 counties in New River Valley.

15.3 Describe the methods that you will use to identify potential subjects:

As mentioned previously above (15.2), we will identify participants through use of flyers placed at strategic locations and through targeted listservs, emails and social media posts.

15.4 Describe materials that you will be use to recruit subjects. Attach copies of these documents with this protocol in Protocol Management and be sure to include the IRB protocol number on each document.

- *For flyers, attach the final copy of printed flyers.*

- *For Virginia Tech News, Facebook postings and ads, newspaper ads, websites, MTurk/SONA/online survey systems, etc., attach the final wording and graphics to be used.*
- *For email recruitments, please include the subject line.*
- *For advertisements meant for audio broadcast, please submit the wording of the advertisement prior to taping (to avoid having to re-record with approved language) and submit the final recorded version for IRB review before use.*
- *Describe any compensation to subjects. Separate compensation into appropriate categories, such as: reimbursement for expenses, time and effort, and additional incentives for study participation. For each category, specify the amount (including any pro-rated amount), schedule, and method of payment.*

A draft copy of our recruitment flyer is uploaded. This flyer will be posted at strategic locations throughout Blacksburg and the surrounding area. We will seek permission at each site as necessary before posting or hanging a flyer. We also plan to use this same advertisement for emails and a modified version for social media posts (that will be submitted for approval at a later date as an Addendum). Emails will use the subject line "Volunteers needed for study on bone health of long-distance runners". Participants will be compensated $25 for completing the baseline visit and first experimental condition (7 days). Participants will be compensated an additional $75 for completing the second experimental condition. This is a total of $100 compensation for completing all testing visits and experimental conditions. Participants that do not complete the second condition will receive information about body composition, BMD, and aerobic fitness (VO2max, running economy), in addition to the $25 compensation. Given the cross-over design of this study, data cannot be used for participants that do not complete both conditions. The experimental conditions will be separated by 3 weeks of their normal diet and exercise routine and not require any lab visits or intervention. The total time commitment for this study will be 19 days. Payment in the form of cash will be scheduled after each individual participant completes each experimental condition.

16.0 Withdrawal of Subjects

16.1 Describe circumstances under which you anticipate subjects could be withdrawn from the research without their consent:

Participants could be withdrawn from the study if they are not showing up for appointments and/or exercise sessions, are not consuming the provided diets, or are not completing or complying with all procedures. They also may be withdrawn if they develop and injury or illness that would prevent them from doing everything that is expected for the study or which might compromise their health.

16.2 If applicable, describe any procedures for orderly termination (e.g., discontinuation of a study drug or debriefing after a behavioral intervention):

If a participant is not complying with the study, the PI or another member of the study staff will first discuss these difficulties with the participant and explain the importance of adhering to the intervention for the purpose of the study. If it is determined that the participant be terminated or discontinued from the study for reasons as described above, the PI will mitigate issues leading to these problems. The participant will be provided any information which is available to them (baseline body composition, fitness testing, BMD). It will then be suggested that the study personnel part ways with the participant.

16.3 Describe procedures that you will follow when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection (e.g., participant declines to continue with regular blood draws, but continues with periodic behavioral questionnaires):

Any participant can discontinue participation at any point without consequence.

17.0 Risks to Subjects

17.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Include for the IRB's consideration a description of the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, privacy, and economic risks. Do not indicate "No risk" or "N/A." Instead, for studies with very low risk (e.g., anonymous online questionnaire on a mundane topic) indicate "The investigators are not aware of any risks from participation in this study." or "No more than risks than are found in everyday life." The example consent form presents a tabular method for risk information, which you can also use here. Common risk types include:

- *Physical (e.g., potential for pain, discomfort, infection)*
- *Psychological (e.g., potential for stress, discomfort, and/or embarrassment)*
- *Social (e.g., potential for discrimination or stigmatization and disruption of personal and family relationships)*
- *Legal (e.g., potential for disclosure of illegal activity, negligence)*
- *Privacy (e.g., potential for personal information being accessed, used, or disclosed without the subjects' knowledge or consent, breach of confidentiality/security)*
- *Economic (e.g., potential for individuals to lose access to economic services, employment, insurability)*

DXA Scan: The amount of radiation that subjects will receive in the DXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount subjects will receive at the exam (including total body, femur, and lumbar spine) is equal to 1/20 of a chest x-ray. The more radiation an individual receives over the course of their lifetime, the more likely that individual's risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, however, the exact increase in such risk is not known.

Blood draws: Slight discomfort may be expected during blood draws. Risk of developing a small bruise or blood clot in the vein, risk of fainting or dizziness, risk of infection and risk of bleeding are also possible. Universal Precautions will be followed for collection, handling, processing, and disposal of items that may have come into contact with bodily fluids during the collection of blood. Blood draws will be

performed by a research phlebotomist (J Rinehart) trained and experienced in the blood draw procedure and in handling minor emergencies such as dizziness and fainting. In case of an emergency, 911 will be called.

Running Economy, Exercise Capacity (VO2max), and Treadmill Runs: There is a small risk of orthopedic injury, treadmill falls or cardiovascular complications that could require a participant to go to the hospital. This includes a heart attack, or even death. In studies involving people with heart disease, the risk of hospitalization was 1 in 500 tests (<0.20%). The risk of heart attack was 1 in 2,500 tests (0.04%) and death, 1 in 10,000 tests (0.01%). The risks are likely to be lower in young, healthy participants who are involved in running and other exercise activities. Only experienced staff members will conduct these tests and subjects will be monitored throughout the test for signs of problems based on standards of the American College of Sports Medicine (ACSM). There is a possibility some subjects will be tired after this test and could have sore muscles for a few days. Risks associated with treadmill running will be minimized by recruiting only participants who have current experience with long-distance running at a high volume (at least 30 miles per week) and frequency (at least 5 days per week) to meet the exercise demands of the protocol.

Continuous Glucose Monitoring. The placement of the device will require that a sensor is inserted into the back of the participant's upper arm. Placement of the sensor may induce some pain during the insertion, inflammation, redness, swelling, minor bleeding and/or minor infection at the site. This will all be minimized by having a trained individual perform the procedure which will take place in aseptic conditions. There is also a possibility a participant may experience these symptoms as a result of contact between the adhesive pad of the sensor and the skin; allergic reactions can also develop in response to the adhesive used to keep the device in place.
If any of these symptoms occur, the participant will be informed that he/she has the ability to remove the CGM and these issues will clear up within a short time period.

Dietary Intervention: Experimental diets will induce a state of low energy availability through dietary energy restriction. Potential risks associated with energy restriction include loss of lean body mass (LBM), suboptimal macronutrient and micronutrient intake, hypoglycemia, feelings of weakness and mood disturbances. These risks will be minimized by providing a daily multivitamin to participants for the duration of the 5-days of energy restriction. Menus will also be designed to provide 20% of total calories from protein to help preserve LBM. To minimize risk of hypoglycemia, diets will provide 55% of total calories from carbohydrates and a snack will be provided to participants containing approximately 30g carbohydrate to be consumed prior to the treadmill run. Additionally, menus will be designed to prioritize foods with low energy density that promote satiety (e.g., high fiber foods) to reduce physical and psychological burden of energy restriction. Participants with existing menstrual disturbances, LEA, and/or low BMD will be excluded from the study to prevent worsening existing conditions. Given the short-duration of this study and the washout period of approximately 24 days between LEA conditions, it is unlikely the acute energy restriction will result in any long-term consequences.

High-Impact Loading Exercises (Jumping): Potential risks associated with high-impact loading exercises include musculoskeletal injuries and excessive bone strain. These risks will be minimized by excluding participants with recent history of bone stress injuries or low BMD (z-score <-2.0). Additionally, the exercise sessions will be supervised by an NSCA-certified strength and conditioning specialist (T. Sterringer) with an emphasis on proper form and safety techniques. The participants will be informed to contact the PI or any member of the research team in the case of any injury or excessive joint or muscle strain.

17.2 Indicate the measures you will use to minimize risks and monitor subjects for safety. (e.g., asking a subject at regular intervals to rate how they are feeling from 1 to 10, or to slowly crouch in order to check their balance.) Indicate the measures you will use to minimize risks and monitor subjects for safety. (e.g., asking a subject at regular intervals to rate how they are feeling from 1 to 10, or to slowly crouch in order to check their balance.)

Weight loss will be expected during the two, 5-day intervention periods because the participants will be in a state of low EA (15 kcal/kgFFM/d). Previous studies (Ihle, Loucks 2004, and Loucks, Thuma 2003) observed an average weight reduction of 2.0-2.2 kg and 1.1-1.2 kg in young women (average age 21 years) in response to EA treatments of 10 and 20 kcal/kgFFM/d, respectively, over a 5-day period. This study will use a safety endpoint of excessive weight loss defined as 5-lbs (approximately 2.3 kg) over the 5-day energy-restricted intervention phases.

Other safety measures addressed in Section 17.1

17.3 If applicable, indicate which procedures might have risks to the subjects that are currently unforeseeable. This will be rare, and usually applicable when testing a new drug or device or a new use of an existing drug or device:

It is possible that participants could develop soreness in legs by participating in our high-volume running regimen on 5 consecutive days and during the 5-day intervention with high-impact jumping. It is also possible an unknown allergy to foods contained in the controlled diets could be identified during the study. These events, however, are not likely. To minimize the potential, we will only recruit participants with current long-distance running experience at a high volume (at least 30 miles per week) and frequency (at least 5 days per week) to meet the exercise demands of the protocol.

17.4 If applicable, indicate which procedures might have risks to an embryo or fetus should the subject be or become pregnant:

Dietary restriction is a risk to the development of the fetus should the subject become pregnant during the study intervention. Pregnancy tests will be performed at baseline testing (specifically before the DXA) and after the 3-week washout period on Day 1 of the second experimental condition, to ensure the participant is not pregnant. Progesterone will be measured to ensure menstrual status. Participation in the study would end if it were determined the participant has become pregnant.

17.5 *If applicable, describe risks to others who are not subjects (e.g., collection of sensitive health data that might affect sexual partners if disclosed, mandatory reporting of abuse, DNA testing that might affect family members or relationships)*:

N/A

18.0 Potential Benefits to Subjects

18.1 *Describe the potential benefits that individual subjects might experience from participating in the research. Include the probability, magnitude, and duration of the potential benefits, as this will be useful to the IRB's risk:benefit analysis. Do not include benefits to society or others. Do not list monetary or non-monetary compensation for participation, as this is not a benefit These should be included in section 2 or 3 of this document*:

Participants will gain information about their bone mineral density, body composition and running fitness (VO2max and running economy). They will also learn how to properly perform high-impact loading exercises.

18.2 *If applicable, specify that there are no anticipated direct benefits for participants*:

N/A

19.0 Data Management and Confidentiality

19.1 *Describe procedures that you will use for quality control to ensure validity of collected data*:

Dr. Larson-Meyer has extensive experience performing data collection the procedures (or similar procedures) as does Dr. Elaina Marinik. Dr. Marinik and Trisha Sterringer are ISCD Certified Bone Densitometry Technologists. Additionally, T. Sterringer is a NSCA Certified Strength and Conditioning Specialist and has experience or been trained on performing the procedures detailed in this protocol. The research team will ensure all study personnel will be properly trained to perform all procedures according to standard protocol. Specific quality control measures will be employed to ensure valid indirect calorimetry data are collected during the exercise economy, VO2max, and exercise energy expenditure tests; These standards are included on data collection sheets for use by members of the study team.

19.2 Describe any existing data or biospecimens you will obtain as part of this study. Include:

- *Variables or samples to be obtained*
- *Source of the data or specimens*
- *Your authorization to access or receive the data or biospecimens*
- *Whether the data or biospecimens are publicly available*
- *Whether the data or specimens you receive will contain identifiers*

N/A

19.3 Describe the steps that you will take to handle and secure study data during data collection, storage, use, and transmission. Include information about training of study staff, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, separation of identifiers and data, etc.:

We will do everything that we can to make sure that study records are kept private. Each participant will be assigned a unique participant code as explained in section 9.4. All data recording sheets and spread sheets will use the subjects' study code. They will not contain the participants' name or date of birth. These will be compiled in a patient research file/chart and stored in a locked file cabinet organized by their unique study code. Their name will be listed only on the phone screening log (which is to be blackened out after they are assigned a participant code), informed consent and on a master participant list that includes the randomization key. The master participant list will be kept in a separate electronic file than the data files; both will be password-protected. The study consents will be kept together in a separate file in a separate location in a locked office. Only authorized study personnel will have access to study data. Results of the study may be published and/or presented at professional conferences. The participants' name or other personal information that would identify them will not be used. All blood collected and post-processed serum and plasma samples will be labeled with the participants unique study code (plus the study visit and date and time of sample collection) and stored in a secure freezer in a locked laboratory until analysis as mentioned in section 9.2. Archives may be kept for up to five years following study analysis. Training of study personnel including graduate students on procedures to ensure secure collection and storage of study data will occur before study initiation.

19.4 For multi-site studies, describe how data or specimens will be handled and secured for each site (e.g., central or disseminated data storage, data coordinating center):

N/A

19.5 Describe the plan for data disposition following the conclusion of the study (e.g., long term maintenance of data, data destruction methods).

- *What information will be included in the long term storage of data or specimens?*
- *How long will the data or specimens be stored?*

- *Where and how data or specimens will be stored?*
- *Who will have access to the data or specimens during long term storage?*
- *Who is responsible for receipt or transmission of the data or specimens?*
- *How will data or specimens be shared or transported?*
- *When and how will personal identifiers be destroyed?*

Telephone screening forms (that contain participants' names) will be shredded immediately after all study participants are recruited. Personal information, primary and secondary endpoints and safety data will be kept indefinitely in a secured electronic location by the PI. Personal information will be kept in a separate file than de-identified data. Blood and urine samples labeled with the patients' unique study code may be stored in a laboratory freezer in a locked laboratory for up to five years following the completion of the analyses; only authorized study personnel will have access to freezer samples. The PI will be responsible for transmission of all data or achieved specimens. Although is not anticipated that any data will need to be transported or shared, this would be done only using de-identified data with samples sent using a secure mechanism.

20.0 Provisions to Protect the Privacy Interests of Subjects

20.1 *Describe the steps that you will take to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on with whom they interact or to whom they provide personal information (e.g., collecting the minimal amount of private information required to complete the study, protecting the data once it is obtained)*:

To ensure privacy interests of all interested and enrolled participants, only the minimal amount of personal information and health history will be obtained using a standard health history form and the Low Energy Availability in Females Questionnaire. This data will be kept in participant files labeled with only the participants' study code in a secured file in a locked room. The data for all participants who do not participate in the study will be destroyed by shredding. The data for participants who do enroll will be entered into an electronic data base using the participants assigned unique study code. Any and all original data collection sheets with the participants' name or identifying information will also be destroyed following entry into the database.

20.2 *Describe steps that you will take to make subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures (e.g., use of a same gender investigator to place sensors on the torso, a private changing area if clothing must be changed, sensitivity when discussing pregnancy testing with subjects, making it clear on surveys that participants can discontinue at any time, not asking questions about private or sensitive issues unless necessary for the research)*:

Study participants will be informed during the baseline screening and exercise sessions that they can discontinue the study and/or exercise session at any time without penalty. All questionnaires and anthropometric testing will be performed by trained research personnel in a private setting. Assignment of same-sex researchers will be employed if necessary; however, all study personnel will be trained to exhibit professional behavior and sensitivity when collecting personal health or medical data or when performing body composition or other testing.

20.3 Describe how you plan to access existing sources of information about the subjects (e.g., medical records, grades) and how you will protect participant privacy through the data security plan:

N/A

20.4 Describe any required reporting that might occur as a result of your research questions, study populations, and data collection methods. Examples for Virginia and Virginia Tech include:

- ***Any*** *suspicions (e.g., circumstantial, disclosed) of child abuse (physical, emotional, sexual) and neglect*
- *Sexual discrimination and/or sexual violence that involves a student*
- *Disclosure or signs of intention to harm oneself (i.e., suicidal ideation and/or plan)*
- *Disclosure or signs of desire to harm others (i.e., homicidal ideation and/or plan)*
- *Suspected abuse, neglect or exploitation of vulnerable adults (e.g., individuals with a disability, elderly persons)*

N/A

21.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Safety monitoring is required when research involves greater than minimal risk and is sometimes appropriate for other studies.

21.1 Describe:

- *The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe (e.g., periodic reporting to the IRB, establishing a data monitoring committee, reporting data monitoring committee findings to the IRB and the sponsor).*
- *What data you will review, including safety data, unexpected events, and data that show the ability to produce the intended results.*
- *How the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with subjects).*

- *The frequency of data collection, including when safety data collection starts.*
- *Who will review the safety data and with what frequency.*
- *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
- *Any conditions that will trigger an immediate suspension of the research (e.g., a serious adverse event).*

The data safety monitoring plan (DSMP) for this study focuses on close monitoring by the principal investigator (PI) and research staff along with prompt reporting of excessive adverse events and any serious adverse events (AEs) to the Institutional Review Board. All serious AEs will be reported by the PI within 48 hours of occurrence to the IRB and the sponsor.

The safety data monitored will include data related to the blood collections, fitness testing and the supervised exercise sessions. Specific safety data include any reports of pain, excess swelling, redness or bruising after the blood draws at the needle insertion site, feelings of light headedness, chest tightness or pain or fatigue on exertion during exercise testing procedures, and symptoms of muscle soreness, joint pain, or unexpected events/issues during the 5 days of consecutive running and 5 days of consecutive running with impact loading exercises. Data will be collected and documented in the participant's chart if a situation arises or when observed by a member of the research team or reported by a participant during a study visits or during supervised resistance training sessions using a general TBD incident reporting form. Safety data will also include excessive changes in body weight or interstitial glucose concentration that will measured throughout the 5 days of energy restriction in the experimental conditions.

The graduate student in charge of the project (T. Sterringer) will consult with Drs. Larson-Meyer and Marinik and be responsible for assembling the data, producing reports, and assuring that all parties obtain copies of these reports. Reports will be submitted annually to the VT IRB for review.

Safety Data collection will start when the first participant is screened and enrolled. The study team will be informed to discuss any observed or reported unusual, excessive or unexpected events immediately with the PI. The PI and/or authorized study personnel will review study charts and ongoing data collected on all participants on a weekly basis to ensure safety. In our small study, it is unlikely that use of statistics would be necessary to determine if excessive events were occurring; however, paired t-tests could be used if appropriate. We do not anticipate that there would be any specific events, other than the unexpected, that would trigger the suspension of our study.

22.0 Compensation for Research Related Injury

22.1 If the research involves more than minimal risk to subjects, describe the available compensation in the event of research-related injury, if any:

Participants will not be provided any form of compensation for medical treatment or other damages (for example lost wages, time lost from work, etc.). If a participant

becomes injured or sick from the research, they will be referred to a clinic or to their personal health care provider. Medical treatment may be provided at their expense or at the expense of their insurance company.

22.2 Provide a copy of contract language, if any, relevant to compensation for research-related injury. At Virginia Tech, this is most common for sponsored research:

N/A

23.0 Economic Burden to Subjects

23.1 Describe any costs that subjects might be responsible for because of participation in the research, including any uncompensated costs for items such as transportation, missed work, and childcare:

The participant will be responsible for costs that may include purchase of athletic clothes or shoes to participate in the running and impact loading exercise sessions or the uncompensated cost that might include transportation, missed work, or childcare.

24.0 Consent Process

24.1 Indicate the process by which you will obtain consent for study participation. Please upload all consent, parental permission, and assent forms, documents, and scripts referenced in this section to Protocol Management.

Describe the following:

- *Where the consent process will take place (e.g., clinic waiting area, classroom, online)*
- *The time interval between sharing the consent information with the prospective subject and obtaining consent. For lab, interview, and focus group studies, the Virginia Tech IRB prefers that subjects have at least 24 hours to review the consent form and study information before the appointment where consent will be obtained. For simple online survey studies, you can typically present the consent information immediately before subjects begin participation.*
- *If applicable, processes to ensure ongoing consent or assent (e.g., for multiple sessions; for research in which a minor will turn 18 during the study; for longitudinal research with minors who will later be asked to provide or affirm their assent).*
- *Please review "SOP: Informed Consent Process for Research (HRP-090)" for recommended procedure. Describe your process, being sure to include:*
    - *The name and role of all study personnel who will be trained and certified by the PI to conduct the consent process*
    - *The time that will be devoted to the consent discussion*

- *Steps that you will take to minimize the possibility of coercion or undue influence*
- *Steps that you will take to gauge or ensure the subjects' understanding*

Participants will be first screened over the phone (phone script and screening form uploaded). The phone screening will include an overview of the study, the time commitment and the possible risks to participation. After explaining the study and before conducting the phone screen, a study team member will describe the purpose of the phone screen and what type of data will be collected, and then ask that the participant provide verbal permission to conduct the phone screening. They will also be informed that they may refuse to answer any and all questions. An informed consent form will be emailed (or mailed) to the participant following the phone screening and at least 24 hours in advance of coming to the laboratory for the screening visit (email template uploaded).

At the start of the screening visit, a team member will provide potential participants with a written copy of the study consent form and review the document with the participant. Participants will be encouraged to ask questions and seek clarification during the phone screening. The participant will then be encouraged to ask questions before providing written consent. As much time as necessary will be devoted to address participant concerns. Once the participant is ready to sign, she will be allowed to sign in a private room near the door where they may also exit the lab if they no longer wish to participate. Screening and informed consent will be performed by the doctoral student in charge of the study (T. Sterringer). Participants will be encouraged to ask questions and seek clarification during the phone screening and before signing the consent at the beginning of the laboratory screening visit.

These steps including time to review the consent before the screening visit, time with study staff to review the protocol and address concerns, and time to sign the consent in a private setting and close to a laboratory exit will help minimize the possibility of coercion or undue influence.

To help gauge the participant's understanding, the team member will ask the participant to explain the study, when they need to notify the team about the start of menstruation, how often they will be asked to consume the meals and snacks provided by the research team, what foods and beverages they can consume outside of the ones provided by the research team, what physical activities they can do outside of the ones conducted in the NEM lab, and how often they would need to come to the lab to run.

Non-English Speaking Subjects

- *Indicate what language(s) other than English are understood by prospective subjects or representatives.*
- *If non-English speakers will be recruited, describe the process you will use to ensure that the oral and/or written consent information provided will be in a language that they understand.*
- *If you translate consent forms and study materials, please provide a certified translation of the form as well as the certification document.*
- *Indicate the spoken language that study personnel obtaining consent will use. Describe how you will assess fluency of personnel obtaining consent to ensure that the translation is accurate.*

Only English-speaking participants will be recruited for the study.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

- *Review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to ensure you have provided sufficient information for the IRB to make these determinations (i.e., that it meets the criteria for a waiver or alteration of the consent process).*

N/A

Subjects who are not yet adults (minors: infants, children, teenagers)

- *Describe the criteria that you will use to determine legal age for consent to treatments or procedures involved in the research under the applicable law of the jurisdiction in which the research will be conducted (e.g., in Virginia, individuals under the age of 18 years).*
  - *For research conducted in Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "minor."*
  - *For research conducted outside of the state, please describe the legal requirements for the definition of "minor."*
- *Describe the process for obtaining parental permission.*
  - *Permission from one parent is acceptable for studies that involve no greater than minimal risk OR involve greater than minimal risk but present the prospect of direct benefit to the minor subject.*
  - *Permission from both parents is required in all other cases (unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the minor).*
- *Describe whether you will obtain permission from individuals other than parents or Legally Authorized Representatives, and if so, who will be allowed to provide permission. Describe the process you will use to determine these individuals' authority to consent to the minor's general medical care.*
- *Indicate whether you will obtain assent from all, some, or none of the minors. If you will obtain assent from some minors, indicate which minors will be required to assent. Consider chronological age and intellectual capacity when determining who will be required to provide assent (e.g., infants are unable to assent. However, teenagers are likely able to read and sign an assent form).*
- *When assent of minors is obtained, describe whether and how you will document it. Will minors sign an assent form or give verbal assent?*
- *Attach parental permission and minor assent forms or scripts in Protocol Management.*

N/A

Adults Unable to Consent

- *Describe the process you will use to determine whether an individual adult is capable of consent.*
- *List the individuals from whom you will obtain permission in order of priority (e.g., durable power of attorney for health care, court appointed guardian for health care decisions, spouse, and non-minor child).*
    - *For research conducted in the Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "legally authorized representative."*
    - *For research conducted outside of Virginia, please describe the legal requirements for obtaining permission from a legally authorized representative in the state where the research will occur.*
- *Describe the process for assent of the subjects.*
    - *Indicate whether you will require assent from all, some, or none of the subjects. If some, indicate which subjects will be required to assent and which will not.*
    - *If you will not obtain assent from some or all subjects, please provide justification for not obtaining assent.*
    - *Describe whether and how you will document assent.*

N/A

25.0 Process to Document Consent in Writing

25.1 *Consult "SOP: Written Documentation of Consent (HRP-091)" for recommended procedures, and describe whether and how consent of the subject will be documented in writing*:

Individuals who respond to the advertisements will be contacted by phone where they will be informed of the general plan of the study and all specific procedures included in the study (previously outlined in section 12). Participants will then be given a chance to ask questions regarding study procedures and risks. Those still interested will be screened over the phone to determine eligibility based on current training status, running frequency and volume, dietary restrictions, medication use, injury history, and other criteria outlined in section 12.2 (see uploaded Screening form). Eligible individuals will be sent a copy of the consent form via email to review prior to coming to the lab. They will then be given a chance to ask any questions either by email or during their scheduled screening/baseline visit. Those still interested will be asked to sign the consent during their first visit, before any data is collected. This information is detailed in section 24 above. A copy of the informed consent will be sent to all participants.

25.2 *If the research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, you can request that the IRB waive the requirement to obtain written documentation of consent (e.g., consent to participate is indicated by pressing a button for an online questionnaire – after the consent information is presented and before the questionnaire begins)*:

Waiver of written consent to perform the phone screening is requested. The phone screening form has been uploaded.

25.3 *If you will document consent in writing, attach a consent document with places for signatures. If you will obtain consent, but not document consent in writing, please attach the consent script or text. Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information. You should use "TEMPLATE CONSENT DOCUMENT (HRP-502)"to create the consent document or script*:

See the attached participant consent form

26.0 Resources Available

26.1 *Describe the resources available to conduct the research. For example, as appropriate:*

- *Describe the PI's availability to supervise the research.*
- *Justify the feasibility of recruiting the required number of suitable subjects within the agreed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?*
- *Describe the time that you will devote to conducting and completing the research.*
- *Describe your facilities.*
- *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated or unanticipated consequence of participation in the research.*
- *Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions (e.g., training plans, detailed study notebooks).*

The PI is a Professor in the Department of Human Nutrition, Foods and Exercise at Virginia Tech. She currently has a 33% research appointment and oversees four doctoral students. She has previously served as a research dietitian and research exercise scientist at the National Institute of Diabetes & Digestive & Kidney Diseases in Phoenix and the Pennington Biomedical Research Center in Baton Rouge, LA, respectively, and has experience conducting exercise training studies and controlled feeding trials. The PI will dedicate time to this study and ensure the doctoral student is adequately trained and performs all aspects of the study according to protocol and procedures. The doctoral student, Trisha Sterringer, will be in charge of participant recruitment and enrollment, protocol execution, data

collection and the day-to-day aspects of the study. T. Sterringer is a registered dietitian (RD), NSCA certified strength and conditioning specialist, and ISCD Certified Bone Densitometry Technologist. She has previous experience conducting energy availability studies in competitive athletes at Case Western Reserve University involving indirect calorimetry, and assessments of dietary intake, exercise energy expenditure, and body composition. Trisha Sterringer will be the doctoral student in charge of the study as part of her PhD dissertation, however, additional undergraduate research assistants may be added to the study in the future. Dr. Elaina Marinik has extensive experience in study management and coordination at Virginia Tech and will be involved in the study to assist as needed. There are several key laboratories in the HNFE Department that will be utilized for this proposed study as described below.

The Nutrition and Exercise Metabolism (NEM) Laboratory is directed by DE Larson-Meyer and located on the Virginia Tech Corporate Research Center campus. Major equipment items in the NEM Laboratory include Parvomedics TrueOne 2400 metabolic cart, private room with a table for completing questionnaires, Woodway treadmill, and refrigerator and freezer storage. Free parking is available on-site and restrooms, showers, and changing facilities are also available.

The Laboratory for Eating Behaviors and Weight Management (Director: Brenda Davy) is located in Wallace Hall and encompasses a ~600 sq ft Metabolic Kitchen with a ~900 sq ft research Dining Laboratory area and a research dietitian computer workstation (for dietary analysis software), reach-in freezer, and refrigerator for storing meals to be consumed off-site, and an additional ~250 sq ft space housing stadiometers, scales, tables for completing questionnaires, a private room for measuring anthropometrics, and a file storage area. Also located in Wallace Hall 233 is a Lunar iDXA (GE Healthcare) and space for sample processing.

The Human Integrative Physiology Laboratory is located on the Corporate Research Campus at the Garvin Innovation Center. The major equipment items in this laboratory include Lunar Prodigy DXA (GE Healthcare) and space for sample processing (i.e., wet lab areas). Additional research space is also available in Wallace Hall for sample processing (i.e., wet lab areas) and storage (-80 freezers).

The Metabolic Core at Virginia Tech will perform the majority of the biochemical analyses. This core laboratory is housed in the Integrated Life Sciences building and includes a 140 sq ft. laboratory space dedicated to biochemical assays. This Core laboratory has a BioTek Synergy 2, a multi-mode microplate reader equipped with Gen5 software capable of measurements of absorbance utilizing a monochrometer for wavelength selection from 200nm to 999nnm, and for fluorescence with excitation and emission filters for luminescence using a liquid-filled light guide.

27.0 Multi-Site Research

Contact the HRPP for multi-site research (involving multiple institutions) and the details required for this section will be provided. Otherwise, indicate N/A.

N/A

Appendix B: Study 1 Approved Informed Consent

Title of Research Study: The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners (Protocol # 22-168)

Principal Investigator: Enette Larson-Meyer, PhD, RD, FACSM, **enette@vt.edu**, 540-231-1025

Other Study Personnel: *Trisha Sterringer, MS, RD, CSCS, tsterringer@vt.edu; Elaina Marinik, PhD emarinik@vt.edu*

Key Information: The following is a short summary of our study to help you decide whether or not to be a part of the study. More detailed information is listed later on in this form.

The reason for this study is to determine the efficacy of adding brief, high-impact loading exercises (jumping) to normal running training on protecting bone health over a 5-day period in generally healthy, female long-distance runners eating a low-calorie diet. There will be two, 5-day periods that participants will compete. In one phase, participants will complete 50 jumping exercises in addition to a treadmill run. In the other phase, participants will only complete a treadmill run. Meals and snacks for both 5-day periods will be provided to participants.

Why am I being invited to take part in a research study?

We invite you to take part in a research study because you are a trained, female long-distance runner between the ages of 18-35y. You will be eligible to participate in this study if you have a BMI between 18.5-30 kg/m^2 and have a normal menstrual cycle without the use of contraceptives. Additionally, you are invited to participate in this study if you are able to run on 5 consecutive days for at least 60 minutes each day. You will not be eligible to participate if you have had a bone fracture in the last 6 months, have a recent history of a diagnosed eating disorder, use contraceptives or certain medications that could potentially affect bone metabolism, are pregnant, lactating, or have a thyroid condition.

What should I know about being in a research study?

- Someone will explain this research study to you
- Whether or not you take part is up to you
- You can choose not to take part
- You can agree to take part and later change your mind
- Your decision will not be held against you
- You can ask all the questions you want before you decide

Why is this research being done?

Many long-distance runners struggle to consume enough calories each day to match the number of calories they are burning during exercise and as part of daily living. Undereating for long periods of time is a serious concern because it can have negative effects on general health and sports performance. One of these long-term consequences is related to bone health. With undereating, bone can start to be broken down faster than it can be rebuilt. Even though it can take months and even years for bone to be seriously affected, this may lead to weak and brittle bones later in life if it is left untreated. Typically, athletes are recommended to increase the number of calories they eat to prevent negative health concerns. However, not all athletes may be willing or able to increase their calories based on their

performance goals, nutrition knowledge, or concerns with food security insecurity. This means that alternative strategies need to be investigated to counteract the negative effects of undereating on bone health. This study proposes to evaluate the effect of adding short bouts of high-impact jumping exercises to typical endurance training to promote healthy bone metabolism. This will specifically involve completing two phases of a supervised treadmill run and consuming a reduced-calorie diet (provided by the research team) on 5 consecutive days. In one of the phases, you will also perform 5 sets of 10 jumping exercises on 5 consecutive days in addition to the treadmill run. Results of the study will serve as an important *first step* in helping exercise and medical professionals understand more about how to protect and manage the bone health of female long-distance runners.

How long will the research last and what will I need to do?

The time commitment for this study will be 2 weeks of an experimental trial separated by approximately 3 weeks of your normal lifestyle, in addition to screening and baseline visits (19 days total). This will include: a) a screening visit at the *Human Integrative Physiology Laboratory;* b) a baseline testing visit at the *Nutrition and Exercise Metabolism Laboratory*; and c) your participation in two, 5-day experimental trials that will be separated by 1 menstrual cycle which will involve eating a reduced-calorie diet (that we will provide) and completing two running programs, one with jumping exercises and one without. The screening visits will include taking your blood, performing tests of your aerobic capacity and exercise efficiency, and estimating your body composition. You will also record your food intake for 3 days and wear a smart watch (provided by the research team) for 3 days before the study begins and for 10 days during the experimental phases of the study. Additionally, you will wear a continuous glucose monitor and record the time of all meals and snacks during the 5-day experimental phases. Your blood will be drawn 5 times throughout the study (once at baseline and before and after each experimental phase). These tests are summarized in the table on page 7. The actual time and frequency of your visits may depend on your schedule and available appointment times.

During your first screening visit, you will determine whether you would like to participate by reviewing and discussing with a member of the research team what is involved in the study and the risks and benefits of doing so. We will ask you to complete some questions about your health history and eating and exercise habits. We will then measure your height and weight and ask you complete a pregnancy test before we perform a Dual-Energy X-ray Absorptiometry (DXA) scan to estimate your body composition and bone density.

During your baseline laboratory visit, we will ask you to come to the lab in the morning after not eating since 10PM the night before. At the Human Integrative Physiology lab, we will collect blood samples. At the Nutrition and Exercise Metabolism lab, we will have you perform a 12-20 min exercise test on the treadmill to measure your maximal aerobic capacity (Vo2max) during running. This visit collectively will take approximately 1 hour to complete.

3-days of usual diet and exercise. After completing the baseline testing visit, you will record the foods you usually eat over a 3-day period (2 days during the week, 1 day on the weekend). We will also ask you to complete a vitamin D questionnaire and wear a physical activity monitor to track the calories you burn each day.

Two 5-day experimental phases. The two, 5-day experimental phases will take place over a 5-week period. You will be scheduled to come in on the third or fourth day after you predict your monthly cycle

will begin to start the 5-days of consecutive running. If your monthly cycle starts late or early, you will be asked to notify the research team and the date for your first run will be shifted. You will also be scheduled to come in the day before each experimental phase (day 2 or 3 of your monthly cycle) to for a blood draw and to have a continuous glucose monitor placed on your arm. For this visit, we will ask you to come to the lab the next morning after not eating since 10PM the night before. If there is a conflict with your or the study staffs' schedule, blood will be drawn on the following morning. The day after your blood draw is complete, you will begin the first 5-day experimental phase. This will involve coming into the laboratory on 5 consecutive days to complete endurance training sessions on the treadmill under the guidance of a researcher who is also a certified trainer. In one phase, you will run on the treadmill for approximately 50-65 minutes depending on your running efficiency. In the other phase, you will complete 5 sets of 10 jumping exercises in the morning, and then return in the afternoon or early evening to run on the treadmill at a moderate pace for 50-65 minutes. You will also be provided with reduced-calorie meals and snacks to consume daily for the 5-day period and asked to record the time you eat each food on a mealtime log. We will ask that you a) not engage in any additional exercise, b) consume any additional foods or beverages (other than water and non-calorie beverages) outside of the ones provided during the 5-day programs, c) consume your meals and snacks around the same time each day, d) record the time you consume all foods and beverages during the experimental phases, and e) return all food packaging and uneaten food to the research team. Once you have completed the 5-day experimental phase, you will come to the lab the next morning after not eating since 10PM the night before to have a blood sample collected and the continuous glucose monitor removed.

After you complete the first experimental phase and have your blood sample collected on the following morning, you will resume your normal diet and exercise training until the start of your next menstrual period (approximately 3 weeks). The second experimental phase will look almost identical to the first phase. You will be asked to come into the lab on the morning after the first or second day of your menstrual period to have a blood sample collected and the continuous glucose monitor device placed. The following day, you will start the second 5-day experimental phase involving the consumption of a reduced-calorie diet and daily treadmill running with or without the jumping exercises (depending on whether you had them in the first phase or not). Once you complete the second phase, you will come into the lab the following morning to have a final blood sample collected and the continuous glucose monitor removed.

Please see "Overview of Testing Procedures" on page 7 for an outline of what will be completed throughout the study.

Is there any way being in this study could be bad for me?

There are some small risks to participating in the study resulting from reduced calorie intake that include loss of body weight, loss of muscle mass, increased hunger, mood disturbances and suboptimal micronutrient intake. Other small risks include sore muscles from the endurance training program, fainting or getting a small infection from giving blood samples, receiving a small amount of radiation from the DXA scans, or feeling extremely tired after the exercise tests.

> More detailed information about the risks of this study can be found under **"Is there any way being in this study could be bad for me? (Detailed Risks)".**

Will being in this study help me in any way?

We cannot promise any health or performance benefits to you or others from your taking part in this research. However, possible benefits may include receiving information about your body composition, bone density, aerobic fitness, and blood test. Please note, you should not consider your participation as a wellness or medical exam, and there will be no direct medical benefit to you. You should discuss any concerns about your health information with your health care provider.

Will I be paid for participating?

You will be compensated a total of $100 for completing this study. You will be compensated $25 for completing both the baseline visit and the first one-week diet and exercise phase. You will be compensated an additional $75 for completing the second diet and exercise phases. Cash payments will be provided after you complete the last day of each experimental phase.

What happens if I do not want to be in this research?

Participation in this research is completely up to you. You can decide to participate or not to participate.

If you are a student or employee, the decision whether to participate or not will have no effect on your grades, your employment, or your relationship with Virginia Tech.

Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, contact Enette Larson-Meyer, the Principal Investigator of the study, at enette@vt.edu or 540-231-1025.

This research has been reviewed and approved by the Virginia Tech Institutional Review Board (IRB). You may communicate with them at 540-231-3732 or irb@vt.edu if:

- You have questions about your rights as a research subject
- Your questions, concerns, or complaints are not being answered by the research team
- You cannot reach the research team
- You want to talk to someone besides the research team to provide feedback about this research

How many people will be studied?

We plan to include about 20 women in this research study.

What happens if I say "Yes, I want to be in this research"?

If you say yes to participating, you will be enrolled in a 5-week endurance running study that will involve two, 5-day endurance training phases separated by approximately 3 weeks of your normal diet and exercise. During the 5-day endurance training phases, reduced-calorie meals and snacks will be provided to you and you will be asked to come into the lab on daily to perform a treadmill run for 5 consecutive days with jumping exercises and 5 consecutive days without jumping. The running phase you start with

will be chosen by chance, like flipping a coin. Neither you nor the study team will choose which phase you start with. You will have an equal chance of starting with either phase.

In addition, you can expect that the following will take at each stage of the study. Also, please refer to "Overview of Testing Procedures" on page 7 which further outlines details of the study.

Baseline Screening Visit (approximately 1 hour)

Informed Consent: You will be provided an informed consent form by email or mail following the phone screening and before coming to the laboratory. You will review this form in-person with one of the researchers and have a chance to ask questions. You will need to sign this form before you can take part in the research.

Health History: You will be asked to complete a health and medical history questionnaire. This questionnaire is used to screen for health problems or reasons you should not participate in this study. If you have existing medical conditions that prohibit you from completing the exercise protocol, a history of menstrual disturbances, or are currently pregnant or lactating, you are not eligible to participate.

Energy Status: You will be asked to complete a questionnaire that assess risk of undereating in female athletes. This questionnaire is used to screen for risk of undereating, medication use, menstrual function, and injury history. If your score indicates "high risk", you will not be eligible to participate.

Urine Sample and Pregnancy Test: You will be asked to provide a small amount of urine before the DXA scan. This will be used to evaluate how hydrated you are. Additionally, you will be required to have a pregnancy test before the DXA scan. The urine sample will be used to perform this pregnancy test. If you are pregnant or the test indicates that you are pregnant, you will not be able to participate in this study.

Body Weight and Composition: Your height and weight will be measured on a digital scale. You will then lie on a hospital-type bed and a small amount of X-ray will be passed through your body to determine the amount of bone, muscle, and fat in your body. This unit is called a DXA scan. This test takes approximately 30 minutes which will involve a whole-body scan and two shorter scans of the hip and lower back (lumbar spine). There is no pain associated with the procedure.

Baseline Laboratory Testing Session (approximately 1 hour)

Blood Draw: Blood will be drawn at 5 timepoints throughout the study. The first blood draw will be taken at the baseline laboratory visit. Blood will be taken from a vein in your arm to measure hormones that reflect health status. These include a CBC (complete blood count) and thyroid-stimulating hormone. Samples of your blood (serum and plasma) will also be kept (banked) for a maximum of 10 years for possible measurement of other related factors that are not known to the researchers that may be important for bone health or general health outcomes**.**

Aerobic Capacity: You will begin running at a self-selected warm-up pace for 5 minutes. After the warm-up phase, the speed or grade of the treadmill will be increased every minute until you are too tired to continue. This will require that you wear a special face mask and nose clip to measure the air you are breathing in and out during the test. The full test will last for 12-20 minutes. After the test, we will offer you water, juice and/or a snack**.**

Normal Diet and Exercise (3 days)

Dietary Record: You will be asked to record all the food and beverages you have each day for 3 days in a row. We will ask you to record your food and beverages on 2 days during the week and 1 day on the weekend. You will also be asked to complete a vitamin D questionnaire with questions related to your food intake and sun exposure.

Physical Activity: You will be asked to wear a smart watch that will be provided by the research team during the 3 days that you complete your food record. ***You will arrange a time to drop off the smart watch and dietary records before the endurance training study begins.***

Experimental Phases

Blood Draw: The remaining 4 blood draws will be taken during the experimental phases. Blood will be taken from a vein in your arm on days 1 and 7 of each phase (see table on page 7) to measure hormones and particles in the blood that are involved with bone health. These include progesterone, N-terminal propeptide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, C-terminal telopeptide of type 1 collage, parathyroid hormone, estradiol, insulin-like growth factor, hepcidin, insulin, markers of iron status, and thyroid hormones. Samples of your blood (serum and plasma) will also be kept (banked) for a maximum of 10 years for possible measurement of other related factors that are not known to the researchers that may be important for bone health**.**

Physical Activity and Continuous Glucose Monitoring: You will be asked to wear a smart watch that will be provided by the research team as well as a device called a continuous glucose monitor (CGM) during the time that you complete the running and reduced-calorie diet phases. The placement of the CGM device will require a sensor being inserted into the back of your upper arm. When the sensor is placed by the researcher, you can expect a sensation similar to a needle insertion for a blood draw. You may experience some discomfort during the insertion. This will take place under aseptic conditions; however, some pain, inflammation, redness/rash, swelling, minor bleeding and/or minor infection at the site is possible. This will be minimized by having a trained individual perform the procedure. You may also experience these symptoms as a result of contact between the adhesive pad of the sensor and your skin; allergic reactions can develop in response to the adhesive used to keep the device in place. If any of these symptoms occur, you have the ability to remove the CGM if you so desire, and the symptoms will clear up within a short period of time. You will wear the smart watch and the CGM for 7 days during the experimental phases.

Pregnancy test: You will be asked to complete a pregnancy test before starting the second experimental phase (Day 1 of the second reduced-calorie diet and running phase).

Mealtime Log: You will be asked to record the time you consume all meals, snacks, and beverages throughout the 5-day experimental phases. We will ask that you return all uneaten food and food packaging to the research team.

Table of Tests and Procedures at Baseline and During the Two Experimental Phases

Tests and Procedures			**Reduced-Calorie Diet and Running Phases (x2)**	

	Baseline	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
			Controlled Running with/without Jumping					
Body weight	x	x	x	x	x	x	x	x
Body composition and bone mineral density by DXA. *Note: a pregnancy test by urine is required before DXA*	x							
Baseline Screening Questionnaires	x							
Continuous glucose monitoring			x	x	x	x	x	
Blood								
CBC and TSH	x							
Progesterone and vitamin D		x						
Hormones and markers of bone health (N-terminal propeptide of type 1 procollagen, undercarboxylated osteocalcin, sclerostin, C-terminal telopeptide of type 1 collagen, parathyroid hormone, estradiol, insulin-like growth factor, hepcidin, ferritin, iron, leptin, cortisol, nesfatin-1, insulin, thyroid hormones)		x						x
Nutrition and Exercise Tracking								
Food records 3-day	x							
Vitamin D questionnaire	x							
Mealtime Log			x	x	x	x	x	
Exercise Tracking (smart watch)	x		x	x	x	x	x	
Fitness Testing								
Aerobic fitness (VO_{2max})	x							
Running efficiency			x				x	
Treadmill running			x	x	x	x	x	

Note: The experimental phases where you will consume a low-calorie diet and run on a treadmill will be completed on days 2-6 and repeated one time for a total of 10 days throughout the study.

What are my responsibilities if I take part in this research?

If you take part in this research, you will be responsible to:

- Give an accurate history of any health problems that you have or had or medicine that you take before the study begins.
- Tell the investigators of any discomfort or unusual feelings before, during, or after any of the study sessions.
- Be on time and attend all scheduled visits, including endurance training sessions.
- Follow all participant instructions for each endurance training session.
- Eat only the provided meals and snacks and only drink water or non-calorie beverages (e.g., black coffee, unsweetened tea).
- Refrain from all physical activity outside of the supervised exercise sessions. This includes additional running, lifting weights, exercise classes, high-intensity interval training, hiking, going

for a walk for leisure, or any other physical activity or exercise. Activities such as getting dressed, walking to your car, bathing, cooking, etc. are permitted.

What happens if I say yes, but I change my mind later?

You can leave the research at any time, for any reason, without penalty.

Is there any way being in this study could be bad for me? (Detailed risks)

DXA Scan: You will be exposed to a small amount of radiation as part of the body composition testing during the DXA scan. The amount of radiation that you will receive in the DXA scans is far less than the amount that the Food and Drug Administration (FDA) allows per year. The amount you will receive from each scan is equal to 1/20th of a chest X-ray. The more radiation you receive over your lifetime, the more your risk increases in developing certain kinds of cancer. The radiation in this study is not expected to greatly increase these risks. The exact increase in this risk is not known.

Blood Draws: During each blood draw, you may experience slight discomfort. When the needle initially enters your skin, there will be a small pinch and it may hurt for a short time. The risks of having your blood drawn include development of a small bruise, development of a blood clot or infection in the vein, fainting, and/or dizziness. Universal Precautions will be followed for collection, handling, processing, and disposal of items that may have come into contact with bodily fluids during the collection of your blood.

Exercise Efficiency and Aerobic Capacity: There is a small risk of injury (e.g., sprained ankle), complications requiring you to go to the hospital, heart attack, or even death. In studies involving people with heart disease, the risk of hospitalization was 1 in 500 tests (<0.20%). The risk of heart attack was 1 in 2,500 tests (0.04%) and death, 1 in 10,000 tests (0.01%). The risks are likely to be lower in young, healthy individuals. Only experienced staff members will conduct these tests and you will be monitored throughout the test for signs of problems. You will be tired after this test and may have sore muscles for a few days.

Low-Calorie Diets: Potential risks associated with consuming a low-calorie diet include weight loss, loss of muscle mass, mood disturbances, and low intake of vitamins and minerals. These risks will be minimized by providing a daily multivitamin to be taken on the 10 days when a low-calorie diet will be consumed. Meals and snacks will also be designed so that 20% of total calories come from protein to help protect muscle mass. It will be encouraged to eat the provided carbohydrate-based snack before the endurance training session each day to reduce feeling of fatigue or lightheadedness. Significant weight loss, decreases in muscle mass, and micronutrient deficiency are unlikely given the short-duration of this study.

Endurance Training: Risks associated with running on the treadmill include falls, stepping off of the belt, and post-exercise movement illusion (treadmill buzz). With any physical activity, there is a chance of muscle injury, ligament and tendon injury, as well as skeletal injury. Abnormal increases or decreases in blood pressure or cardiac arrhythmia are also possible but unlikely in young, healthy individuals with a background in endurance training. You are encouraged to let the researchers know if you develop any discomfort during or as the result of your treadmill runs, including excessive joint or muscle soreness, injury, or extreme fatigue.

High-Impact Jumping Exercises: Potential risks associated with high-impact jumping exercises include muscle injury, ligament and tendon injury, and skeletal injury. A low volume of jumps will be performed in this study (5 sets of 10 jumps per day) with a 60 second rest between each set of 10 jumps to reduce risk of excess strain on joints and bones. Jumps will be performed on a platform that measures force. Additionally, all jumps will be supervised by a NSCA-certified strength and conditioning specialist who will monitor movements for proper form and safety techniques. You are encouraged to let the researchers know if you develop any discomfort during or as the result of your jumping exercises, including excessive joint or muscle soreness or injury.

Continuous Glucose Monitoring: The placement of this device will require that a sensor is inserted into the back of your upper arm. Placement of the sensor may cause some pain during the insertion or lead to inflammation, redness, swelling, minor bleeding and/or minor infection at the site. This will all be minimized by having a trained individual perform the procedure which will take place in aseptic conditions. There is also a possibility that you may experience these symptoms as a result of contact between the adhesive pad of the sensor and the skin; allergic reactions can also develop in response to the adhesive used to keep the device in place. If any of these symptoms occur, you have the ability to remove the CGM and these issues will clear up within a short time period.

What happens to the information collected for the research?

We will make every effort to limit the use and disclosure of your personal information, including study data and medical history data, only to people who have a need to review this information. We cannot promise complete confidentiality. Organizations that may inspect and copy your information include the IRB, Human Research Protection Program, and other authorized representatives of Virginia Tech. If identifiers are removed from your private information or from samples collected as part of this research, that de-identified information or those de-identified samples could be used for future research studies or distributed to another investigator for future research studies without your additional informed consent.

We may publish the results of this research, but we will keep private your name and any information that could identify you. We protect your information from disclosure to others as the law requires. We cannot promise complete secrecy.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Data or specimens collected in this research might have your identifying information removed and used for future research or given to another researcher for future research without your consent.

Can I be removed from the research without my OK?

The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include:

- It is in your best interest
- If the researchers are unable to obtain measurements that are necessary for the study
- You develop an injury that does not allow you to fully participate in the study
- You become pregnant
- You are unable or unwilling to consume only the provided meals and snacks during the experimental phases

- You are unable to show up for the endurance training sessions or are unable to keep other scheduled appointments

What else do I need to know?

We will tell you about any new information that might affect your health, welfare, or choice to stay in the research.

If you become injured or sick from the research study that you are participating in, you will be referred to a clinic or to your personal physician or health care provider. Generally, this care will be billed to you, your insurance, or other third party. Virginia Tech has no program to pay for medical care for research-related injuries.

We will offer to share your individual test results with you. You may accept or decline these results.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research. We will provide you with a signed copy of this form for your records.

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

Appendix C: Study 1 Recruitment Materials

Volunteers Needed for Research Study on Bone Health in Female Runners

Female volunteers are needed for a study evaluating the effect of jumping exercises on bone health in long-distance runners eating a reduced-calorie diet.

IRB #22-168

You may be able to participate if you:

- Are a woman in good general health
- Are 18-35 years old
- Are a non-smoker
- Have a BMI between 18.5-25.0 kg/m2
- Have a regular menstrual cycle and are not on birth control (e.g., "the pill", IUD, and other birth control medications)
- Can run on 5 consecutive days for at least 60 minutes each day
- Not taking medications that could affect study results
- Not pregnant or lactating
- Are willing to complete supervised treadmill runs on 5 consecutive days on two occasions
- Are willing to eat a reduced-calorie diet that will be provided by the study team

You will receive information on your body composition, bone density, VO2max, running efficiency and $100 for your time.

This study will require visiting the lab on 17 days, 5 blood draws, consumption of meals provided by the research team, and participation in two phases of supervised treadmill runs and jumping exercises on 5 consecutive days in Blacksburg, VA.
Measurements will include body composition, bone density, physical activity, aerobic capacity, exercise efficiency, and dietary intake. You will also be asked to complete health screening questionnaires and wear a continuous glucose monitor.

VIRGINIA TECH

FOR MORE INFORMATION
Email VTNEMLab@gmail.com

The Effect of Impact Loading on Bone Biomarkers in Energy-Restricted Female Runners (IRB #22-168)

Volunteers are needed for a study to examine the effect of jumping exercise on bone health in long-distance female runners eating a reduced-calorie diet.

You may be able to participate if you meet the following criteria:

- Woman in good general health
- Age 18-35 years old
- Not on birth control (including oral contraceptives, injections, or IUD)
- Has a regular menstrual cycle
- BMI between 18.5-25.0 kg/m2
- Can run on 5 consecutive days for 60-90 minutes each day
- Non-smoker
- Not pregnant or lactating

This study involves completing supervised treadmill runs on 5 consecutive days on two occasions. During one of those occasions, you will also be asked to perform jumping exercises. The two occasions will be separated by 1 menstrual cycle (approximately 3 weeks). Reduced-calorie meals and snacks will be provided during both 5-day periods.

Study measurements will include 5 fasted blood draws, body composition, bone density, VO2max, and exercise efficiency. Total time commitment will be 16 non-consecutive days of lab visits.

Participants will receive information on body composition, bone density, VO2max, running efficiency, and $100 for your time.

Please contact Trisha Sterringer (VTNEMLab@gmail.com) for more information.

Email To Send to Interested and Qualified Participants *after* the Phone Screening

Subject Line: "Bone Health in Female Runners Consent for your Consideration (IRB #22-168)"

Dear Participant [Insert Name],

Thank you again for your time to complete the phone screen for our study *The Effect of Impact-Loading on Bone Biomarkers in Energy-Restricted Females Runners (IRB #22-168)*. As promised, I am attaching a copy of the consent for the study for your careful consideration. The consent form provides a general overview of why we are doing the study, what we expect to get out of it, what you would do if you were to decide to participate and the potential risks to participating.
This copy is for your review only. You do not need to print it. If you decide to participate in the study, we will provide a hard copy for you to sign at the beginning of your screening visit which [*Insert A or B here] [(A) is scheduled at XX time on X date] or [(B) you can schedule when you are ready by replying to this email]. During your scheduled screening visit, we have set aside time at the start to discuss the consent and address any questions or concerns you have. You may also email questions before your scheduled visit by replying to this email.
Again, thank you for your interest in the study. We look forward to [(A) seeing you] or [(B) hearing from you].

Sincerely,
Trisha Sterringer, MS, RD, CSCS

Appendix D: Study 1 Questionnaires

HEALTH HISTORY SCREENING QUESTIONNAIRE

Please fill out as completely and accurately as possible.

Date _____/_____/_____

Name:_____ Ethnicity:_____

Address:_____City:_____State:_____Zip:_____

Age:_____ Phone #:_____ Alt #:_____

Email:_____@_____

CARDIOVASCULAR HEALTH HISTORY

Have you ever been diagnosed with or had any of the following?

Heart Attack?		Yes		No
Heart Surgery ?		Yes		No
Cerebrovascular accident?		Yes		No
Transient Ischemic Attack (TIA)?	Yes		No	
Carotid Artery Disease?	Yes		No	
Cardiac Catheterization?		Yes		No
Coronary Angioplasty?	Yes		No	
Pacemaker/Implantable Cardiac Device?		Yes		No
Irregular Heart Rate/Heart Rhythm Disturbance?		Yes		No
Atrial Fibrillation?		Yes		No
Heart Valve Disease?		Yes		No
Heart Failure?		Yes		No
Heart Murmur?	Yes		No	
Heart Transplantation?	Yes		No	
Congenital Heart Disease?		Yes		No

Have you ever experienced any of the following symptoms:

Chest discomfort with exertion? Yes No

Unreasonable breathlessness? Yes No

Dizziness, fainting, or blackouts? Yes No

Syncope (loss of consciousness)? Yes No

Hypoxia (low oxygen levels)? Yes No

Do you currently take heart medications? Yes No

If yes, what?_____

Have you been diagnosed with diabetes (Type 1 or Type 2) or problems with blood sugar levels? Yes No

If yes, please note Type 1 or Type 2_____

If you circled yes to any of the above statements in this section, consult your physician or appropriate healthcare provider before engaging in exercise. You may need to use a facility with a* ***medically qualified staff.*

CARDIOVASCULAR RISK FACTORS

Are you a male over 45 years old? Yes No

Are you a female over 55 years old? Yes No

Have you had a hysterectomy with or without ovary removal? Yes No

Are you postmenopausal? Yes No

Do you currently smoke or have you quit within the last six months? Yes No

Is your blood pressure greater than 140/90 mm Hg? Yes No I Don't Know

If known, what is your blood pressure? _____/_____ mm Hg

Do you currently take blood pressure medications? Yes No

Do you currently take any medications for your heart? Yes No

Is your blood cholesterol level greater than 200 mg/dl? Yes No I Don't Know

Do you know your cholesterol or triglyceride levels? Yes No

If yes, Total Cholesterol _____ HDL _____

LDL _____ Triglycerides_____

Do you have a close blood relative who has suffered a heart attack or had any kind of heart surgery before the age of 55 (for father or brother) or age 65 (for mother or sister)? Yes No

Are you more than 20 pounds overweight? Yes No I Don't Know

Are you physically inactive (i.e., do you get less than 30 minutes of physical activity less than three times a week)? Yes No

Have you had a recent surgery (in the past 2 years)? Yes No

If yes, please explain_____

_____

Have you had an exercise stress test, cardiac catherization, or echocardiogram? Yes No

If yes, please explain_____

_____

If you circled yes to two or more of the statements in the above section you should consult your physician or other healthcare provider before engaging in exercise. You might benefit from using a facility with a **professionally qualified exercise program.*

To the best of our knowledge, do you have any of the above fore-mentioned risk factors? Yes No**

*** You should be able to exercise safely without consulting your physician or other healthcare provider in a self-guided program or almost any facility that meets your exercise program needs.*

To the best of my knowledge, the information I have provided above is an accurate assessment of my health and medical history.

_____ _____ _____

Name of Participant **Participant's Signature** **Date**

_____ _____ _____

Name of Administering Staff **Signature of Staff Member** **Date**

Please stop here. The remainder of this Health History Screening Questionnaire will be administered to you by one of our staff.

Staff: Please administer the remaining portion of the Health History Survey.

GENERAL MEDICAL HISTORY

(this portion of the questionnaire is completed by oral interview of the participant by study staff)

Provide the following information:

Height:_____ Weight:_____ BMI (calculated):_____

Hip circ.:_____ Waist circ.:_____

Circle One

Do you drink alcohol? Yes No

If yes, how many drinks per week?_____

Are you taking any prescription or over-the-counter medication? Yes No

If yes, what medication and what dosage?_____

_____

Do you take any vitamins, supplements, performance enhancers or herbal/homeopathic medications?

Yes No

If yes, what type and what dosage?_____

_____

Have you been on a recent diet or a specific diet prescribed by a healthcare provider? Yes No

If yes, please explain_____

Have you been on a recent self-prescribed diet or a specific eating plan?

Yes No

If yes, please explain_____

For how long? _____

Have you been diagnosed with asthma, exercise-induced asthma, reactive airway disease, chronic obstructive pulmonary disease, or any other respiratory disease?

Yes No

If yes, please describe:_____

_____

Have you ever been diagnosed with cancer? Yes No

If yes, please describe when and what type:_____

_____

Have you ever undergone a lymphectomy? Yes No

If yes, please describe when and why?_____

Do you have musculoskeletal problems that limit your physical activity such as walking? Yes No

Do you have any bone, joint, ligament or tendon problems that bother you or cause discomfort on a regular basis? Examples include persistent knee or back pain. If yes, please describe _____ Yes No

_____

_____

Do you have concerns about your safety when you exercise or exert yourself? Yes No

Have you ever experienced burning or cramping sensations in your lower legs when walking short distances? Yes No

Have you ever been told that you were anemic? Yes No

If yes, how long ago? _____

Do you have other (recent, previous, current?) health problems, illnesses, diseases, infections, surgeries, allergies or hospitalizations including an eating disorder? _____

_____

Do you have any other health problems, illnesses, diseases, infections, surgeries, allergies, hospitalizations? Yes No

If yes, please explain_____

_____

Are you claustrophobic? Yes No

Do you have problems with having your blood drawn? Yes No

Do you have a fear of needles? Yes No

Do you have problems having your blood pressure taken in either arm? Yes No

FAMILY HISTORY

Please check all that apply

Family Member	High Blood Pressure	Diabetes Type I or II	Heart Disease	Obesity	Comments
Mother					If yes, was it before the age of 65? **Yes No**
Father					If yes, was it before the age of 65? **Yes No**
Sibling					Gender: Age:

Sibling					Gender: Age:
Paternal Grandmother					Age:
Paternal Grandfather					Age:
Maternal Grandmother					Age:
Maternal Grandfather					Age:

FOR FEMALES ONLY:

Are you pre-____, peri-____ or post-____ menopausal?
When was your last menstrual period? _____
How frequently do your menstrual periods come? _____

Are you using oral contraception (the Pill) or any form of estrogen only or combined hormonal contraception/ therapy, including creams, patches, or subdermal treatments? Yes No

If yes, what type?_____ Dosage?_____

If you are premenopausal:

Are you pregnant? Yes No I Don't Know

Could you be pregnant? Yes No I Don't Know

Are you trying to become pregnant? Yes No

If you are peri- or postmenopausal:

For how long?_____

Have you had a hysterectomy w/ or w/out ovary removal? Yes No

Are you currently taking any Hormone Replacement Therapy? Yes No

If yes, what type?_____How long?_____Dosage_

Do you have any nutrition-related disorders that such as lactose intolerance, irritable bowel, food allerges, problems gaining weight, etc.

_____
_____

Administered by _____ (name of staff admistering form)

Name:_____ IRB #22-168
(blacken out after assigning Study Code)

Screening for LOAD Study

Date:_____ **Screener:** _____**Study Code:**_____
Age:_____ **Email:**_____ **Phone:**_____
Reported: Height_____ in. Weight_____ lbs. BMI:_____ kg/m²

Consider yourself in good general health? **Yes** **No** **Not sure**

Pregnant or Lactating? **Yes** **No**

Currently trying to gain or lose body weight? **Yes** **No** **Not currently**
Reason _____
How long attempting? _____

<u>Endurance Training:</u>

1. How long have you been running long distance?

- ☐ Less than 1 year, _____ months or weeks
- ☐ 1-3 years
- ☐ 3-5 years
- ☐ 5-10 years
- ☐ 10 years+

2. On average, how many days a week do you currently run?

3. On average, how many miles do you currently run each week?

- ☐ Less than 20
- ☐ 20-30
- ☐ 30-40
- ☐ 40-50
- ☐ More than 50

If athlete doesn't fit above criteria, please explain:

4. On average, what is the distance of your normal training run (not including long runs)?

5. Do you currently engage in any cross-training (resistance training, swimming, cycling, etc.)? **Yes** **No**

If **yes**, how often?

6. Are you currently training for any races or athletic events **Yes** **No**

If **yes**,

Race date:
Race distance:

7. Do you have a heart or lung condition that would prevent you from participating in the study which will require daily exercise? **Yes** **No**

8. Are you currently dealing with any injury that would prevent you from running or engaging in your normal training? **Yes** **No**

If **yes**,

When the injury occurred:
Injury:

<u>Dietary Habits:</u>

Special diet/food preference: Vegetarian/vegan ____ Gluten-Free ____ Low-Carb ____

Paleo____ Other? _____

Do you have any food allergies or sensitivities? **Yes** **No**
If yes, which foods? _____
_____

<u>Medications & Supplements:</u>

Thyroid Hormone Replacement (e.g., Synthroid)	**Yes**	**No**
Anticonvulsants	**Yes**	**No**
Hormone Replacement	**Yes**	**No**
Birth Control	**Yes**	**No**

Dietary Supplements (Multi-vitamins, brand, dose, etc.):

_____
_____

Do you have normal menstrual cycles? **Yes** **No**

If yes, how frequently do they occur? _____
If yes, approximately how long do your menstrual cycles last? _____
If yes, when was your last period? _____

Have you ever been anemic? **Yes** **No**
If yes, when and any circumstances? _____

Have you ever had a blood or thyroid disorder? **Yes** **No**
If yes, when and any circumstances? _____

Have you ever been diagnosed with an eating disorder? **Yes** **No**
If yes, when and any circumstances? _____

Willing to participate in a supervised exercise program that would begin on the third or fourth day of your monthly cycle? **Yes** **No**

Willing to participate in supervised running program that will require you to run on 5 consecutive days in the NEM lab for approximately 60 minutes each day? **Yes** **No**
(5 consecutive days on 2 occasions)

Willing to participate in supervised jumping program? **Yes** **No**
(5 consecutive days on a randomly assigned occasion)

Willing to consume a reduced-calorie diet? **Yes** **No**
(5 consecutive days on 2 occasions)

Comfortable with body composition testing? **Yes** **No**

Fear of needles or difficulty getting blood drawn? **Yes** **No**

Experience running on a treadmill **Yes** **No**

Appendix E: Study 1 Experimental Menus

Meal	Menu A	Menu B
Breakfast	• Low-sugar instant oatmeal • Nonfat plain Greek yogurt with blueberries and granola	• Whole wheat bagel with cream cheese • Nonfat plain Greek yogurt with blueberries and granola
Lunch	• Salad: Quinoa, lettuce, red cabbage, carrots, slivered almonds, sesame ginger dressing • Wheat crackers	• Salad: Brown rice, lettuce, cucumber, red pepper, feta cheese, low-fat Greek dressing • Wheat crackers
Pre-Run Snack *1-2 h before run*	• Pretzels (30g CHO)	• Pretzels (30g CHO)
Dinner	• Commercial frozen dinner with chicken • Whole wheat bread and butter • Dark chocolate square	• Commercial frozen dinner with beef • Whole wheat bread and butter • Dark chocolate square

Example of rotating menus for controlled diets for Study 1. *Menu A consumed on D1, D3, and D5. Menu B consumed on D2 and D4. Menus provided 30 kcal·kgFFM^{-1}·d^{-1} with 55% total kcal from carbohydrate (CHO), 20% from protein, and 25% from fat. Food items adjusted as necessary based on individual preferences to increase adherence.*

Jumping Intervention
LOAD – Nutrition & Exercise Metabolism Laboratory

JUMP DAY 1

Procedure:

1. Calibrate the metabolic cart after the heater has warmed up for at least 30 minutes.
2. Set up the mouthpiece for the metabolic cart with the spit tube and black plug on opposite sides so that the air collection tube is on the participant's left side (Image 1).
3. Place the wooden box at the back of the treadmill so that it is flush against the machine.
4. Place the force plates approximately 3-6 inches in front of the wooden box (adjust distance as necessary based on participant preference and mechanics).
5. Place the padded mats directly in front of the force plates (Image 2)
6. Connect the force plates to each other, the external battery, and the USB adapter. Connect the USB adapter to the NEM laptop.
7. Open the NeuLog app on the NEM laptop.
    a. Click "Run Experiment" in the upper left corner of the dashboard (Image 3).
    b. In Experiment Setup, select the following (Image 4):
        i. Duration: 10 minutes
        ii. Rate: 20 per second
        iii. Trigger: Off
        iv. Display while recording: checked
    c. On the lefthand side, check that the force plates are reading 0 N. If they are not at zero, click on the force plate an follow instructions below (Image 5):
        i. Click "Extra Command" > Reset
        ii. The plate should then read zero and you are ready to begin the experiment.
8. Weigh participant without shoes.
9. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
10. Demonstrate to the participant how to perform the jumps. Use the following instructions.
    a. Step off of the box so that both feet land at about the same time with one foot on each force plate. Then, using the momentum from the downward movement, jump straight up into the air and land with one foot on each force plate. That is considered one rep.
    b. Land on the balls of your feet. Avoid landing on your heels.
    c. Leave a slight micro-bend in your knees when you land. Avoid buckling or locking your knees in a straight position.
    d. Make sure you give yourself enough room on the step down to avoid catching your heel on the box and falling forward.
    e. Jump high enough that you make it a few inches off of the ground.
11. Have the participant practice 3-5 jumps and provide feedback on form.
12. Place the facemask on the participant connected to the metabolic cart.
13. Have the participant practice 3-5 jumps with the equipment on and provide feedback on form.
    a. Instruct the participant to hold the tube with their left hand as they jump to avoid tripping or getting the tube caught during the movement.
14. Start the participant's smartwatch for "Cardio".

15. Start the Polar app on your personal cell phone. Check to make sure the HR monitor is connected to the polar app. Select "functional training" as the exercise modality and press to begin.
16. Once all equipment (mouthpiece and nose clip) is on the participant, begin the exercise test on Parvo. Have the participant stand on the box and breathe normally for 4 minutes to collect steady state breathing before the jumps.
17. After 4 minutes, begin the NeuLog application to start recording the ground reaction force.
    a. On the NeuLog app, click "Record" experiment to begin the force plate recordings. **Do not start the force plate recording until you are ready to begin.** The app will automatically stop at 10 minutes and the last jumps may not be recorded if the participant exceeds 10 minutes.
18. Have the participant perform 10 repetitions and then 60 second rest. Repeat 4 more times for a total of 50 jumps (5 x 10 reps with 60 sec rest between sets). The test should take approximately 7-10 minutes.
    a. If the participant completes the 4th set and there is less than 90 seconds left on the force plate timer, stop the NeuLog application, save the file for the first 4 sets, and then restart the NeuLog app again (repeat step 6). Failure to do this may result in lost data for the last repetitions of jumps if there is not enough time for the 10-minute recording.
19. After the fifth set of jumps, collect one minute of recovery breathing. A summary of the protocol is shown below (figure 1).
20. Stop the experiment on NeuLog and save the csv file
    a. Click "Stop" then "Export"
    b. Save file as LOAD# JUMP D1
    c. Email file to tsterringer@vt.edu and vtnemlab@gmail.com with the title "LOAD# JUMP D1"
    d. Save the csv file to DropBox by creating a new folder for the participant in the GRF folder (*DropBox > LOAD Study > Data > GRF)*
        i. Name the file: LOAD##_GRF_D1

    PLEASE NOTE: Unsaved NeuLog data cannot be retrieved. Confirm that the email has been sent or the file has been saved BEFORE exiting out of the NeuLog experiment window.
21. Stop the watch and polar app. Remove HR monitor. Record the HR max and average from the polar app on the data sheet.
22. Save the metabolic report csv file on the desktop and in DropBox
    a. Name the file: LOAD##_JUMP
    b. Desktop: *Desktop > Data> LOAD > GRF*
    c. DropBox: *DropBox > LOAD Study > Data > JUMP - Cart*

JUMP DAY 2-5

Procedure:

1. Set-up force plates in the weight room against the wall. Repeat steps 4-7 from Day 1 protocol for setting up force plates and experiment in NeuLog application.
2. Weigh participant without shoes.
3. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
4. Start the participant's smartwatch for Cardio.
5. Start the polar app for Fitness Training.
6. Start the experiment in the NeuLog application

7. Have the participant perform 5 sets of 10 jumps with a 60 second rest between each set.
8. Stop the NeuLog experiment after the final set. Save csv file to desktop and email copies to tsterringer@vt.edu and VTNEMLab@gmail.com. Save csv file to dropbox.
9. Stop watch and polar app.
10. Remove HR monitor.

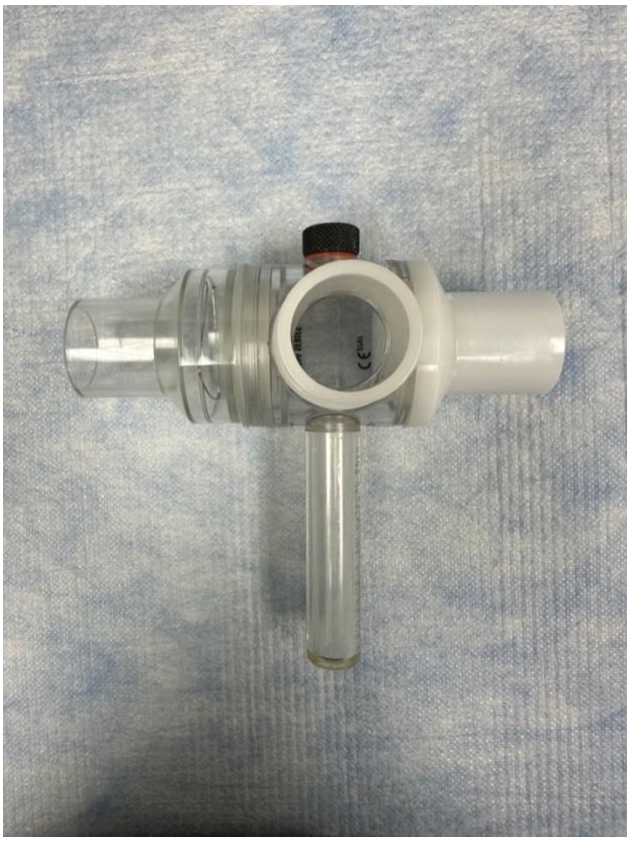

Image 1. Mouthpiece set-up for air collection during jumping exercises.

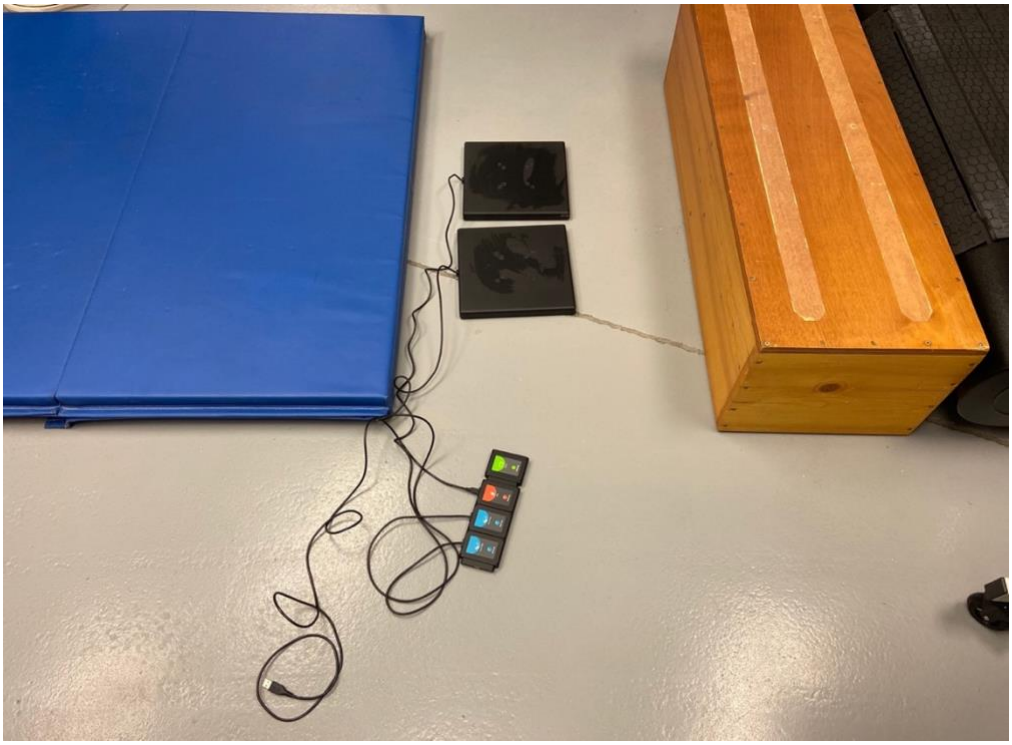

Image 2. Force plate set-up for jumps on day 1 with metabolic cart.

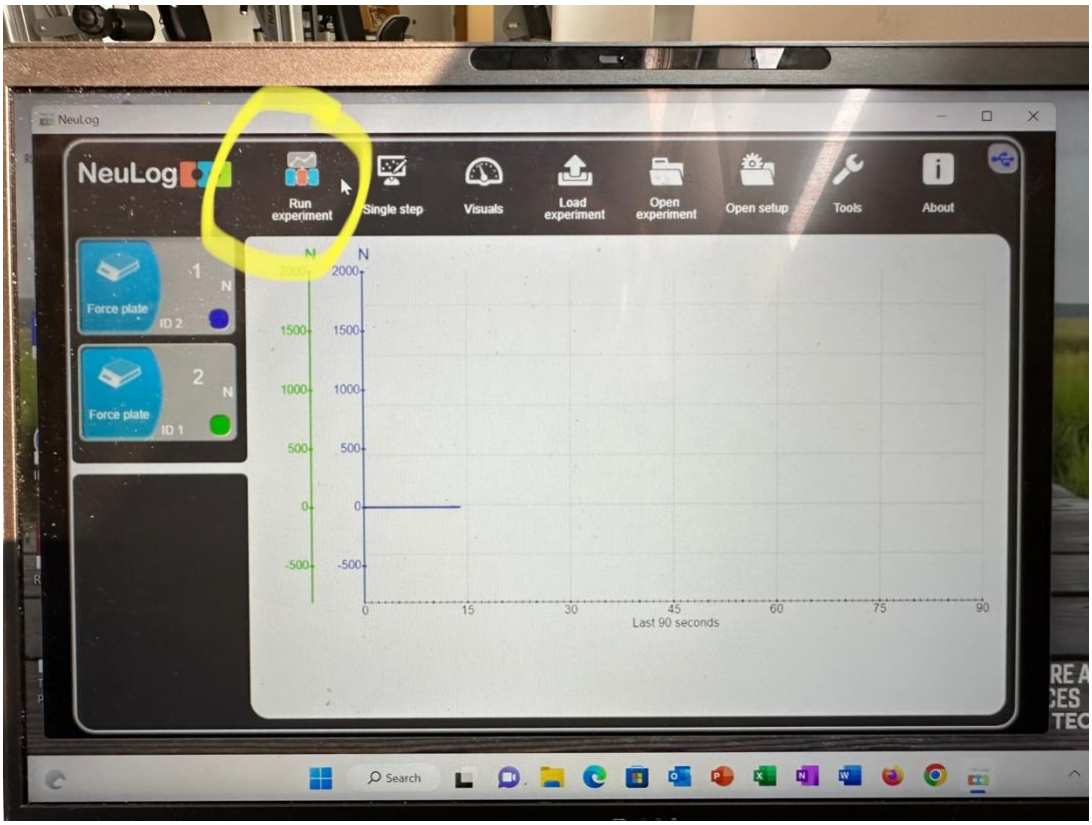

Image 3. Run Experiment

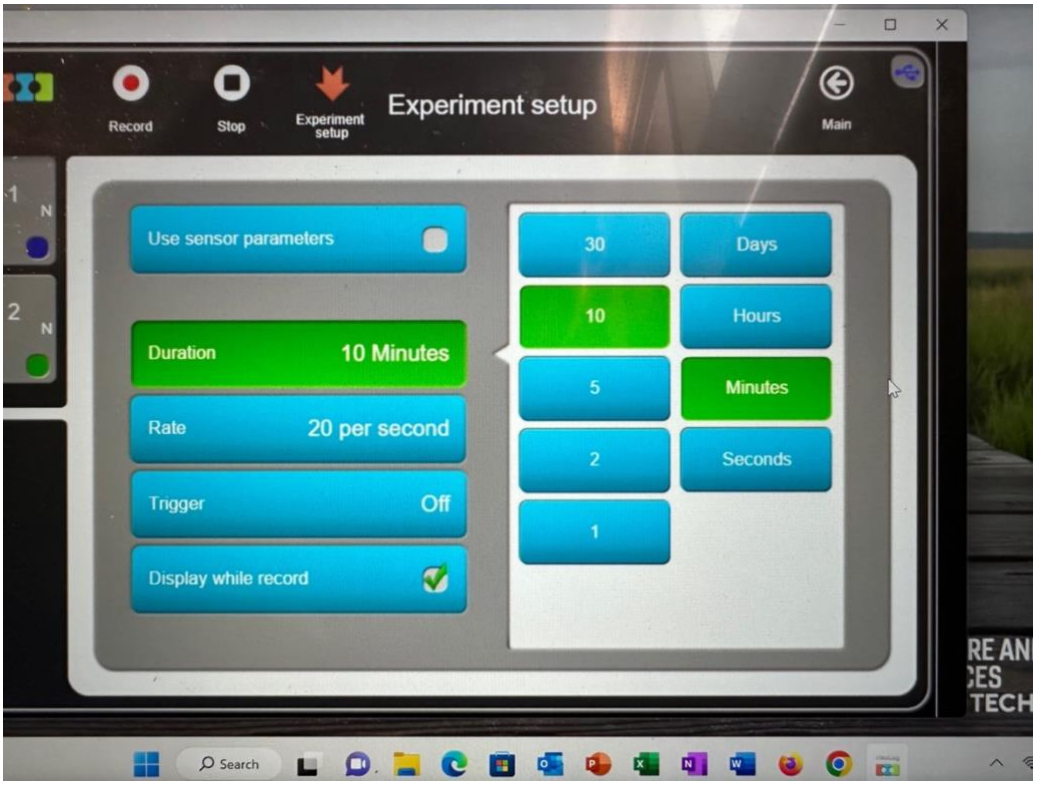

Image 4. Experiment setup

Image 5. Resetting the force plates to zero.

Figure 1. Checklist for Day 1 using metabolic cart

Time	Check	Details	Notes
45-60 min prior to testing		Start metabolic cart	
		Set-up facemask	
		Set-up box and force plates	
		Set-up the experiment in the NeuLog application	
Start of the appointment, before testing begins		Weigh participant without shoes	
		Wet and place HR monitor on participant	
		Demonstrate jumps	
		Allow participant to practice jumps without equipment	
		Place facemask on participant and connect to metabolic cart	
		Allow participant to practice jumping with the equipment on	
		Start the participant's watch for "cardio"	
		Start the polar app on cell phone for "functional training"	
Start the experiment		Place nose clip on participant and begin the test on Parvo	
		Collect 4 minutes of steady state breathing	
		Have participant start jumping at 4:00	
		Participant performs 5 sets of 10 jumps with a 60-sec rest between sets	
		After the last set, collect one minute of recovery breath on the cart	
Once jumps are complete		Stop the experiment on NeuLog and save the file in DropBox	
		Stop the watch	
		Stop polar app and record the HR max and avg on data sheet	
		Remove HR monitor	
		Save the metabolic text report to dropbox	

Smart Watch Set-up & Data Retrieval
Virginia Tech – Nutrition & Exercise Metabolism Lab

Providing the Smart Watch

1. Select smart watch and enter watch and wearing information on Garmin device tracking sheet Excel spreadsheet (found in Dropbox)
2. Sync watch with computer using USB cable and Garmin Express PC app
3. Log into Garmin Connect account associated with smart watch # - on website, with email and password on Excel spreadsheet under "Devices" tab
4. Click on picture of watch at the top and select "Device Settings"
5. Click on "Activity Tracking" tab
6. Enter Stride Length test results* - distance in feet and # of steps; Click on "Save Settings"
7. Click on "User settings" tab
8. Enter in info below; Click on "Save Settings"
    a. Birthdate
    b. Gender
    c. Height
    d. Weight
9. Click on "General" tab
10. Select Right or Left wrist; Click on "Save Settings"
11. Exit out of webpage and return to Garmin Express PC app
12. Sync the watch again to update settings and log back in to Garmin Connect to double-check settings

*Stride length test – Participant stands behind carpet line in the hallway, have participant walk 20 steps, place piece of tape at the back of the foot of the 20th step, and count the number of carpet squares (24" each) and measure the distance between the back of the piece of tape to the start of the carpet square and add together. Enter distance and # of steps into Activity Tracking on Garmin Connect website.

Retrieving the Smart Watch and Recorded Data

1. Collect smart watch, USB cable, and box from participant after the 3- or 5-day collection period
2. Connect smart watch to the computer using the USB cable
3. Open the Garmin Express app and allow smart watch to sync
4. When Garmin data file appears, copy the folder to the Smart Watch raw data folder on Dropbox
5. Click on option to load data and log into the Garmin Connect website account associated with the smart watch number
6. Verify that the data for the collection period looks correct and has uploaded to the account.

7. Contact Trisha Sterringer (tsterringer@vt.edu, 440-2215653) that someone has completed activity tracking
    a. Which participant
    b. Which watch
    c. What dates

Setting Up User Profile—STAFF ONLY

1. Hold button B down
2. Select ⚙
3. Choose “User Profile”
4. Select option to change

Setting Custom Stride Length

Garmin Connect Web

1. Connect device to computer with USB cable.
2. Sign into **Garmin Connect**
3. Select the device icon ⌚ (upper right corner)
4. Select your device from the list
5. Select **Activity Tracking**
6. Under Custom Stride Length, toggle **Walking** button to turn it on
7. Enter the known distance from your walk
8. Enter the number of steps it took to cover that distance
9. Scroll down and select **Save Settings**
10. Sync your device with Garmin Express or with the Garmin Connect app, if paired to a smartphone

Customizing Device

Activities and App Settings—STAFF ONLY

1. Hold button B
2. Select ⚙
3. Select “Activities and Apps”
4. Select chosen activity
5. Select “activity settings”
6. Definitions of settings that can be changed
    - Alerts: notifications during activity
    - Auto lap: creates a lap without pushing the button at a predetermined distance
    - Auto pause: stops recording when under a certain speed or stopped

- Auto rest: stops recording during pool swim, creates rest interval
- Auto scroll: flips through data screens during activity
- Auto set: starts and stops strength sets during activity
- Data screens: customize the data screens during activity
- Edit weight: add weight to an exercise set during strength
- GPS: sets mode for GPS antenna
- Pool size: sets size of pool for pool swimming
- Vibration alerts: notify to inhale or exhale during breathwork - NO VIBRATION FEEDBACK

Treadmill Runs
LOAD – Nutrition & Exercise Metabolism Laboratory

PRESCRIPTION RUN

Procedure:

23. Calibrate the metabolic cart after the heater has warmed up for at least 30 minutes.
24. Weigh participant without shoes.
25. Record the time of the participant's last meal/snack.
26. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
27. Place the facemask on the participant connected to the metabolic cart.
28. Start the participant's smartwatch for "treadmill run".
29. Begin the running economy test at a speed of 5.5mph and 0% grade. Increase pace by 0.5mph in 4-minute stages. Record the RPE during the last minute of each stage. Collect HR every 20 seconds during the last 2 minutes.
30. When the economy test is complete, proceed to estimate target pace.
31. Increase incline to 1% grade and set the speed to the target pace estimated using the ACSM equation. If the participant seems more efficient based on the economy test, increase the pace to a speed that seems close to the target 65-70% VO_2max. Each phase will be 4 minutes with the last 2 minutes of steady state data collected.
    a. **If VO_2 is reached:** Do not end the test until the 4-min stage is complete.
    b. **If VO_2 is exceeded:** Do not adjust the speed until the 4-min stage is complete. Then reduce the speed as necessary.
    c. **DO NOT stop the test or adjust the speed until the entire 4-min stage is complete**.
32. When the test is complete, remove facemask and save excel file. In the notes of the metabolic test, include the paces used for the economy test and prescription stages. Save file in the data folder on DropBox or OneDrive.
33. Provide a weighed water bottle. Instruct the participant to consume as much water as they would like during the remainder of the run. When the run is complete, reweight the bottle.
34. Record the time when the run begins and start a timer. Pause the timer any time the participant needs to use the restroom or take a break. Collect HR every 5 min.
35. Open the prescription excel file (*DropBox > LOAD Study > Study Forms > Data Collection Sheets > Prescription Spreadsheet Template*). Enter the last 2 minutes of data for the VO_2 and REE/min. The excel sheet will calculate the remaining duration of the run.
36. At the end of the run, remove HR monitor and stop smartwatch. Weigh the water bottle to calculate amount of water consumed.
37. Provide lunchbox.

RUN ON DAYS 2-4

Procedure:

11. Weigh participant without shoes.
12. Record the time of the participant's last meal/snack.
13. Provide water bottle with the same amount of water that was consumed on day 1. Instruct participant to consume the entire bottle during the run.

14. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
15. Start the participant's smartwatch for Treadmill Run.
16. Record the time when the run begins and start a timer. Pause the timer any time the participant needs to use the restroom or take a break.
17. Begin with a 5-minute warm-up at a self-selected pace of 5.5, 6.0, or 6.5 mph at 0% grade. After warm-up, increase speed to prescription pace and grade to 1%. Collect HR and RPE every 5 min.
18. At the end of the run, remove heart rate monitor and stop smartwatch.

DAY 5 RUN

Procedure:

1. Calibrate the metabolic cart after the heater has warmed up for at least 30 minutes.
2. Weigh participant without shoes.
3. Record the time of the participant's last meal/snack.
4. Provide water bottle with the same amount of water that was consumed on day 1. Instruct participant to consume the entire bottle during the run.
5. Place a heart rate monitor on the participant by first wetting the electrode strip and skin of the participant. The HR monitor should fit snuggly against the participant's skin without any gaps.
6. Place the facemask on the participant connected to the metabolic cart. Set the exercise testing to "submaximal" and then begin the test.
7. Start the participant's smartwatch for Treadmill Run.
8. Begin the running economy test at a speed of 5.5mph and 0% grade. Increase pace by 0.5mph in 4-minute stages.
9. When economy test is complete, remove facemask and save excel file (*Desktop > LOAD > Economy).*
    a. BEFORE SAVING: In the notes section on the economy test put the time the test ended and the speed of the 3 stages for the economy test.
        i. Example: "Test ended at 12:01. Economy stages 5.5, 6.0, 6.5 mph at 0% grade."
    b. Print a copy of the metabolic report.
    c. Save the excel file on the cart computer.
        i. Location: *Desktop > LOAD > Economy*
        ii. File Name: ParticipantID_Economy_Pre/Post_RUN/JUMP
    d. Save the excel file on DropBox or OneDrive
        i. Location: DropBox > LOAD study > Data > Economy > Participant ID
10. The participant can then start running again at the target pace. Be sure to record the time when the run begins and start a timer. Pause the timer any time the participant needs to use the restroom or take a break.
11. Calculate the remaining duration for the run using the steps below:
    a. Open the Prescription Spreadsheet Template on DropBox (*DropBox > LOAD Study > Study Forms > Data Collection Sheets > Prescription Spreadsheet Template*)
    b. the last 2 minutes of data for the VO_2 and the spreadsheet will automatically calculate the remaining duration for the run.
12. Collect HR every 5 min.
13. At the end of the run, remove heart rate monitor and stop smartwatch.

Blood Draw
LOAD – Nutrition & Exercise Metabolism Laboratory

Supplies

ALL BLOOD DRAW VISITS

- 2 Chuck pads
- Nitrile gloves
- Tourniquet
- CVS – Closed venous system ("butterfly")
- Vacutainer holder
- 2 Alcohol preps
- Coban
- Gauze pads

SCREENING

- 1 4mL purple top tube
- 1 8.5mL red/gray "tiger" top tube
- Non-sterile urine cup
- Pregnancy test
- 1 small biohazard bag (for Lab Corp)
- Lab Corp sample requisition form

PRE/POST INTERVENTION

- 1 4mL purple top tube
- 2 8.5mL red/gray "tiger" top tube (1 for Lab Corp, 1 for storage)
- 2 small biohazard bags (for Lab Corp; one frozen one room temp)
- 2 Lab Corp sample requisition forms (one for frozen, one for room temp samples)
- 2 purple cryovials (for Lab Corp)
- 6 orange top cryovials (for storage)

ADDITIONAL FOR BEFORE SECOND INTERVENTION

- Non-sterile urine cup
- Pregnancy test

Blood Collection Procedure

SCREENING

1. Set up for blood draw.
2. Label 4mL purple and 8.5mL red/gray "tiger" top tubes with subject ID# (LOAD ##), DOB, collection time, & date.
3. Once fasting blood sample is collected, invert all tubes 6-8 times.
4. Allow red/gray "tiger" top tube to sit for at least 15 min at room temp. 4mL purple top tube rests on ice.
5. Centrifuge 4mL purple top and 8.5mL red/gray "tiger" top tubes after 15 min (gray top tubes should be clotted) at 2500 rpm for 15 min (or 3500 rpm for 13 min) at 4°C. Ensure centrifuge is balanced.
6. Store 8.5mL red/gray top (ensure stopper is between the two layers) and 4mL purple top tubes in small biohazard bag at room temp.

PRE/POST INTERVENTION

1. Set up for blood draw.
2. Label both 8.5mL red/gray "tiger" top tubes with subject ID# (LOAD ##), DOB, collection time, & date. Label purple cryovials (for Lab Corp) with subject ID# and timepoint, DOB, collection time and date, and "PTH frozen from EDTA" OR "INSULIN frozen from SST".

Label storage cryovials (orange top) with subject ID#, collection date, P/S, study timepoint (1 plasma, 5 serum).

3. Once fasting blood sample is collected, invert all tubes 6-8 times.
4. Allow red/gray top tubes to sit for at least 15 min at room temp. 4mL purple top tube rests on ice.
5. Centrifuge 4mL purple top and red/gray top tubes after 15 min (red/gray top tubes should be clotted) at 2500 rpm for 15 min (or 3500 rpm for 13 min) at 4°C. Ensure centrifuge is balanced.
6. Using transfer pipet, transfer ~1mL plasma (4mL purple top) into the purple cryovial labeled PTH for Lab Corp. Transfer the remaining plasma into the one storage cryovial (orange) labeled for plasma. Identify the red/gray "tiger" top with the greater volume of serum. This one will be used for Lab Corp. Using a new pipet, transfer ~1mL serum into the purple cryovial labeled INSULIN for Lab Corp. Reseal the red/gray "tiger" top tube for Lab Corp. Transfer serum from the second red/grey "tiger" top tube into 5 serum labeled cryovials. Place the 2 purple cryovials for Lab Corp into the -80 freezer. Store the 6 orange cryovials in the -80 freezer. Indicate the # of cryovials collected on the LOAD blood collection form as well as the storage box and slots (also on freezer blood sample tracking sheet).

LabCorp Requisition Form

1. Enter date specimen collected, mm/dd/yyyy
2. Enter time specimen collected using a 24-hour clock, hh:mm
3. Enter patient initials, LOAD
4. Enter patient number, study number
5. Enter sex, F
6. Enter patient date of birth, mm/dd/yyyy
7. Mark Fasting or Nonfasting at top of the page
8. Enter patient visit name, study timepoint (SCREEN, PRE/POST A or B)
9. "X": Required tests based on timepoint
    a. SCREEN: CBC, TSH
    b. PRE/POST A and B
        i. Frozen: Insulin, PTH
        ii. Room Temp: Cortisol, Iron, Ferritin
10. Place order form in the side pouch of the blood sample biohazard bag.
11. For frozen samples: Wait at least 30 minutes for samples to freeze completely. Place frozen samples in blood sample biohazard bag and place inside of the Lab Corp frozen specimen samples box with an icepack on the top and bottom of the sample (small gel ice packs located in top shelf of standing freezer).

Courier Service

1. **Preferred: Sign in to LabCorp Link account to schedule pick-up online. or Call LabCorp at 540-563-9852, opt 3 ASAP to schedule a pick-up for account #45050060 at 1872 Pratt Dr., Suite 1575, Garvin Building.
2. At pick-up time or earlier, place biohazard bag into the collection box in the hall. Key to collection box in Elaina's upper side desk drawer, labeled LabCorp.

A report will usually be ready the following day via LabCorp Link. Save results on Dropbox. Print results and place in subject's study folder in Garvin.
If trouble with report, contact Suzanne Carlisle, carlis1@labcorp.com or Loretta Ashcraft, ashcral@labcorp.com.
Print and place lab report in participant subject folder.

Continuous Glucose Monitor
Virginia Tech – Nutrition & Exercise Metabolism Lab

Forms: CGM participant tracking sheet, CGM device tracking spreadsheet

Supplies

-Application:
CGM sensor
CGM reader
Chuck pad
Nitrile gloves
Alcohol wipe
Sharps container
Tegaderm tape, 2 patches

-Removal:
Gloves
Alcohol wipe
Small biohazard bag

Set-up

- Select CGM reader, sensor box
- Record serial # and participant information on CGM device tracking sheet
- Open sensor box and remove Sensor Pack, Sensor Applicator, and alcohol wipe
- Check that Sensor Pack and Applicator serial #'s match

Sensor application

- Select site on the back of the mid-upper arm. Determine if participant is a side sleeper and avoid the arm that is slept on regularly.
- Technician put on gloves
- Clean site with alcohol wipe, let dry.
- Take off lid to Sensor Pack and unscrew cap from Sensor Applicator
- Line up dark marks on Sensor Applicator and Sensor Pack. Press down firmly on Sensor Applicator until it comes to a stop.
- Lift Sensor Applicator out of Sensor Pack
- Place Sensor Applicator over site. Hold participant's arm and push down firmly until there is a "click" from the Sensor; remove Applicator.
- Apply Tegaderm tape, if needed. Provide extra Tegaderm tape, if necessary.
- Discard Applicator in Sharps biohazard container.
- Activate sensor by scanning with the reader*. Turn on reader by pressing the Home Button, select "+Start New Sensor", hold the reader within 1.5 inches of the sensor, listen for "beep". Wait 2 min, press "yes" to rescan the sensor, hold reader within 1.5 inches of the sensor; listen for "beep"; press OK to go back to the Home Screen when reader has Sensor Working message.
- Clean reader with damp microfiber cloth before storing

Data collection

- Turn reader on; use the same one that started the data collection.
- Select “Get Sensor Data”
- Hold reader within 1.5 inches of the sensor; listen for “beep”

Sensor removal

- Loosen sensor adhesive (remove Tegaderm tape first, if worn) and slowly remove from skin.
- Place sensor in Sharps/biohazard container
- Clean participant’s arm with alcohol wipe

Data download

- Connect reader to computer using yellow USB cable
- Sign in to HNFE’s Clinical Studies LibreView.com account
- Select “Upload device” and select “Create Report Linked to Patient” for new participant
- For the first collection, create participant profile, includes first and last name as ID# and 01/01/birth year as DOB. All following testing, search for the specific participant.
- Download raw glucose data Excel spreadsheet on laptop/PC and back up on a thumb drive

Notes

- Provide participant with CGM tracking sheet and instructions
- Do not wear CGM during DXA
- Cannot collect multiple CGM sensor data on one reader. Once data is collected, it must be uploaded to a computer/LibreView account before another sensor is read.
- First reading should appear ~1 hour after sensor activation
- Review data for overnight sleep compression artifact (~ <50 mg/dL)

***Setting up the Reader**

- Press Home Button to turn on the reader.
- Select preferred language, if prompted, and press OK
- Set the current date and press next
- Set the current time and press next
- Set the target glucose range (most literature indicates 70-180 mg/dL); this can be changed in the software for participant reports. Press next.
- Press done to return to Home screen
- Turn on sound in settings, if needed

Appendix G: Study 2 Virginia Tech Institutional Review Board Research Protocol

Division of Scholarly Integrity and Research Compliance
Institutional Review Board
North End Center, Suite 4120 (MC 0497)
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732
irb@vt.edu
http://www.research.vt.edu/sirc/hrpp

VIRGINIA TECH

MEMORANDUM

DATE: January 25, 2024

TO: Enette Larson-Meyer, Trisha Marie Sterringer

FROM: Virginia Tech Institutional Review Board (FWA00000572)

PROTOCOL TITLE: Changes in Energy Availability, Body Composition, and Bone Density in Male Division I Soccer Players During an Athletic Season

IRB NUMBER: **22-901**

Effective January 25, 2024, the Virginia Tech Institution Review Board (IRB) approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

https://secure.research.vt.edu/external/irb/responsibilities.htm

(Please review responsibilities before beginning your research.)

PROTOCOL INFORMATION:

Approved As:	**Full Review**
Protocol Approval Date:	**January 8, 2024**
Protocol Expiration Date:	**January 7, 2025**
Continuing Review Due Date*:	**December 23, 2024**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

ASSOCIATED FUNDING:

The table on the following page indicates whether grant proposals are related to this protocol, and which of the listed proposals, if any, have been compared to this protocol, if required.

PROTOCOL TITLE:

Include the full protocol title.

Changes in Energy Availability, Body Composition, and Bone Density of Male Division I Soccer Players During an Athletic Season

PROTOCOL NUMBER:

Include the number assigned in Protocol Management (verify this has been added before submitting protocol to HRPP).

22-901

PRINCIPAL INVESTIGATOR:

Full Name and Degrees: Dawnine Enette Larson-Meyer, PhD
Department: Human Nutrition, Foods, and Exercise
Telephone Number: 540-231-1025
Email Address: enette@vt.edu

FUNDING:

Sponsor(s): no sponsor
Funded already or in the proposal phase?: Proposal phase
Is Virginia Tech the primary awardee or the coordinating center of this grant or contract? If not, list the primary institution: N/A

VERSION NUMBER/DATE:

Include the version number and date of this protocol. Versions should start at 1.0.

Version 4.0

March 13, 2023

REVISION HISTORY:

Use this table to keep track of changes. Add more rows as needed.

Revision #	Version Date	Brief Summary of Changes (i.e., the different sections)	Consent Change?
1	11/17/22	Section 8.2 – Added locations for lab visits and PPE description for urine analysis Section 8.4 – Clarification on diet and exercise logs and questionnaires Section 9.1 – Clarification of computer referenced	Yes

		Section 9.3 – Justification for sharing data with coaches Section 10.1 – Justification for sharing data with coaches Section 12.1 – Eligibility confirmation clarified Section 13.1 – Ancillary reviews clarified Section 14.1 – Minimum number of subjects needed added to this section Section 16.1 – Reasons for subject removal Section 16.2 – Clarification to subject removal process Section 16.3 – Added procedure for when participants enroll but withdraw before completion Section 17.1 and 17.2 – Further explanations of potential risks related to DXA and confidentiality Section 19.2 – Added training data and wellness report	
2	12/13/22	Section 13.1 (page 22) – Added detail to how only authorized Radford personnel will be able to distinguish enrolled participants. Section 8.4 – Added detail about how demographic and anthropometric data will be collected. Section 17.1 and 17.2 – Added detail to how DXA scan history will be verified.	No
3	3/13/23	Section 19.2 and table 1 – Added fitness tests to the pre- and post-season data collection. Section 24.1 – Added how previously enrolled participants will be re-consented based on the addition of the fitness tests.	Yes
4	3/20/23	Section 24.1 (page 35) – More detail added to re-consent documentation	No
5	1/23/24	Section 12.1 (page 21) and Section 19.2 (page 29) Removed Radford collaborators from protocol	No

Table of Contents

28.0 Study Summary

Study Title	Changes in Energy Availability, Body Composition, and Bone Density in Male Division I Soccer Players During an Athletic Season
Study Design	Observational Study
Primary Objective	Estimate changes in energy availability, body composition, and bone density of Male Division I Soccer players during a non-championship spring season.
Secondary Objective(s)	Explore the association of energy availability on changes in body composition and bone density. Evaluate motivations and methods used by male soccer players to alter body composition. Explore changes in athlete wellness scores and the potential relationship with EA throughout Spring season.
Study Population	Male Division I Soccer players
Sample Size	40
Research Intervention(s)/ Investigational Agent(s)	This study is observational in nature. Body composition, bone density, and dietary intake will be assessed at two timepoints. The first testing session will take place before the spring season begins, and the second testing session will take place near the end of spring season. Participants will follow the team-administered training program.
Study Duration for Individual Participants	There will be 3 appointments for consent, start of season testing, and end of season testing. Participants will also track 3 days of their normal diet on two occasions.
Acronyms and Definitions	DXA, Dual-Energy X-ray Absorptiometry; BMD, bone mineral density; BMC, bone mineral composition; CBDT, certified bone densitometry technologists; EA, energy availability; NEM Laboratory, Nutrition and Exercise Metabolism Laboratory; NCAA, National Collegiate Athletic Association

29.0 Objectives

29.1 Describe the purpose, specific aims, or objectives of this study:

1) Estimate changes in energy availability (EA), body composition, and bone mineral density (BMD) of Male Division I Soccer players in response to a non-championship spring season.
2) Explore the association between EA and changes in body composition and BMD.
3) Evaluate the motivations and methods used by male soccer players to alter body composition.
Exploratory Aim: Evaluate changes in athlete wellness scores and its potential relationship with EA throughout the Spring season.

29.2 State the hypotheses to be tested:

Energy availability, total and lean body mass, and bone mineral composition will increase during the Spring season, whereas body fat will decrease.

Post-season energy availability will be positively associated with changes in total and lean body mass.

Use of dietary supplements will be higher in athletes that want to gain total and/or lean body mass.

30.0 Background

30.1 Summarize the relevant prior research on this topic and gaps in current knowledge within the field of study:

Adequate energy intake is essential for athlete health and performance. Failure to consume enough calories to meet those energy needs can result in low energy availability (EA). EA is the term used to describe the amount of energy that is available to support acts of daily living and normal bodily function after accounting for energy expended during exercise (exercise energy expenditure; EEE). This is calculated by subtracting EEE from dietary energy intake (EI) and is commonly reported relative to fat-free mass (FFM) as kcal/kgFFM/day (EA = [EI – EEE] / FFM). Low EA is a concern for athletes because it may increase injury risk or impair sports performance, and results in health consequences such as mood and hormone disturbances, decreased bone mineral density, gastrointestinal issues, loss of menstruation, and weakened immune system (Mountjoy, 2018). In female athletes, 30 kcal/kgFFM/day is the suggested threshold for low EA. However, there is no accepted cutoff for low EA in male athletes who appear to be more resilient to the effects of low EA compared to women (Areta, 2021).

Although the calculation of EA seems straightforward, there are many challenges to accurately assessing EA in free-living athletes that could result in errors of EA reporting (Burke, 2018). Additionally, EA can fluctuate in an athlete depending on the competitive season and training periodization. Furthermore, there is mixed evidence on how EA changes in response to a competitive season. In a study of 88 athletes involved in five different non-weight and weight-sensitive sports, 12.5% were found to have clinically low EA during the preparatory phase of the season. However, by the end of the season EA was higher across all five sports and no athletes reported clinically low EA (Jesus 2022). In a study of Division I female soccer players, EA was found to be significantly lower mid-season compared to pre- and post-season (Reed 2013). However, EA recovered by post-season and was not significantly different form pre-season levels. Interestingly, EI was lower in the post-season compared to pre-season, but EA was not significantly low due to a concurrent decrease in EEE in the post-season. Few studies investigating EA in male athletes have been conducted and no study to date has examined changes in EA in collegiate male soccer players over the course of an athletic season.

Previous studies have also found changes in body composition over an athletic season. Analiza et al. (2017) observed fifty-seven athletes (39 men, 18 women) during a competitive season, and found significant increases in FFM and decreases in fat mass (FM) between the preparatory and competitive phases of the season. FFM increased in the male athletes 1.3 ± 1.8 kg during the season. Another study by Analiza et al. (2017) of eighty athletes (54 men, 26 women) found the male athletes had a significant increase in body mass (0.7 ± 2.5 kg), FFM (1.1 ± 1.7 kg), and bone mineral composition (BMC; 0.047 ± 0.097 g/cm), and a decrease in percent FM (0.6 ± 1.6%) over the course of an athletic season. Walker et al. (2022) observed similar results in 46 professional

Australian soccer players. From pre-season to mid-season, there was a significant increase in lean mass and decrease in FM. In a study of Division II women's lacrosse players (n=20), BMC and z-scores (an indication of bone density compared to age-matched population) were significantly higher during the championship season compared to the off-season and pre-season (Zabriskie 2019).

National Collegiate Athletic Association (NCAA) Division I soccer athletes participate in two seasons each academic year. The traditional championship season is played in the fall, and a "non-championship" season is played in the Spring. Athletes have significantly less mandatory training sessions and matches during the non-championship seasons compared to the championship season in the fall. This is an important consideration when evaluating low EA risk because the level of EA may be different in a championship versus non-championship season.
A majority of existing studies have looked at changes that occur during off-season (where there is no supervised training) and championship seasons. No studies to date have examined the changes in EA, body composition, or BMD in male collegiate soccer players during a non-championship season.

30.2 *Describe any relevant preliminary data*:

N/A

30.3 *Based on the existing literature, provide the scientific or scholarly rationale for and significance of your research and how will it add to existing knowledge*:

The NCAA limits athlete playing time in the spring season for men's soccer athletes to no more than 4 hours/day, 8 hours/week, and at least 2 mandatory days off each week (NCAA, 2022). This is different from the championship season in the fall where athletes are permitted to train up to 4 hours/day, 20 hours/week, and are only required to have one day off per week. This drastic difference in training and match volume is likely to result in a lower EEE in the spring compared to the fall. One of the biggest differences between fall and spring soccer seasons is the number of matches played. During the fall soccer season, teams play an average of 20 games or more depending on post-season play. Most teams will play two matches per week during the fall. With only 5 games in the spring season, teams play approximately only one match every two weeks. Previous studies estimate soccer players expend approximately 1300-1800 kilocalories (kcal) per match depending on aerobic fitness and playing position (Hulton, 2022). In a pilot study of Korean Male Collegiate Soccer Players (n=12) during a pre-competition training period, average EEE during a 7-day period was found to be 1,747 kcal/day (Lee, 2020). The NCAA also limits the number of supervised training hours in the spring to 8 hours/wk with two rest days, compared to 20 hours/wk and one rest day in the fall. It is likely the EEE of collegiate soccer players in the spring is lower compared to EEE in the fall.

The training goals during spring are also different from the objectives in the fall. During spring season there is often a greater emphasis placed on building muscle strength and hypertrophy, as opposed to conditioning and field play in the fall. There is also a desire in some athletes, especially men, to gain weight or lean body mass (Garthe 2011). In a survey of 115 male and 88 female Division I athletes, men were significantly more likely to use supplements related to weight/muscle gain including protein powders,

androstenedione, dehydroepiandrosterone (DHEA), creatine, hydromethylbutyrate (HMB), and weight gainers compared to female athletes (Froiland 2004). Weight gainer supplement use was reported by 10.3% of male athletes in this study.

Existing research on changes in EA and body composition during a competitive season should not be generalized to a non-championship season based on the difference in training volume and potential body composition goals. This study aims to investigate the changes that occur in athletes during a non-championship season and what, if any, methods athletes use to alter body composition.

Findings from this proposed study will be of interest to sports dietitians and strength coaches based on its potential to improve fueling strategies and training during the non-championship spring season.

31.0 Study Endpoints

*31.1 Describe the primary and secondary **study** endpoints. See links below for discussion of study endpoints and how they may differ from study objectives. These are most common in clinical trials but are sometimes applicable to other types of biomedical research, as well as social, behavioral, or educational research. See link below for a discussion.*

https://docs.google.com/document/d/1Wocz7K7a0hCQJPPO_khh5l1SQQjhGDDGHzcOPRHR5Tw/edit?usp=sharing

Primary Endpoints

Change EA

Change body composition (i.e., total body mass, fat mass, lean body mass, fat free mass)

Secondary Endpoints

Change in bone mineral density (BMD), bone mineral composition, and BMD z-score in total body, dual femur and lumbar spine.

Change in muscle strength

Change in wellness scores

*31.2 Describe any primary or secondary **safety** endpoints. These should be included for all studies that are greater than minimal risk. (Minimal risk: The probability and magnitude of harm or discomfort anticipated in the research that are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.)*:

Participants will be exposed to a small amount of radiation during the DXA scan. There is no specific guideline for how long an individual must wait between DXA scans. The International Society for Clinical Densitometry recommends using an approach called "ALARA" (As Low As Reasonably Achievable). This includes strategies such as limiting time of exposure, maximizing distance from the radiation source, and using shielding to reduce exposure of tissues (ISCD, 2005). To ensure participant safety, participants will be restricted from having a DXA scan if they have had one in the previous 4 weeks.

32.0 Study Design and Statistical Analysis Plan

32.1 Describe the basic study design/approach (e.g., qualitative study using five focus groups of first year students to describe assimilation into the university community; randomized controlled trial of a behavioral change intervention to increase dietary intake of whole grains; pre- post-test evaluation of new pedagogical techniques to improve adult literacy):

This is an observational study that will estimate changes in EA and body composition of male division I soccer players over a non-championship spring season.

32.2 Describe corresponding data analysis plan/approach (e.g., content analysis of focus group transcripts; descriptive analysis followed by linear regression modeling; nonparametric analysis of pre- and post-test measures):

Data will be analyzed using IBM SPSS statistics software. One-sample t-test will be used to assess EA compared to previously published data. Paired t-tests will be used to compare measurements taken at the start and end of season. Pearson or Spearman's coefficient will be used to examine relationships between variables. Repeated measures ANOVA with post hoc analysis and multiple regression analysis will be used to determine association of changes in EA on body composition and bone density. Descriptive statistics will be applied to evaluate motivations and approaches to alter body composition. Data will be summarized as mean ± 1 standard deviation. The significance level will be set a priori at $p<0.05$.

33.0 Setting

33.1 Describe the sites or locations where your research team will conduct the research. Consider each of the items listed below:

- *Identify where your research team will identify and recruit potential subjects.*
- *Identify where the team will perform the research procedures.*
- *Describe the composition and involvement of any community advisory board(s).*
- *For research conducted in other locations, describe:*
  - *Site-specific regulations or customs affecting the research at those locations.*
  - *Local scientific and ethical review structure at those locations.*
    Examples include work in other cultures or ethnic groups (within or outside of the U.S.) and work with churches. The HRPP will provide additional guidance for international research.

The research will be conducted at Radford University and Virginia Tech. All members of the Radford University men's soccer team are eligible to participate, providing they meet eligibility criteria. Participants will be recruited before the start of the Spring season.

Research procedures and appointments will be conducted in the Human Integrative Physiology (HIP) Laboratory in the Garvin Innovation Building, 233 Wallace Hall, Nutrition and Exercise Metabolism Laboratory (NEM), and Radford University athletics facility.

34.0 Study Intervention(s)/Investigational Agent(s)

7.1 Describe the study interventions (including behavioral interventions) and/or investigational agents (e.g., drugs or devices) to be used in this study. Consider each of the items listed below:

- *Drug/Device Handling: If the research involves drugs or devices, describe your plans to store, handle, and administer the drugs or devices so that they will be used only on subjects, and only by authorized investigators.*
- *Describe whether any of the following will be used: microwaves, X-rays, DEXA scans, general anesthesia, or sedation*
- *If control of the drugs or devices used in this protocol will be accomplished by following an established, approved organizational SOP (e.g., Research Pharmacy SOP for the Control of Investigational Drugs, etc.), please reference the SOP in this section.*

The Research does not involve administration of drugs. The research does involve use of Dual-energy x-ray absorptiometry (DXA/DEXA) scans that will be performed at 2 timepoints during the study (start of season, end of season). DXA scans will be performed by an ISCD Certified Bone Densitometry Technologist (T. Sterringer).

34.2 List the name of all drugs (including any vitamins, supplements, herbs, or nicotine) to be used in the study. Indicate whether they have FDA approval, and list any limitations for their use:

N/A

34.3 List all devices, how they will be used, their purpose in the study, and if they will be used in a manner consistent with their approved uses. If they will be used in ways that are not yet FDA approved, indicate whether they need an IDE or a determination that they are exempt from the IDE Determination. If a determination of significant risk or non-significant risk is needed for any of the devices, include the researcher's recommendation for each of those devices:

The medical equipment used in this study is the DXA.

The DXA will be used to assess total body composition and bone mineral density. Total body, dual-femur, and lumbar spine BMD will be measured in all subjects via DXA scan.

The device is FDA approved and the research will involve employment of this device for approved uses. Scans will be performed only by members of the research staff who are trained and certified bone densitometry technologists (CBDT) through the International Society of Clinical Densitometry (T. Sterringer).

34.4 If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

- *Identify the holder of the IND/IDE/abbreviated IDE.*
- *Explain procedures followed to comply with sponsor requirements for FDA regulated research for the following:*

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	***X***	***X***	
21 CFR 54	***X***	***X***	
21 CFR 210	***X***		
21 CFR 211	***X***		
21 CFR 312	***X***		
21 CFR 812		***X***	***X***
21 CFR 820		***X***	

N/A

35.0 Procedures Involved

35.1 Describe and explain the study design:

This observational study aims to recruit 40 male soccer players from the Radford University men's soccer team to evaluate changes in energy availability, body composition, and BMD over a non-championship Spring season.

As outlined in the table, eligible participants will undergo two testing visits over the Spring season. Testing visits will take place at the start of the season and towards the end of the Spring season, with no intervention during the season. Following informed consent, participants will undergo a DXA scan to determine body composition and BMD. Participants will then be asked to track 3 days of their normal diet using food intake records, in addition to tracking their exercise outside of team training. Exercise movement during on field training throughout the Spring season will be tracked using Sports Performance Tracking (SPT) GPS-enabled devices. These devices are currently worn by all athletes on the team and are used as part of normal, routine training. They will also be worn throughout the entire Spring season. Participants will also complete an electronic daily wellness report during spring training. This report asks questions related to the participant's sleep, stress level, fatigue, muscle soreness, and hydration. These wellness reports are completed by all athletes on the team as part of normal care by the coaches and training staff. Near the end of Spring season, participants will undergo a final DXA scan and complete a 3-day food intake record, exercise log, and daily wellness report. Participants will also be asked to complete a questionnaire to assess motivations and methods used to alter body composition during Spring season.

Table 1. Overview of Data Collection			
	Start of Season	During Season	End of Season
Height	x		
Body mass (weight)	x		x
Body Composition by DXA	x		x
BMD (total body, dual femur, lumbar spine) by DXA	x		x
Urine sample for hydration testing	x		x
Food intake records (3 days)	x		x
Exercise log (3 days)	x		x
Fitness tests	x		x
Movement during field training (SPT device)	x	x	x
Daily Wellness Report	x	x	x
Questionnaire – motivations and methods to alter body composition			x

35.2 Provide a description of:

- *All research procedures being performed*
- *If the study has more than one procedure, session, and/or subject population, describe each procedure, session, and/or study population separately. For complex studies, you are encouraged to include a figure or chart.*

Informed Consent

Informed Consent: Participants will be provided an informed consent form following the study presentation at a team talk at the start of the Spring semester and in advance of coming to the laboratory as outlined in section 11.0. They will be encouraged to ask questions in person to the team dietitian (T. Sterringer) or by email or individual appointment prior to signing the consent form during the first lab appointment.

Start of Season Testing Visit (Location: Human Integrative Physiology Lab, 1872 Pratt Drive on Corporate Research Center Campus)

Body Weight Height and Composition: Body weight and height will be measured on a digital physician's scale. Percent body fat, lean body mass, and fat-free mass will be measured in all subjects via DXA scan.

Bone Mineral Density: Total body, dual-femur, and lumbar spine BMD will be measured in all subjects via DXA scan. Scans will be conducted by T. Sterringer who is an ISCD Certified Bone Density Technologist. During this procedure, participants will lie on their back and be advised to stay as still as possible. Total duration for all DXA scans will be approximately 15 minutes.

Urine sample: All participants will be required to provide a small cup of urine immediately before the DXA at baseline. Urine will be collected using a wide-mouth urine specimen cup. Research personnel will wear gloves at all times when handling and analyzing the sample. The urine will be evaluated for hydration status via specific density using a portable refractometer (Fisher Scientific). After USG has

been collected, the urine will be discarded, and the sample cup will be placed in a biohazard waste bin. The urine sample will not be used for any other purposes.

Dietary Intake: At the start of the season, participants will be instructed on completing food records for 3 days. Food records will be analyzed using ESHA Food Processor, a dietary analysis software program.

Exercise Log: Participants will be asked to record any exercise outside of scheduled team training sessions or matches during a 3-day period at the start of the spring season. Exercise logs will be used to estimate EEE over the 3-day period.

<u>During Spring Season</u>
Exercise Movement: The SPT GPS-enabled device will be used to capture exercise movement for on-field training and matches during the Spring season. The GameTraka analysis platform will be used to download the data from the SPT device. The device captures metrics such as total distance, meters/minutes, speed, heart rate, sprint efforts, etc. Exact GPS location is not tracked.

Wellness Report: Electronic daily wellness reports will be completed by participants throughout the Spring season. These wellness reports are a requirement for all athletes on the men's soccer team, regardless of their participation in this study. The questions asked in this wellness report are related to stress, sleep, fatigue, muscle soreness, mood, and hydration. Stress, fatigue, reduced recovery (i.e., muscle soreness), and mood disturbances have been associated with low EA symptoms.

<u>End of Season Testing Visit (Location: Human Integrative Physiology Lab, 1872 Pratt Drive on Corporate Research Center Campus)</u>
Body Weight Height and Composition: Body weight and height will be measured on a digital physician's scale. Percent body fat, lean body mass, and fat-free mass will be measured in all subjects via DXA scan.

Bone Mineral Density: Total body, dual-femur, and lumbar spine BMD will be measured in all subjects via DXA scan. Scans will be conducted by T. Sterringer who is an ISCD Certified Bone Density Technologist. During this procedure, participants will lie on their back and be advised to stay as still as possible. Total duration for all DXA scans will be approximately 15 minutes.

Urine sample: All participants will be required to provide a small cup of urine immediately before the DXA at baseline. The urine will be evaluated for hydration status via specific density using a refractometer. The same safety procedures will be followed for handling, analyzing, and discarding the urine sample as specified during visit 1.

Dietary Intake: Near the end of the season, participants will be asked to complete food records for 3 days. Food records will be analyzed using ESHA Food Processor, a dietary analysis software program.

Exercise Log: Participants will be asked to record any exercise outside of scheduled team training sessions or matches during a 3-day period near the end of the spring season. Exercise logs will be used to estimate EEE over the 3-day period.

Questionnaire: Participants will be asked to complete a questionnaire related to motivations and methods taken to alter body composition during the Spring season.

35.3 Describe:

- *Procedures or safeguards intended to reduce the probability and magnitude of risks. (For example: Reducing the risk of injury in a virtual reality study either by having the subjects sit during the study or by providing an obstacle-free space for walking.)*
- *Be sure to describe all drugs and devices used in the research, when they will be administered or used, and their purpose.*
- *Methods used to collect data about subjects. Please upload all data collection forms to Protocol Management. Some common examples are:*
  - *Screening questionnaires*
  - *Survey(s), including online surveys*
  - *Demographic questionnaire(s)*
  - *Interview guide(s), e.g., questions or pool of questions for semi-structured interviews*
  - *Focus group guide(s)*
  - *Other documents used to collect data*

The following safeguards will be employed to reduce the probability and magnitude of risks associated with study participation. The specific risks are highlighted in Section 17.

Questionnaire and Study Logs: The final questionnaire will be collected with the participant sitting in a private setting. Dietary intake and exercise logs will be placed in each participant's study file in a locked cabinet in Dr Larson-Meyer's Laboratory with access limited to study personnel only.

DXA scan: Participants will be exposed to a very low dose of ionizing radiation as part of the DXA scan. DXA procedure will be performed by trained staff. Participants will be informed of the risk of radiation exposure prior to study enrollment.

35.4 What data will you collect during the study and how you will obtain them? Please include descriptions of electronic data collection, database matching, and app-based data collection:

Anthropometric and basic demographic (age) data will be recorded on data sheets and manually entered into a database (excel format) on a secure computer. Anthropometric data (height and weight) will be collected by the ISCD certified bone technologist before the DXA scan (T. Sterringer). Height will be measured using a stadiometer and weight will be measured using a digital physician's scale. Body composition will be estimated using DXA and performed by T. Sterringer who is an ISCD certified bone technologist. Age will be collected before the DXA scan by T. Sterringer. Participants will track dietary intake and exercise using hand-written food intake records and exercise logs, respectively. Food intake records and exercise logs will be entered manually into ESHA Food Processor and the data will be exported electronically from ESHA Food Processor into excel spreadsheets. ESHA Food Processor has been reviewed and approved for use by ITPALs. The Nutrition and Body Composition Questionnaire will be completed in a paper copy format at the second lab visit. The SPT device will collect data from synced satellites and to a tablet near the game/training field. The data from the tablet can be then transferred

to a computer using USB connection and exported to excel files using the GameTraka analysis platform. Radford University uses a third-party platform (GPS DataViz) to collect wellness report responses. The athletic trainer will provide the research team with only the responses for the student athletes enrolled in the study. This data will be provided in an excel document with identifiers removed (study codes only).

35.5 Who will transcribe or code audio and/or video recordings?:

N/A

35.6 Include a description of any deception to be used in the study. Include justification for the use of deception (why the deception is necessary), describe the debriefing process, and describe how the study meets <u>all</u> the following criteria for alteration of consent (deception is considered an alteration of informed consent):

- *The research involves no more than minimal risk to the subjects*
- *The alteration will not adversely affect the rights and welfare of the subjects*
- *The research could not practicably be carried out without the alteration/deception*
- *(Optional but encouraged in most cases) Subjects will be provided with additional pertinent information after participation (i.e., debriefing for studies involving deception)*

N/A

35.7 If the study involves long-term follow-up (once all research related procedures are complete), describe what data will be collected during the follow up period and when it will occur:

N/A

36.0 Data and Specimen Long Term Storage and Use

36.1 If you will store data or specimens for future use, describe where you will store the data or specimens, how long they will be stored, and how and by whom the data or specimens will be accessed:

All data will be stored in a locked cabinet in Dr. Larson-Meyer's laboratory. Access to the lab is restricted to authorized personnel only. Identifiable information including study key will be stored on a Virginia Tech secured and managed machine within the NEM laboratory. De-identified data will be stored on Virginia Tech Google Drive, which is a cloud service that is secured and managed through Virginia Tech.

Access to the Google Drive will be restricted to only authorized study personnel. The study key and data sets will be password protected. All de-identified data will be kept indefinitely.

Urine samples will be discarded immediately after hydration testing at lab visits 1 and 2. No samples will be retained for future analysis.

36.2 *For specimens, list the data to be stored or associated with each specimen*:

N/A

36.3 *Describe the procedures to release data or specimens outside of the research team, including the process to request a release, approvals required for release, who can obtain data or specimens, and what data will be provided with specimens*:

De-identified aggregate data (total energy intake, body mass, bone density, lean body mass, and fat mass) will be shared with the athletic trainer and strength and conditioning staff for sports performance only. Summarized energy intake is being shared to inform coaching staff on the energy status of their players. Low EA negatively affects athlete health and performance. These results may help guide future decisions related to athlete fueling and nutrition education during an athletic season to prevent low energy intake. Body composition (body mass, lean body mass, and fat mass) and bone density will be provided to inform coaching staff on the changes that occur in their athletes during a non-championship season. This will provide insight into the effectiveness of the training performed during the Spring. Negative changes in body composition may indicate the training program is not beneficial to athletic progress.

36.4 *Describe the identifiers to be included with stored data or specimens, as well as any key or code that could be used to make them identifiable. Describe where the code will be stored, who will have access to it, and when it will be destroyed*:

Study Codes using a combination of letters and numbers will be used to de-identify subjects from their personal information. No obvious identifiers will be stored with the data; the data spreadsheet, however will include each participants' age and starting weight as part of the de-identified data. Original de-identified data collection sheets will be stored in a locked file cabinet as mentioned previously as part of study records; scans of some de-identified information may be kept in a password-protected electronic file that is accessible only to research personnel. During the active phase of the study, a master document (key) that will contain the participants name and assigned study code will be kept in a password-secured file that will be accessible only to authorized study personnel. The key will be destroyed 12 months after collection of data from the last participant. De-identified data may be kept indefinitely.

36.5 *Please select the identifiers you will obtain (whether directly from participants or from another source), including but not limited to:*

☒	*Name*
☒	*Geographical subdivisions smaller than a state, including street address, city, county, precinct, zip code, and equivalent geocodes (note, the initial three digits of a zip code are not considered identifiable)*
☒	*Elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death, and single year of age over 89 and all elements of dates (including year) indicative of such age (note, such ages and elements may be aggregated into a single category of age 90+)*
☒	*Phone numbers*
☐	*Fax numbers*
☒	*Electronic mail addresses (e-mail)*
☐	*Social Security numbers*
☐	*Medical record numbers*
☐	*Health plan beneficiary numbers*
☐	*Account numbers*
☐	*Certificate/license numbers*
☐	*Vehicle identifiers and serial numbers, including license plate numbers*
☐	*Device identifiers and serial numbers*
☐	*Web Universal Resource Locators (URLs)*
☐	*Internet protocol (IP) address numbers*
☐	*Biometric identifiers, including finger and voice prints (audio recording)*
☐	*Full face photographic images and any comparable images (including video recording)*
☐	*Student record number or identification number*
☐	*User name for online or computer accounts*
☐	*Any other unique identifying number, characteristic, or code (note this does not mean the unique code assigned by the investigator to code the data)***:** Click here to explain.

37.0 Sharing of Results with Subjects

37.1 Describe whether you will share results (study results or individual subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings) with subjects or others (e.g., the subject's primary care physician). If so, describe how you will share the results and include this information as part of the consent document. Upload materials you will use to explain the results to subjects:

At the conclusion of the study or when the participants' involvement in the study ends, interested participants will be provided with individual results related to their body composition, BMD, and dietary intake. These data will be summarized on a TBD summary document. Participant can arrange to pick this information up from T Sterringer at The Radford Athletic Training Facility, Dr Larson-Meyer's lab or another arranged meeting location or have (upon request) it mailed to them at a provided address. This form will be submitted as an Addendum before it is provided to the first participant. Athletes who are not interested in this information will not be provided a summary of this data.

De-identified aggregate data (total energy intake, body mass, bone density, lean body mass, and fat mass) will be shared with the athletic trainer and coaching staff as outlined in Section 9.3. No identifiable individual data will be shared. The data will be presented on a TBD summary document and provided to the select personnel at an in-person meeting after study conclusion. This form will be submitted as an Addendum before it is provided to the coaches and athletic trainer.

The participants will also be notified when a summary of the study findings is published if an active email address is on file.

38.0 Study Timelines

38.1 Describe:

- *The duration of an individual subject's participation in the study (for example, 1 hour, 2-4 weeks, 3-5 years).*
- *The amount of time expected to enroll all study subjects (weeks, months, years, etc.)*
- *The amount of time expected for the investigators to complete this study including primary data analyses.*

At the start of Spring season, a recruitment presentation will be given to the Radford University men's soccer team that will include the athletic trainer, registered dietitian, and strength and conditioning coach. Information regarding details of participation will be discussed. Consent forms will be available for those interested in participating.

The duration of an individual's participation in this study will be the entire spring season including approximately 4 days at the start of the season (1 lab visit and 3 days of nutrition and exercise tracking) and 4 days near the end of the season (1 lab visit and 3 days of nutrition and exercise tracking).

Participants will begin the study at the same timepoint at the start of the Spring season. The investigators will complete primary data analyses within the following year but all analyses of study data may not occur for up to ten years following study completion.

39.0 Inclusion and Exclusion Criteria

39.1 Describe how you will screen individuals for eligibility. When will screening occur and what procedures will you use? Upload any screening scripts or surveys to Protocol Management:

Following the recruitment presentation, potential participants will be made fully aware of the eligibility criteria, time commitment, possible risks and their right to withdraw from the study at any time. A consent form will be distributed after the recruitment presentation for review. An inclusion age of 18 will be set.

Participants will be verbally screened for inclusion. They will be asked if they are eligible for full team participation and if they are 18 years or older. Team participation eligibility and age will be confirmed by the team dietitian (T. Sterringer).

39.2 *Describe the eligibility criteria that define who will be included and who will be excluded from enrollment for each procedure of your study. Include any geographic criteria (e.g., Virginia Tech undergraduate students, a national sample of adults with engineering degrees, minors aged 8-12 in the New River Valley, university faculty in Virginia and Paris, France)*:

All eligible players over the age of 18 may be included. Athletes will not be considered eligible if they have current injuries that prohibit full, unrestricted participation in training and matches, or if they are ineligible for full team participation for any other reason (i.e., academic or disciplinary suspension).

39.3 *Indicate specifically whether you will include or exclude each of the following special populations: (You may not include members of these populations as subjects in your research unless you indicate them in the description of your subject population.)*

- *Minors, as defined by state law where the study is performed (infants, children, teenagers)*
- *Pregnant women (can be included in minimal risk studies by mentioning in section 13.1)*
- *Prisoners (including all incarcerated individuals)*
- *Adults not capable to consent on their own behalf*

None of the above will participate.

40.0 Vulnerable Populations

40.1 *If the research involves individuals who are vulnerable to coercion or undue influence, please describe additional safeguards you will include to protect their rights and welfare. Consider the applicable items listed below:*

- *If the research involves Virginia Tech students, indicate whether these are students of any of the investigators. If so, describe whether the activities will take place during class time as part of the curriculum and the steps you will take to reduce the possibility that students feel obliged to participate in order to improve their course grade. The HRPP can provide further guidance as needed. Describe whether you will request access to student records (e.g., SAT, GPA, GRE scores).*
- *If the research involves employees of Virginia Tech or the research sponsor, describe steps you will take to ensure that the employees are freely participating and describe how their data will be protected from inspection by their supervisors.*
- *If the research involves Virginia Tech NCAA athletes, you must obtain approval from the athletic department.*
- *For research involving Montgomery County Public Schools, you must obtain county approval (after obtaining contingent Virginia Tech approval). Other locales have different requirements; please check on these and describe here. Approval is typically granted by the superintendent, principal, and classroom teacher (in that order). Approval by an individual teacher is insufficient. School approval, in the form of a letter or a memorandum should be uploaded as a supporting document.*

- *If the research involves pregnant women, review "CHECKLIST: Pregnant Women (HRP-412)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves prisoners, review "CHECKLIST: Prisoners (HRP-415)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves persons who have not attained the legal age for consent to treatments or procedures involved in the research (minors), review the "CHECKLIST: Minors (HRP-416)" to ensure that you have provided sufficient information in this protocol.*
- *If the research involves cognitively impaired adults, review "CHECKLIST: Cognitively Impaired Adults (HRP-417)" to ensure that you have provided sufficient information in this protocol.*

Coaches will be informed not to influence player study participation through play time incentives or training punishments. Their decision to participate will have no effect on playing time or their relationship with Radford University.

The research team will ensure only the listed Radford personnel will be aware of enrollment status by having all interactions with players (lab visits and consent meetings) in the athletic training room which is in a separate building from the coaching offices. Coaches will not be on any emails corresponding about the study between the Virginia Tech and Radford University study personnel.

Others will not be aware of enrollment status unless the participant voluntarily shares that information with their coaches or fellow players. Participants will be responsible for their own transportation to lab visits; therefore, coaches will not be aware of who is enrolled. All athletes complete the daily wellness reports and wear training vests at practice, so there would be no way to distinguish players that are enrolled.

41.0 Number of Subjects

41.1 Indicate the total number of subjects to be enrolled and how this number was determined (e.g., sample size calculation [show], number of available subjects in a finite pool, number of tests funding award would allow):

A study sample of 40 participants was chosen based on the roster size of the Radford University men's soccer team (currently 40 men). This will allow any interested athlete to participate.

We anticipate a minimum of 11 athletes will be needed to answer the primary research aim based on a similar study of Division I Female Soccer players which found as significant difference in FFM over a Spring season (Purdom 2020). However, in observational studies, we can better understand individual responses within participants when there are a greater number of participants. We do not anticipate a minimum number of participants required for our exploratory aim of motivations and methods to change body composition.

41.2 *If this is a multi-site study, indicate the number of subjects to be enrolled at this site and the total to be enrolled from all sites*:

N/A

41.3 *If applicable, indicate the number of potential subjects you expect to screen for enrollment, and the number of subjects you will need to complete the research procedures*:

N/A

41.4 *If the study has more than one procedure, indicate the total number of subjects to undergo each procedure separately*:

All enrolled participants will undergo all outlined procedures.

42.0 Recruitment Methods

42.1 *Describe when, where, and how you will recruit potential subjects*:

Participants will be recruited by the team dietitian and study personnel (T. Sterringer) during a presentation at the start of the season at Radford University (see section 11.0).

42.2 *Describe the source of subjects (for example, clinic patients with specific conditions, students in the library, community members at a gathering, or members of a local gym)*:

We will recruit participants from the Radford University men's soccer team.

42.3 *Describe the methods that you will use to identify potential subjects*:

All active roster players (age 18 or older) that are free from injury and are eligible for full team participation in the Spring will be eligible to participate.

42.4 *Describe materials that you will be use to recruit subjects. Attach copies of these documents with this protocol in Protocol Management and be sure to include the IRB protocol number on each document.*

- *For flyers, attach the final copy of printed flyers.*

- *For Virginia Tech News, Facebook postings and ads, newspaper ads, websites, MTurk/SONA/online survey systems, etc., attach the final wording and graphics to be used.*
- *For email recruitments, please include the subject line.*
- *For advertisements meant for audio broadcast, please submit the wording of the advertisement prior to taping (to avoid having to re-record with approved language) and submit the final recorded version for IRB review before use.*
- *Describe any compensation to subjects. Separate compensation into appropriate categories, such as: reimbursement for expenses, time and effort, and additional incentives for study participation. For each category, specify the amount (including any pro-rated amount), schedule, and method of payment.*

A brief presentation will be given to the team at the start of the Spring season which will describe the study purpose and details of involvement. They will be informed that there is no direct compensation for travel expenses to Virginia Tech for lab appointments. There will be no financial compensation for participating in the study.

43.0 Withdrawal of Subjects

43.1 Describe circumstances under which you anticipate subjects could be withdrawn from the research without their consent:

Participants could be withdrawn from the study if it is within their best interest, the researchers are unable to obtain measurements that are necessary for the study, they are not showing up for appointments or are not completing or complying with all procedures, or if they become injured or ineligible for team participation.

43.2 If applicable, describe any procedures for orderly termination (e.g., discontinuation of a study drug or debriefing after a behavioral intervention):

If a participant is not complying with the study, the PI or another member of the study staff will first discuss these difficulties with the participant and explain the importance of adhering to the intervention for the purpose of the study. If it is determined that the participant be terminated or discontinued from the study for reasons as described above, the PI will mitigate issues leading to these problems. It will be the decision of the PI whether the participant may remain in the study. If the PI determines the participant should not continue the study due to any of the previously listed reasons in Section 16.1, a member of the study team will thank the participant for their time commitment to this point and explain to the participant why they are being removed from the study. The participant will be provided any information which is available to them (body composition, BMD, dietary intake).

43.3 Describe procedures that you will follow when subjects withdraw from the research, including partial withdrawal from procedures with continued data collection (e.g.,

participant declines to continue with regular blood draws, but continues with periodic behavioral questionnaires):

Any participant can discontinue participation at any point without consequence. All data that has been collected will be used up until the point of study withdrawal. If a participant elects to not complete or participant in a certain aspect of the study, data collected for all other procedures will still be included in the dataset.
They can withdraw from the study by contacting any member of the research personnel.

44.0 Risks to Subjects

44.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related the subjects' participation in the research. Include for the IRB's consideration a description of the probability, magnitude, duration, and reversibility of the risks. Consider physical, psychological, social, legal, privacy, and economic risks. Do not indicate "No risk" or "N/A." Instead, for studies with very low risk (e.g., anonymous online questionnaire on a mundane topic) indicate "The investigators are not aware of any risks from participation in this study." or "No more than risks than are found in everyday life." The example consent form presents a tabular method for risk information, which you can also use here. Common risk types include:

- *Physical (e.g., potential for pain, discomfort, infection)*
- *Psychological (e.g., potential for stress, discomfort, and/or embarrassment)*
- *Social (e.g., potential for discrimination or stigmatization and disruption of personal and family relationships)*
- *Legal (e.g., potential for disclosure of illegal activity, negligence)*
- *Privacy (e.g., potential for personal information being accessed, used, or disclosed without the subjects' knowledge or consent, breach of confidentiality/security)*
- *Economic (e.g., potential for individuals to lose access to economic services, employment, insurability)*

DXA Scan: The amount of radiation that subjects will receive in the DXA exam is less than the amount permitted by the Food and Drug Administration (FDA) per year. The amount subjects will receive at the exam (including total body, femur, and lumbar spine) is equal to 1/20 of a chest x-ray. The more radiation an individual receives over the course of their lifetime, the more likely that individual's risk increases in developing cancerous tumors. The radiation in this study is not expected to greatly increase these risks, however, the exact increase in such risk is not known. History of previous DXA scan(s) will be determined by self-report before the DXA scan is conducted by T. Sterringer.

Confidentiality: Data collection forms and questionnaires will be stored in a locked file cabinet within the NEM laboratory. The lab and file cabinet have restricted access to authorized personnel only, however, total confidentiality cannot be guaranteed. There is also a risk of an electronic security breach for electronic data.

44.2 Indicate the measures you will use to minimize risks and monitor subjects for safety. (e.g., asking a subject at regular intervals to rate how they are feeling from 1 to 10, or to slowly crouch in order to check their balance.) Indicate the measures you will use to

minimize risks and monitor subjects for safety. (e.g., asking a subject at regular intervals to rate how they are feeling from 1 to 10, or to slowly crouch in order to check their balance.)

During the DXA scan, the technologist will monitor the participant and check with them before beginning any procedures to ensure they are comfortable. The scan will be performed by an ISCD certified technologist (T. Sterringer). The technologist will use the "ALARA" (As Low As Reasonably Achievable) approach. This includes limiting time of exposure by not repeating DXA measurements unless the researchers determine it is necessary to obtain a required measurement (e.g., scan was performed incorrectly due to positioning or machine error resulting in inaccurate results and a re-scan is required). History of previous DXA scan(s) will be determined by verbal self-report. The technologist will ask the participant prior to the scan if they have ever had a DXA scan previously and the date of their last scan.

All sports-related training is monitored by the team athletic trainer and strength and conditioning staff as part of normal care.

Participant files, questionnaires, and consent forms will be stored in the NEM laboratory in a locked file cabinet only accessible by authorized personnel. Excel files and electronic data will be password-protected and only accessibly by authorized research personnel. Password-protected electronic data will be stored on Virginia Tech Google Drive and access will be restricted to only the authorized study personnel. No identifiable data will be stored in Google Drive.

Other safety measures addressed in Section 17.1

44.3 *If applicable, indicate which procedures might have risks to the subjects that are currently unforeseeable. This will be rare, and usually applicable when testing a new drug or device or a new use of an existing drug or device*:

N/A

44.4 *If applicable, indicate which procedures might have risks to an embryo or fetus should the subject be or become pregnant*:

N/A

44.5 *If applicable, describe risks to others who are not subjects (e.g., collection of sensitive health data that might affect sexual partners if disclosed, mandatory reporting of abuse, DNA testing that might affect family members or relationships)*:

N/A

45.0 Potential Benefits to Subjects

45.1 *Describe the potential benefits that individual subjects might experience from participating in the research. Include the probability, magnitude, and duration of the potential benefits, as this will be useful to the IRB's risk:benefit analysis. Do not include benefits to society or others. Do not list monetary or non-monetary compensation for participation, as this is not a benefit These should be included in section 2 or 3 of this document*:

Participants will gain information about their bone mineral density, changes in body composition, and dietary intake. This information may be of interest to participants who want to improve athletic performance.

45.2 *If applicable, specify that there are no anticipated direct benefits for participants*:

N/A

46.0 Data Management and Confidentiality

46.1 *Describe procedures that you will use for quality control to ensure validity of collected data*:

Dr. Larson-Meyer has extensive experience performing data collection the procedures (or similar procedures) outlined in this proposal. Trisha Sterringer is an ISCD Certified Bone Densitometry Technologists. Additionally, Dr. Larson-Meyer and T. Sterringer are registered dietitians and have experience with assessing dietary intake, EEE, and EA in athletes.

46.2 *Describe any existing data or biospecimens you will obtain as part of this study. Include:*

- *Variables or samples to be obtained*
- *Source of the data or specimens*
- *Your authorization to access or receive the data or biospecimens*
- *Whether the data or biospecimens are publicly available*
- *Whether the data or specimens you receive will contain identifiers*

Exercise during the Spring season will be collected by the Strength and Conditioning Coach (L. Mason) which includes field exercise movement data using SPT training devices and strength training sessions. The SPT devices are worn by all athletes on the Men's Soccer team as part of normal, routine participation. Supervised strength training sessions are mandatory for all athletes on the team. This data is not publicly available and will be retrieved by the team dietitian (T. Sterringer). Data will be received only with study codes, no identifiers.

Wellness reports will be completed daily with questions regarding stress, muscle soreness, fatigue, sleep, and hydration. These wellness reports are completed by all student athletes as part of normal, routine participation. Wellness report results will be collected by the athletic trainer and retrieved by the team dietitian (T. Sterringer). This data is not publicly available. Data will be received only with study codes, no identifiers.

We will obtain fitness test data that was collected at the start and end of Spring season by the Strength and Conditioning Coach (L. Mason). Fitness data will include the Yo-Yo test score and one rep maximum weights for deadlift and bench press. The Yo-Yo tests is a maximal aerobic endurance fitness test used by Radford University Athletics. This data is not publicly available, and it will be received only with study codes.

46.3 Describe the steps that you will take to handle and secure study data during data collection, storage, use, and transmission. Include information about training of study staff, authorization of access, password protection, encryption, physical controls, certificates of confidentiality, separation of identifiers and data, etc.:

We will do everything that we can to make sure that study records are kept private. Each participant will be assigned a unique participant code as explained in section 9.4. All data recording sheets and spread sheets will use the subjects' study code. They will not contain the participants' name or date of birth. These will be compiled in a patient research file/chart and stored in a locked file cabinet organized by their unique study code. Their name will be listed only on the informed consent and on a master participant list that includes the study code key. The master participant list will be kept in a separate electronic file than the data files; both will be password-protected. The study consents will be kept together in a separate file in a separate location in a locked office. Only authorized study personnel will have access to study data. Results of the study may be published and/or presented at professional conferences. The participants' name or other personal information that would identify them will not be used. Training of study personnel including graduate students on procedures to ensure secure collection and storage of study data will occur before study initiation.

46.4 For multi-site studies, describe how data or specimens will be handled and secured for each site (e.g., central or disseminated data storage, data coordinating center):

N/A

46.5 *Describe the plan for data disposition following the conclusion of the study (e.g., long term maintenance of data, data destruction methods).*

- *What information will be included in the long term storage of data or specimens?*
- *How long will the data or specimens be stored?*
- *Where and how data or specimens will be stored?*
- *Who will have access to the data or specimens during long term storage?*
- *Who is responsible for receipt or transmission of the data or specimens?*
- *How will data or specimens be shared or transported?*
- *When and how will personal identifiers be destroyed?*

Personal information, primary and secondary endpoints and safety data will be kept indefinitely in a secured electronic location by the PI. Personal information will be kept in a separate file than de-identified data. Although is not anticipated that any data will need to be transported or shared, this would be done only using de-identified data with samples sent using a secure mechanism.

47.0 Provisions to Protect the Privacy Interests of Subjects

47.1 *Describe the steps that you will take to protect subjects' privacy interests. "Privacy interest" refers to a person's desire to place limits on with whom they interact or to whom they provide personal information (e.g., collecting the minimal amount of private information required to complete the study, protecting the data once it is obtained)*:

A minimal amount of personal information will be obtained using the final questionnaire related to motivations and methods to alter body composition. This data will be kept in participant files labeled with only the participant's study code in a secure locked cabinet. Any and all original data collection sheets with the participants' name or identifying information will be destroyed following entry into the database.

47.2 *Describe steps that you will take to make subjects feel at ease with the research situation in terms of the questions being asked and the procedures being performed. "At ease" does not refer to physical discomfort, but the sense of intrusiveness a subject might experience in response to questions, examinations, and procedures (e.g., use of a same gender investigator to place sensors on the torso, a private changing area if clothing must be changed, sensitivity when discussing pregnancy testing with subjects, making it clear on surveys that participants can discontinue at any time, not asking questions about private or sensitive issues unless necessary for the research)*:

Study participants will be informed during the consent appointment that they can discontinue the study at any time without penalty. All questionnaires and anthropometric testing will be performed by trained research personnel in a private setting.

47.3 Describe how you plan to access existing sources of information about the subjects (e.g., medical records, grades) and how you will protect participant privacy through the data security plan:

N/A

47.4 Describe any required reporting that might occur as a result of your research questions, study populations, and data collection methods. Examples for Virginia and Virginia Tech include:

- ***Any*** *suspicions (e.g., circumstantial, disclosed) of child abuse (physical, emotional, sexual) and neglect*
- *Sexual discrimination and/or sexual violence that involves a student*
- *Disclosure or signs of intention to harm oneself (i.e., suicidal ideation and/or plan)*
- *Disclosure or signs of desire to harm others (i.e., homicidal ideation and/or plan)*
- *Suspected abuse, neglect or exploitation of vulnerable adults (e.g., individuals with a disability, elderly persons)*

N/A

48.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Safety monitoring is required when research involves greater than minimal risk and is sometimes appropriate for other studies.

48.1 Describe:

- *The plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe (e.g., periodic reporting to the IRB, establishing a data monitoring committee, reporting data monitoring committee findings to the IRB and the sponsor).*
- *What data you will review, including safety data, unexpected events, and data that show the ability to produce the intended results.*
- *How the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with subjects).*
- *The frequency of data collection, including when safety data collection starts.*
- *Who will review the safety data and with what frequency.*
- *The statistical tests for analyzing the safety data to determine whether harm is occurring.*
- *Any conditions that will trigger an immediate suspension of the research (e.g., a serious adverse event).*

The data safety monitoring plan (DSMP) for this study focuses on close monitoring by the principal investigator (PI) and research staff along with prompt reporting of excessive adverse events and any serious adverse events (AEs) to the Institutional Review Board. All serious AEs will be reported by the PI within 48 hours of occurrence to the IRB.

The graduate student in charge of the project (T. Sterringer) will consult with Dr. Larson-Meyer and be responsible for assembling the data, producing reports, and assuring that all parties obtain copies of these reports. Reports will be submitted annually to the VT IRB for review.

Safety Data collection will start when the first participant is enrolled. The study team will be informed to discuss any observed or reported unusual, excessive or unexpected events immediately with the PI. The PI and/or authorized study personnel will review study charts and ongoing data collected on all participants on a weekly basis to ensure safety. Paired t-test could be used to determine if excessive events were occurring. We do not anticipate that there would be any specific events, other than the unexpected, that would trigger the suspension of our study.

49.0 Compensation for Research Related Injury

49.1 *If the research involves more than minimal risk to subjects, describe the available compensation in the event of research-related injury, if any*:

Participants will not be provided any form of compensation for medical treatment or other damages (for example lost wages, time lost from work, etc.). If a participant becomes injured or sick from the research, they will be referred to a clinic or to their personal health care provider. Medical treatment may be provided at their expense or at the expense of their insurance company.

49.2 *Provide a copy of contract language, if any, relevant to compensation for research-related injury. At Virginia Tech, this is most common for sponsored research*:

N/A

50.0 Economic Burden to Subjects

50.1 *Describe any costs that subjects might be responsible for because of participation in the research, including any uncompensated costs for items such as transportation, missed work, and childcare*:

The participant will be responsible for costs that may include transportation, missed work, or childcare.

51.0 Consent Process

51.1 Indicate the process by which you will obtain consent for study participation. Please upload all consent, parental permission, and assent forms, documents, and scripts referenced in this section to Protocol Management.

Describe the following:

- *Where the consent process will take place (e.g., clinic waiting area, classroom, online)*
- *The time interval between sharing the consent information with the prospective subject and obtaining consent. For lab, interview, and focus group studies, the Virginia Tech IRB prefers that subjects have at least 24 hours to review the consent form and study information before the appointment where consent will be obtained. For simple online survey studies, you can typically present the consent information immediately before subjects begin participation.*
- *If applicable, processes to ensure ongoing consent or assent (e.g., for multiple sessions; for research in which a minor will turn 18 during the study; for longitudinal research with minors who will later be asked to provide or affirm their assent).*
- *Please review "SOP: Informed Consent Process for Research (HRP-090)" for recommended procedure. Describe your process, being sure to include:*
    - *The name and role of all study personnel who will be trained and certified by the PI to conduct the consent process*
    - *The time that will be devoted to the consent discussion*
    - *Steps that you will take to minimize the possibility of coercion or undue influence*
    - *Steps that you will take to gauge or ensure the subjects' understanding*

Participants will be recruited and screened during a team study presentation. The presentation will include an overview of the study, the time commitment, and the possible risks to participation. The athletic trainer, registered dietitian, and strength and conditioning coach will be present. No other coaching staff will be present during the presentation.

Participants will be encouraged to ask questions and seek clarification during the study presentation and afterward before providing informed consent. Consent forms will be available after the presentation and/or by email for those interested. As much time as necessary will be devoted to address participant concerns. Informed consent will be performed by the doctoral student in charge of the study (T. Sterringer) either in a private setting following the presentation or at the beginning of the first laboratory visit.

These steps including time to review the consent before the screening visit, time with study staff to review the protocol and address concerns, and time to sign the consent in a private setting and close to a laboratory exit will help minimize the possibility of coercion or undue influence.

To help gauge the participant's understanding, the team member will ask the participant to explain the study, how often they will be asked to take time for data collection procedures,

when they would need to track their diet and exercise, and how often they would need to come to the lab.

We are not planning on renewing written informed consent during this study. However, participants can withdrawal from the study at any time by contacting a member of the research team via email, phone call, or in person during training sessions or team meetings.

Enrolled participants will be re-consented by T. Sterringer before the second lab visit for permission to access existing fitness test data that was collected at the start and end of the Spring season. These fitness tests are a requirement for all eligible athletes on the team, regardless of study participation. Re-consent process will be documented by saving the newly signed reconsent forms in the participant's file. A master datasheet will also be kept by the research team to track when a participant has been re-consented which will include the date of re-consent.

Non-English Speaking Subjects

- *Indicate what language(s) other than English are understood by prospective subjects or representatives.*
- *If non-English speakers will be recruited, describe the process you will use to ensure that the oral and/or written consent information provided will be in a language that they understand.*
- *If you translate consent forms and study materials, please provide a certified translation of the form as well as the certification document.*
- *Indicate the spoken language that study personnel obtaining consent will use. Describe how you will assess fluency of personnel obtaining consent to ensure that the translation is accurate.*

Only English-speaking participants will be recruited for the study.

Waiver or Alteration of Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

- *Review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" to ensure you have provided sufficient information for the IRB to make these determinations (i.e., that it meets the criteria for a waiver or alteration of the consent process).*

N/A

Subjects who are not yet adults (minors: infants, children, teenagers)

- *Describe the criteria that you will use to determine legal age for consent to treatments or procedures involved in the research under the applicable law of the*

jurisdiction in which the research will be conducted (e.g., in Virginia, individuals under the age of 18 years).

- *For research conducted in Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "minor."*
- *For research conducted outside of the state, please describe the legal requirements for the definition of "minor."*

- *Describe the process for obtaining parental permission.*
  - *Permission from one parent is acceptable for studies that involve no greater than minimal risk OR involve greater than minimal risk but present the prospect of direct benefit to the minor subject.*
  - *Permission from both parents is required in all other cases (unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the minor).*
- *Describe whether you will obtain permission from individuals other than parents or Legally Authorized Representatives, and if so, who will be allowed to provide permission. Describe the process you will use to determine these individuals' authority to consent to the minor's general medical care.*
- *Indicate whether you will obtain assent from all, some, or none of the minors. If you will obtain assent from some minors, indicate which minors will be required to assent. Consider chronological age and intellectual capacity when determining who will be required to provide assent (e.g., infants are unable to assent. However, teenagers are likely able to read and sign an assent form).*
- *When assent of minors is obtained, describe whether and how you will document it. Will minors sign an assent form or give verbal assent?*
- *Attach parental permission and minor assent forms or scripts in Protocol Management.*

N/A

Adults Unable to Consent

- *Describe the process you will use to determine whether an individual adult is capable of consent.*
- *List the individuals from whom you will obtain permission in order of priority (e.g., durable power of attorney for health care, court appointed guardian for health care decisions, spouse, and non-minor child).*
  - *For research conducted in the Virginia, review "SOP: Legally Authorized Representatives, Minors, and Guardians (HRP-013)" to determine which individuals in the state meet the definition of "legally authorized representative."*
  - *For research conducted outside of Virginia, please describe the legal requirements for obtaining permission from a legally authorized representative in the state where the research will occur.*
- *Describe the process for assent of the subjects.*

- *Indicate whether you will require assent from all, some, or none of the subjects. If some, indicate which subjects will be required to assent and which will not.*
- *If you will not obtain assent from some or all subjects, please provide justification for not obtaining assent.*
- *Describe whether and how you will document assent.*

N/A

52.0 Process to Document Consent in Writing

52.1 *Consult "SOP: Written Documentation of Consent (HRP-091)" for recommended procedures, and describe whether and how consent of the subject will be documented in writing*:

Individuals who are interested in the study after the initial study presentation will be provided an informed consent form for review (previously outlined in section 12). Participants will then be given a chance to ask questions regarding study procedures and risks. Those still interested will be asked to sign the consent at that time or during their first visit, before any data is collected. This information is detailed in section 24 above. A copy of the informed consent will be sent to all participants.

52.2 *If the research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, you can request that the IRB waive the requirement to obtain written documentation of consent (e.g., consent to participate is indicated by pressing a button for an online questionnaire – after the consent information is presented and before the questionnaire begins)*:

N/A

52.3 *If you will document consent in writing, attach a consent document with places for signatures. If you will obtain consent, but not document consent in writing, please attach the consent script or text. Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information. You should use "TEMPLATE CONSENT DOCUMENT (HRP-502)"to create the consent document or script*:

See the attached participant consent form

53.0 Resources Available

53.1 *Describe the resources available to conduct the research. For example, as appropriate:*

- *Describe the PI's availability to supervise the research.*

- *Justify the feasibility of recruiting the required number of suitable subjects within the agreed recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?*
- *Describe the time that you will devote to conducting and completing the research.*
- *Describe your facilities.*
- *Describe the availability of medical or psychological resources that subjects might need as a result of an anticipated or unanticipated consequence of participation in the research.*
- *Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions (e.g., training plans, detailed study notebooks).*

The PI is a Professor in the Department of Human Nutrition, Foods and Exercise at Virginia Tech. She currently has a 33% research appointment and oversees four doctoral students. She has previously served as a research dietitian and research exercise scientist at the National Institute of Diabetes & Digestive & Kidney Diseases in Phoenix and the Pennington Biomedical Research Center in Baton Rouge, LA, respectively, and has experience conducting exercise training studies and controlled feeding trials. The PI will dedicate time to this study and ensure the doctoral student is adequately trained and performs all aspects of the study according to protocol and procedures. The doctoral student, Trisha Sterringer, will be in charge of participant recruitment and enrollment, protocol execution, data collection and the day-to-day aspects of the study. T. Sterringer is a registered dietitian (RD), NSCA certified strength and conditioning specialist, and ISCD Certified Bone Densitometry Technologist. She has previous experience conducting energy availability studies in competitive athletes at Case Western Reserve University involving assessments of dietary intake, exercise energy expenditure, and body composition. Trisha Sterringer will be the doctoral student in charge of the study as part of her PhD dissertation, however, additional undergraduate research assistants may be added to the study in the future.

The Human Integrative Physiology Laboratory is located on the Corporate Research Campus at the Garvin Innovation Center. The major equipment item in this laboratory relevant to this study is the Lunar Prodigy DXA (GE Healthcare)

Wallace Hall is located on the main campus of Virginia Tech. The major equipment item relevant to this study is the Lunar iDXA (GE Healthcare).

In-person informed consent and recruitment presentation will take place at Radford University in Dedmond Center.

Other study procedures may take place at the Nutrition and Exercise Metabolism Laboratory (Dr Larson-Meyer's laboratory) which is also located on the Corporate Research Campus at 2270 Kraft Drive.

54.0 Multi-Site Research

Contact the HRPP for multi-site research (involving multiple institutions) and the details required for this section will be provided. Otherwise, indicate N/A.

N/A

Appendix H: Study 2 Approved Informed Consent

Title of Research Study: Changes in Energy Availability, Body Composition, and Bone Density in Male Division I Soccer Players During an Athletic Season (Protocol # 22-901)

Principal Investigator: Enette Larson-Meyer, PhD, RD, FACSM, **enette@vt.edu**, 540-231-1025
Other Study Personnel: *Trisha Sterringer, MS, RD, CSCS, tsterringer@vt.edu*

Key Information: The following is a short summary of our study to help you decide whether or not to be a part of the study. More detailed information is listed later on in this form.

The reason for this study is to observe the changes in diet, body composition, and bone density during Spring season and the possible effect these changes may have on athlete performance and health. This study does not include an intervention. This means that you will not be asked to consume specific foods or participate in extra exercise or training. Instead, you will participate in your typical (required) training activities but also asked to participate in several additional measurements of your food intake and body composition. Specifically, at the start and end of Spring season, you will be asked to have a body composition and bone density scan, and track food intake and exercise for 3 days. You will also be asked to complete a questionnaire at the end that will ask about intentional things that you did (or did not do) to change body composition or weight.

Why am I being invited to take part in a research study?

We invite you to take part in a research study because you are a male division I soccer player. You will be eligible to participate in this study if you are on the active roster, are not currently experiencing any injuries, and are at least 18 years of age.

What should I know about being in a research study?

- Someone will explain this research study to you
- Whether or not you take part is up to you
- Not participating will have no effect on your grades, playing time, or relationship with Radford University
- You can choose not to take part
- You can agree to take part and later change your mind
- Your decision will not be held against you
- You can ask all the questions you want before you decide

Why is this research being done?

This study proposes to evaluate how food intake and body composition change during Spring season in division I male soccer players. Soccer is an energetically demanding sport and research in male soccer players indicates that it may be difficult for some athletes to adequately meet energy and carbohydrate needs. This study will evaluate food intake, calories burned during exercise, body composition and methods used to alter body composition, signs of wellness, along with GPS technology you are already

familiar with. This data collected will help sports dietitians and medical professionals better understand how to help male soccer players fuel during a non-championship season (i.e., Spring season) to meet their desired body composition and performance goals.

How long will the research last and what will I need to do?

The time commitment for this study will be approximately 8 days over the course of Spring season (4 days at the start Spring training and 4 days near the end). At the start of Spring season, you will be asked to come to Virginia Tech for a lab visit to test body composition and bone density. You will also be asked to track your diet using a food intake record, and any exercise outside of team trainings using an exercise log. When the Spring season is almost over, you will have a second lab appointment at Virginia tech for body composition and bone density testing, and you will be asked to complete a questionnaire about any actions you took in an effort to change your body composition or weight during the spring season. You will also be asked to track your diet and exercise for 3 days at the end of the season. These procedures and tests are summarized in the table on page 4. The actual time and frequency of your visits may depend on your schedule and available appointment times. More detailed information about the study procedures can be found under the section, "What Will I Be Doing in This Study?"

What happens if I say "Yes, I want to be in this research"?

If you say yes to participating, you will be enrolled in an observational study that will involve tests and procedures outlined in sections to follow.

Will being in this study help me in any way?

We cannot promise any health or performance benefits to you or others from your taking part in this research. However, possible benefits may include receiving information about your body composition, bone density, and dietary intake. Please note, you should not consider your participation as a wellness or medical exam, and there will be no direct medical benefit to you. You should discuss any concerns about your health information with your health care provider.

Is there any way being in this study could be bad for me?

Participation in the study has minimal risk. There is potential risk of a small dose of radiation from the DXA scan procedure. More detailed information about the risks of this study can be found under "Is there any way being in this study could be bad for me?"

What happens if I do not want to be in this research?

Participation in this research is completely up to you. You can decide to participate or not to participate.

Your decision whether to participate or not will have no effect on your grades, playing time, or your relationship with Radford University.

Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, contact Enette Larson-Meyer, the Principal Investigator of the study, at enette@vt.edu or 540-231-1025.

This research has been reviewed and approved by the Virginia Tech Institutional Review Board (IRB). You may communicate with them at 540-231-3732 or irb@vt.edu if:

- You have questions about your rights as a research subject
- Your questions, concerns, or complaints are not being answered by the research team
- You cannot reach the research team
- You want to talk to someone besides the research team to provide feedback about this research

How many people will be studied?

We plan to include about 40 participants in this research study.

What will I be doing in this study?

Lab Visit 1 (Start of Spring Season)
Location: Virginia Tech, Human Integrative Physiology Lab, 1872 Pratt Drive. Blacksburg, VA 24060

Informed Consent: You will receive this consent form in hard copy, or by email or mail following the initial study presentation. A member of the research team will review this form with you at the beginning of the first lab visit, and provide you the opportunity to ask questions. You will need to sign this form before you can take part in the research. The consent process will take place in a private setting.

Urine Sample: You will be asked to provide a small amount of urine in a urine cup. This will be used to evaluate how hydrated you are. The urine sample only will be used to check hydration.

Body Weight, Body Composition, and Bone Density: Your height and weight will be measured on a digital scale. You will then lie on a hospital-type bed and a small amount of X-ray will be passed through your body to determine the amount of bone, muscle, and fat in your body. This procedure is called a dual-energy x-ray absorptiometry (DXA) scan. This test takes approximately 15 minutes which will involve a whole-body scan and two shorter scans of the hips and lower back (lumbar spine). There is no pain associated with the procedure.

Dietary and Exercise Record: You will be asked to record all the food and beverages you consume each day for 3 days in a row using paper records. This is the same food record that you have completed in previous seasons. You will also be asked to record all exercise and physical activity outside of team training for the 3 days on a paper exercise log. A member of the research team will explain how to fill out the records.

During Spring Season
All activities during the Spring season will be part of your normal responsibilities as a student athlete on the Radford University Men's Soccer team. You are not required to do anything additional as part of the study. The study team is requesting access to the following data as part of this research study:

Exercise Movement: During all field activity you will wear an activity monitor (Sport Performance Tracking vest) as a normal part of training.

Wellness Report: You will complete a daily wellness report during the spring season. This is the same report you completed during the fall season.

Yo-Yo Test Score: We are requesting access to your yo-yo fitness scores from the start and end of Spring season.

One-Rep Max (1RM): We are requesting access to your 1RM for bench press and deadlift from the start and end of Spring season.

Lab Visit 2 (End of Spring Season)
Location: Virginia Tech, Human Integrative Physiology Lab, 1872 Pratt Drive. Blacksburg, VA 24060

Urine Sample: You will be asked to provide a small amount of urine in a urine cup to evaluate your hydration status. The urine sample will only be used to check hydration status.

Body Weight, Body Composition, and Bone Density: Your height and weight will be measured on a digital scale. You will then have another DXA scan to determine the amount of bone, muscle, and fat in your body. This test takes approximately 15 minutes and will involve a whole-body, hip, and lower back scan. There is no pain associated with the procedure.

Dietary and Exercise Record: You will be asked to record all the food and beverages you consume each day and exercise outside of team training for 3 days in a row using paper records.

Questionnaires: You will be asked to complete a final questionnaire that will assess whether you had goals related to body composition or weight, and any methods you used in an attempt to alter your weight or body composition.

Table of Tests and Procedures:

Table 1. Overview of Data Collection	Start of Season	During Season	End of Season
Height	x		
Weight	x		x
Body Composition by DXA	x		x
Bone Density (total body, hips, lower back) by DXA	x		x
Urine sample for hydration testing	x		x
Food intake records (3 days)	x		x
Exercise log (3 days)	x		x
Fitness tests (Yo-Yo score, 1RM for bench press and deadlift)	x		x
Exercise movement during field training (SPT device)	x	x	x
Daily Wellness Report	x	x	x
Questionnaire – motivations and methods to alter body composition			x

What happens if I say yes, but I change my mind later?

You can leave the research at any time, for any reason, without penalty. Any data collected previously, however, may be used along with data collected with other participants as previously explained.

Is there any way being in this study could be bad for me? (Detailed risks)

Participation in the study has minimal risk. Risks include those associated with a small dose of radiation from the DXA scan and potential breach of confidentiality.

DXA Scan: You will be exposed to a small amount of radiation as part of the body composition testing during the DXA scan. The amount of radiation that you will receive in the DXA scans is far less than the amount that the Food and Drug Administration (FDA) allows per year. The amount you will receive from each scan is equal to 1/20th of a chest X-ray. The more radiation you receive over your lifetime, the more your risk increases in developing certain kinds of cancer. The radiation in this study is not expected to greatly increase these risks. The exact increase in this risk is not known. These potential risks will be minimized by having a certified technologist performing the DXA scans and restricting participants from having a DXA scan if they have had one in the previous 4 weeks.

Confidentiality: There is a potential risk of a confidentiality breach. We will make every effort to limit the use and disclosure of your personal information, including study data, only to people who have a need to review this information. Hard copies of study data will be stored in a locked file cabinet in the Nutrition and Exercise Metabolism Laboratory with restricted access to authorized personnel. Electronic data will be stored in password-protected files on a secure server.

What happens to the information collected for the research?

We will make every effort to limit the use and disclosure of your personal information, including study data, only to people who have a need to review this information. We cannot promise complete confidentiality. Members of the coaching staff (head and assistant coaches, strength and conditioning coach, and athletic trainer) will be provided with select team data related to sport/exercise performance. This will include a statistical summary of changes in weight, lean body mass, fat mass, bone density, and total caloric intake for the team members that participated. Coaching staff will only be provided with de-identified group data. This means coaches will not know your individual results. This data is being provided to inform coaching staff on the physical changes that occurs during spring season and how that potentially impacts sports performance. Organizations that may inspect and copy your information include the IRB, Human Research Protection Program, and other authorized representatives of Virginia Tech. If identifiers are removed from your private information or from samples collected as part of this research, that de-identified information or those de-identified samples could be used for future research studies or distributed to another investigator for future research studies without your additional informed consent.

We may publish the results of this research, but we will keep private your name and any information that could identify you. We protect your information from disclosure to others as the law requires. We cannot promise complete secrecy.

Data collected in this research might have your identifying information removed and used for future research or given to another researcher for future research without your consent.

Can I be removed from the research without my OK?

The person in charge of the research study can remove you from the research study without your approval. Possible reasons for removal include:

- It is in your best interest
- If the researchers are unable to obtain measurements that are necessary for the study
- You are unable to show up for scheduled appointments or completing all procedures
- If you become injured or ineligible for team participation

What else do I need to know?

If you become injured or sick from the research study that you are participating in, you will be referred to a clinic or to your team physician, or personal physician or health care provider. Generally, this care will be billed to you, your insurance, or other third party. Virginia Tech has no program to pay for medical care for research-related injuries.

We will offer to share your individual test results with you. You may accept or decline these results.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research. We will provide you with a signed copy of this form for your records.

Signature of subject	Date
Printed name of subject	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

Appendix I: Study 2 Survey

Participant ID: _____

Nutrition and Body Weight Survey

1. What was your goal during Spring season related to your body weight or composition? *Check all that apply.*

   ☐ Maintain weight

   ☐ Lose weight

   ☐ "Cut" or lose mostly body fat

   ☐ Gain weight

   ☐ Build muscle

   ☐ Other (please describe):

2. Did you achieve your goal?

   ☐ Yes ☐ No ☐ Unsure

3. Check all the methods below that you used specifically to achieve the goal(s) in question 1.

   ☐ Changed the amount of food I was eating

   ☐ Changed the types of food I was eating

   ☐ Started following a new diet or way of eating

   ☐ Took nutrition supplements

   ☐ Participated in extra workouts *in addition* to supervised team training

   ☐ Asked for advice from the team Registered Dietitian

   ☐ Asked for advice from the team Strength and Conditioning Coach

   ☐ Asked advice from a teammate

   ☐ Sought advice from the internet

   ☐ Sought advice from a published book or resource

   ☐ None

   ☐ Other (please describe):

   Please provide additional detail for the method(s) you used:

4. For each of the categories below, please check the box that best describes how you changed your diet during the Spring season to achieve the goal(s) in question 1.

	Increased	Decreased	No Change
Overall calories eaten			
Portion sizes			
Number of meals each day			
Number of snacks each day			
Protein foods			
Fruits and vegetables			
Dietary supplements			

Other (please describe):

5. Did you start following a new diet or eating pattern during Spring season (e.g., keto, low carb, intermittent fasting, high protein, high carb, vegetarian, etc.)?

☐ No

☐ Yes

If yes, please describe the diet you followed:

6. Check all the nutrition supplements you used during Spring season.

☐ None

☐ Protein powder/shakes

☐ Meal replacement shakes (e.g., Boost, Ensure, Carnation Breakfast)

☐ Weight gainers

☐ Appetite suppressants

☐ Multi-vitamin

☐ Supplemental vitamin(s) or mineral(s): (*please list all)* _____

☐ Fish oil or omega-3

☐ Testosterone boosters

☐ Pre-workout

☐ Other: