The Associations Between Testosterone and Bone Mineral Density in Male Collegiate Athletes

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Authorization to Submit Thesis

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Abstract

Testosterone (T) deficiency and low bone mineral density (BMD) are common symptoms among those experiencing relative energy deficiency in sport. However, a lack of research exists on the associations between T concentration and BMD in various male athletes. The purpose of this study was to assess the associations between T concentration and low BMD in male collegiate athletes. Male collegiate cross-country (CC) runners, club rugby (R) athletes, and collegiate track and field (TF) sprinters/jumpers, completed one lab visit at the end of their sports off-season. Participants arrived fasted (8 hours), abstained from exercise (12 hours), and within 60 minutes of waking. Testing included completing an eating disorder risk questionnaire, a 24-hour dietary food recall, 3 dual-energy x-ray absorptiometry (DXA) scans (whole-body, left femur, and lumbar spine), and collection of a saliva sample. Descriptive statistics were performed on dependent variables using measures of central tendency, variability, and frequencies. The statistical hypotheses were examined using analysis of variance (ANOVA), in order to compare the three athletic groups. A post hoc tukey test was then used to determine where the significant differences occurred. Pearson correlations were utilized to assess the associations between T and BMD at whole-body, left femur, left femoral neck, and lumbar spine among all the male athletes and for each sport. Significance was accepted at $p \leq 0.05$. Seven of the ten R athletes were considered to have a high risk of eating disorders because of their responses to the extreme weight control behavioral questions. CC had a higher caloric intake than R but not TF (CC: 3,813±1,239 kcal; R: 2,402±589 kcal; p=0.005). CC had lower whole-body BMD when compared to R but not TF (CC: 1.21±0.03g/cm^2; R: 1.32±0.07g/cm^2; TF: 1.28±0.09g/cm^2, p=0.007, p=0.365, respectively). There were no significant differences in T concentration between groups.

There were no significant associations between T concentration and BMD in CC and TF, but R had significant associations between T concentration and whole-body ($r=0.635$, $p=0.049$), left femur ($r=0.671$, $p=0.034$), and left femoral neck BMD ($r=0.686$, $p=0.028$). Our findings support previous research findings demonstrating low BMD in endurance runners compared to other athletes. However, no associations were observed between T and BMD except for in R athletes. T may not be a great predictor of BMD in male collegiate athletes.

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Chapter 1: Introduction

Female and male athletes both experience health benefits from sport participation. Evidence suggests that those that participate in regular physical activity have reduced risk of chronic diseases and positively effects emotional wellbeing [1,2]. However, a subset of athletes may experience negative health consequences from participating in sports [3]. Many athletes have a high energy expenditure due to long bouts of high intensity training. When an athlete does not consume enough calories to meet their caloric needs, they are in a low energy availability (LEA) state [3]. Energy availability is used to describe the difference between the amount of energy intake and estimated energy expenditure, which is then normalized to a kilogram of lean body mass [3]. LEA may occur intentionally or may be inadvertent due to lack of knowledge regarding the amount and types of foods they should be consuming to meet their energy requirements [3]. In sports that emphasize leanness, athletes often will restrict calories or participate in unhealthy dietary habits in order to lose body weight [3]. Athletes who are in a LEA state can experience negative health and performance consequences. LEA was first discovered among female athletes during investigations of the female athlete triad. In 1992, the female athlete triad was identified as a syndrome consisting of disordered eating, amenorrhea, and osteoporosis [4]. In 2007, the diagnosis of the female athlete triad was changed to identify female athletes within a spectrum of LEA, menstrual dysfunction, and bone mineral density (BMD) [4]. Soon after, the term, relative energy deficiency in Sports (RED-S), was introduced to encompass a broader range of physiological functions that are disturbed during LEA [5]. These physiological functions include: metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular and psychological health [5]. Furthermore, evidence suggests that REDS also affects male

athletes and parallels of the female athlete triad were identified in male athletes including hypogonadotropic hypogonadism (HH), low BMD, and LEA [3,5].

HH is an imbalance of reproductive hormones that lead to decreased-function of the reproductive organs [6]. Previous studies identified that male endurance athletes (cyclists and runners) exhibit lower levels of testosterone (T) than their sedentary counterparts [3,7,8]. After 12 months of training, with the intent to overtrain their participants, studies reported that there was a 40% reduction in T and 43% reduction in sperm count compared to their baseline values among elite male runners [9–11]. Males athletes that exhibited less than 12 nmol/L of testosterone were considered to have the exercise-hypogonadal male condition (EHMC) [3,7]. Athletes with EHMC have testes that are not able to be stimulated adequately or do not respond from the gonadotropin releasing hormone. Thus, the production of T and sperm decrease [7]. Extensive research of EHMC has occurred among endurance athletes due to the sports' emphasis on lean body types and increase risk of LEA [3,7,8,12]. However, limited research exists on the risk and prevalence of EHMC among other various male athletes. In order to provide greater insight of sport differences in T levels, the current study examined athletes who participated in different sports that had different body composition goals. The athletes that were examined in the current study were cross-country (CC) runners, rugby (R) athletes, and track and field (TF) sprinters and jumpers.

Another possible negative outcome that results from LEA is decreased BMD [3]. Low BMD increases the risk of bone stress injuries, especially within the trabecular regions, during their collegiate years and increases risk for osteoporosis later in life [3,13,14]. The most common bone stress injury that occurs are stress fractures [15]. Stress fractures are common bone injuries that inhibit athletes from participating in their training sessions and

competitions [15]. Another detrimental health consequence that can result from low BMD during college aged years (18-25 years) is the increase risk for osteoporosis later in life [3,13]. Most males reach their peak bone density by the age of 20 years-old and the peak bone accrual rates occur between 13-15 years-old [3]. If male athletes do not optimize these ages for accruing BMD and have low BMD during their college years they are at an increased risk for osteoporosis as they age [3,13,14].

Previous literature has demonstrated that male R athletes and TF sprinter/jumpers tend to have higher BMD compared to non-athletic population [16]. In contrast, endurance runners tend to have similar or lower BMD than a non-athletic population, especially in the hip and lumbar spine [3,17]. Differences in BMD between R and TF athletes and CC runners may be due to differences in body composition and total body mass. Evidence has suggested that those with greater body mass exhibit a greater amount of BMD [18]. A R team has multiple positions and different positions requires different body composition to optimize their performance. R forwards are the R athletes that exhibit a greater amount of body mass and tend to have a greater amount of fat and lean body mass because they need to be strong, powerful, and robust to protect their teammates from the opposing team. In contrast, the R backs and TF sprinters/jumpers are athletes that depend on their acceleration and speed to optimize their performance [19,20]. The R backs and TF sprinters/jumpers athletes tend to have a low amount of fat mass but exhibit a greater amount of lean body mass compared to CC runners [19,20]. In order to optimize running economy CC runners tends to exhibit a lower amount of fat mass and lean body mass than R athletes and TF sprinters/jumpers [20,21].

A lack of literature exists on T concentration differences among male athletes with different body composition goals. In the current study, CC runners, R athletes, and TF sprinters/jumpers were assessed because of their different body composition goals to enhance performance in their respective sports. Therefore, the primary aim of the current study was to assess the relationship between T concentrations and BMD among collegiate male CC, R, and TF athletes. The secondary aim of this study was to identify any sport differences in BMD and T levels among these three sports.

Purpose Statement

The purpose of this study was to assess the associations between T and BMD among collegiate male CC, R, and TF athletes.

Research Questions

1. Is there a relationship between T levels and BMD in male collegiate athletes?
2. Are there differences in BMD by sport?
3. Are there differences in T levels by sport?

Hypotheses

1. A positive relationship between T levels and BMD exists among male CC runners, R athletes, and TF sprinter/jumpers.
2. Male cross-country runners exhibit lower levels of T than male R athletes and TF sprinters/jumpers.
3. Male cross-country runners exhibit less BMD than male R athletes and TF sprinters/jumpers.

Delimitations

1. Male athletes (age 18 to 25 years) enrolled full-time at a college or university in the Palouse. All female athletes were excluded from the study.
2. All male athletes who are not part of the Washington State University, University of Idaho, or Lewis-Clark State College's cross-country, track and field, and rugby teams were excluded. A purposive sample consisted of members of the University of Idaho and Lewis-Clark State College men's collegiate cross-country and track and field teams, and Washington State's club rugby team during fall 2019.
3. Individuals with contraindications to exercise based on the American College of Sports Medicine and American Heart Association (ACSM/AHA) risk of stratification including uncontrolled hypertension, currently taking blood pressure medications, or have been diagnosed with cardiovascular disease, stroke, diabetes, thyroid, or kidney dysfunction were not be eligible to participate in the study.
4. Participants did not experience a serious musculoskeletal injury within six months prior of the study. This included injuries involving the bones, muscle, tendons, and other soft tissues that have prevented the athletes from training and/or competition. Examples of serious musculoskeletal injuries included broken bones, muscle strains or ruptures, etc.
5. Participants came to the lab in a fasted state (no consumption of calories for at least 8 hours).
6. Participants came to the lab within one hour of waking up.

Limitations

1. The morning activities of the participants prior to testing were not controlled.

2. Dietary intake was not controlled prior to the fasting period which may have influenced their T results.
3. Training volume and intensity were not controlled which may have influenced their T results.

Assumptions

1. Participants answered the questions truthfully.
2. Participants understood and fulfilled their instructions to the best of their abilities.
3. ASA24® was used to accurately assess dietary intake.
4. DXA was an objective, reliable, and valid instrument for measuring body composition and bone mineral density.
5. EAT-26 was a valid and reliable tool to assess eating disorder risk in athletes.
6. Salivary T was highly correlated with serum T.

Definition of Terms

1. **Appendicular Skeletal Mass Index (ASMI):** An assessment of age-related muscle wasting (sarcopenia). ASMI is estimated by appendicular skeletal muscle mass/ height^2 (kg/m^2) [22].
2. **Body Composition:** A health-related component of physical fitness that relates to the relative amounts of lean body mass and fat mass. Fat mass includes essential fats needed to support daily functioning and excess non-essential fats. Lean body mass includes blood, water, muscles, bones, ligaments, tendons, and organs [23,24].
3. **Bone Mineral Density (BMD):** A measure of the volume of minerals contained in a certain volume of bone. DXA is a tool that can assess BMD [25].

4. **Dual-energy X-ray Absorptiometry (DXA):** The Hologic QDR 4500A is a fan beam X-ray bone densitometer, which uses two different energy levels produced by an energy tune to estimate bone mineral content, BMD, and body composition. DXA is a tool that can diagnose osteopenia and osteoporosis [26].
5. **Eating Disorder:** A mental disorder characterized by serious disturbances in eating patterns consisting of anorexia nervosa, bulimia nervosa, and binge-eating disorder [27].
6. **Energy Availability:** The amount of energy necessary to support the range of body functions involved in optimal health and performance. Energy availability is the difference between dietary caloric consumption and exercise energy expenditure, relative to lean body mass [5].
7. **Exercise Hypogonadal Male Condition (EHMC):** A condition that occurs when the testes are not able to be stimulated adequately or do not respond from the gonadotropin releasing hormone and results in T deficiency. Male athletes that exhibit less than 12 nmol/liter of T concentrations are considered to have EHMC [7].
8. **Female Athlete Triad:** A spectrum of abnormalities of energy availability, low BMD, and menstrual dysfunction in female athletes [4].
9. **Hypogonadotropic Hypogonadism (HH):** Is the failure to stimulate the testes or ovaries through the gonadotropin-releasing hormone, Follicle Stimulating Hormone (FSH), and the Luteinizing Hormone (LH) [6].
10. **Testosterone (T):** A sex hormone that is present both in females and males' reproductive system. Males have higher concentration of T which stimulates the development of male secondary sex characteristics [9].

11. **Visceral Adipose Tissue:** The storage of fat tissue around the internal organs of the trunk, abdomen, and pelvis [28].

Chapter 2: Review of Literature

It is known that there are many benefits for those who choose to participate in sports. Van Boekel et al. (2016) found that among 12th grade high schoolers, the student-athletes have higher GPAs, increased perception of social support from teachers, friends, and family, and increased perception of school safety [29]. Sports also help adolescents improve health and mental states and develop positive character attributes that can be beneficial later in adolescents' life [30]. Sports, under the right conditions can teach good sportsmanship, hard-work, dedication, teamwork, and so forth [30]. Therefore, sports can provide many benefits for youth and adolescents in many different aspects of their lives. However, detrimental consequences can also result from participation in sports [3].

In 1992, ACSM identified a syndrome that occurred among female athletes, the female athlete triad [4]. The female athlete triad consists of three components, low energy availability (LEA), menstrual dysfunction, and low bone mineral density (BMD) [31,32]. Relative energy deficiency in sports (REDS) was later introduced to recognize other impaired physiological functions including metabolic rate, menstrual function, bone health, immunity, protein synthesis, and cardiovascular health [5]. Current studies have presented evidence that male athletes can experience REDS similarly to female athletes, and parallels of the female triad exist in male athletes including hypogonadotropic hypogonadism (HH), low BMD, and LEA [3,5].

The purpose of this study was to assess the relationship between testosterone (T) concentrations and BMD among collegiate male cross-country (CC), rugby (R), and track and field (TF) athletes.

Energy Availability

In 1992, The Task Force on Women's Issues of ACSM defined the syndrome of disordered eating, amenorrhea, and osteoporosis as the female athlete triad [4]. At the time, a female athlete had to be diagnosed simultaneously with all three of the components to be diagnosed with the female athlete triad. In 2007, the female athlete triad's diagnosis and definition was redefined as a spectrum of abnormalities in energy availability, menstrual function, and low BMD [4]. Additionally, energy availability replaced disordered eating because further investigations revealed that female athletes could be in a LEA state with or without eating disorders and display female athlete triad symptoms [4].

LEA is a result of decreased energy intake and/or increased exercise load and the energy requirements are not met by caloric consumption. Energy availability is calculated by the following equation: energy availability = [Energy intake (kcal) – Exercise energy expenditure (kcal)] / fat-free mass (kg) [32]. According to Mountjoy et al. (2014), adequate energy availability is 45 kcal/kg/fat-free mass/day. Less than 30 kcal/kg/fat-free mass/day is considered LEA and various body functions are disturbed [32].

One of the most common symptoms of LEA is hypogonadotropic hypogonadism (HH) [6,33]. HH is the body's inability to adequately stimulate the ovaries or the testes through the gonadotropin releasing hormone, luteinizing hormone, or the follicle stimulating hormone. This causes an imbalance of sex hormone in both female and male athletes. HH impairs the production of estrogen in female athletes and impairs testosterone (T) production in male athletes [3,6,32,33]. Several theories exist on how LEA is associated with T deficiency. The theories are based on hormonal imbalances of cortisol and leptin and their influences on T levels when exposed to LEA conditions [3,9,34]. Roberts et al. (1993)

hypothesize that cortisol decreases the number of luteinizing hormone (LH) receptors on Leydig cells, and consequently decreases the amount of T secreted from these Leydig cells [9]. Gomez-Merino et al. (2002) identified associations between T and leptin concentrations during LEA [34]. Leptin informs the central nervous system the amount of adipose tissue that is present in the body and sends signals to the brain to inhibit hunger. During LEA states, subjects have deceased levels of T and leptin, however, it is unclear how leptin levels are associated with T levels during LEA [35].

These imbalances of sex hormones can also negatively influence BMD. There are estrogen receptors on bone tissue that will signal for a decrease in bone reabsorption by osteoclasts. Therefore, during REDS, female have a decreased production of estrogen and osteoclasts activity increases. Consequently, more bone minerals are being reabsorbed into the blood stream and negatively influences BMD [5,32,36]. Male athletes experience this phenomenon similarly but instead of estrogen being negatively influenced by REDS, the males' T production is hindered [3,13]. A lack of T can also negatively influence BMD because there are androgen receptors on bone tissue that are responsible for signaling for an increase of osteoblast activity [3,13]. Osteoblasts are responsible for signaling for bone formation [37]. Therefore, when there a lack of serum T, there may be a lack of signaling for bone formation and can negatively influence BMD [3,13]. However, there is a lack of evidence that assesses this relationship between T levels and BMD in male collegiate athletes.

Hypogonadotropic Hypogonadism

One of the greatest concerns that can result from LEA is HH [33]. In female athletes, HH causes menstrual dysfunction [5,32]. During a regular menstrual cycle, LH and FSH are

excreted from the pituitary gland [38]. LH and FSH stimulate the ovary to develop a follicle, ovulate and support the corpus luteum through the end of the cycle [39]. When a female athlete has hypothalamic amenorrhea, the production of LH is impaired, and anovulation occurs. Estrogen levels decrease and progesterone levels do not elevate like they should during menstruation [38,40]. Within the general population, 2-5% experience amenorrhea [41]. The range of prevalence of amenorrhea within exercising women is between 1- 61% [41].

Male athletes with HH experience a decrease in T production. The normal concentration of T in males is 12 nmol/liter [7]. Male athletes with values below 12 nmol/liter are considered T deficient [7]. One study examined a group of nine distance runners who ran 81±14 km/week and a control group consisting of 8 men who had not participated in any running 12 months prior to the study. The researcher found that 8 of the nine distance runners (89%) had concentrations of T below 12 nmol/liter and were considered to be T deficient [7]. Meanwhile, in the control group none of the participants were considered T deficient [7]. However, the LH and FSH levels were not significantly different between the long-distance runners and control group [7]. Yet, MacConnie et al. (1986) reported decreased levels of LH among marathon runners who were at a high risk of LEA [42]. Therefore, there is conflicting evidence on whether LH concentrations are affected by LEA and if it results in T deficiency.

According to Tenforde and colleagues (2016) four of five studies with small sample sizes (N = 5 – 11 per study) that included participants who ran greater than 100km/ week reported 10-30% decrease in T levels than the sedentary control group. However, two of six studies that had participants that trained less than 100km/ week had significantly lower T

levels than the sedentary control group [3]. Therefore, the “volume threshold hypothesis” indicates that altered hormone levels in endurance runners occur when running more than 100km/week [43]. It is yet to be determined how critical T deficiency is among male athletes. In a previous study that concerned marathon runners and T deficiency, 14 of the 20 participants were T deficient. However, only two of the 20 participants had severely low T concentrations and suffered from oligospermia [44]. Oligospermia is a condition when males have a low sperm count ($< 15 \times 10^6$ sperm/ml) and the ability to reproduce is inhibited [44]. T also has an important anabolic role within the muscle [45]. Muscle cells have androgen receptors on the cell membrane. When T binds to these androgen receptors, muscle protein synthesis is stimulated which allows athletes to repair and strengthen skeletal muscle after participating in training sessions [46]. The current mechanism is one of the adaptations that occurs from participating in physical activity [46].

Furthermore, males have a decreased production of T as they age and this can negatively influence musculoskeletal health and may result in sarcopenia [47,48]. Sarcopenia is term that characterizes those with muscle wasting and impaired skeletal muscle function [49]. A method to assess risk for sarcopenia is estimating an individual’s appendicular skeletal muscle index (ASMI). Those that have an ASMI less than 7.40 kg/m^2 are considered high risk for sarcopenia [50]. T is important for skeletal muscle health because when it binds to these androgen receptors on the muscle tissues it signals for an increase of protein synthesis which positively influences skeletal muscle health [47,48]. Additionally, T enhances nitrogen retention which allows the body to be more efficient at utilizing dietary protein for protein synthesis [48]. Evidence suggests that older men that have low T have an increased risk for sarcopenia and can result in impaired muscular strength, decreased lean

body mass, and increased fat mass [47,48]. However, Supplementation with T has been shown to improve skeletal muscle strength, increase lean body mass, and decrease fat mass in older men [47,48]. If a male athlete has the EHMC this may increase their risk of sarcopenia later in life due to the lack of T binding to these androgen receptors bone tissue to upregulate protein synthesis and increase nitrogen retention [8,33,47,48]. However, further investigations are needed to determine if risk of sarcopenia is a major concern for athletes with EHMC [7]. In conclusion, reproductive dysfunction, impaired recovery from exercise, and increased risk for sarcopenia may be concerns among those with T deficiency during their collegiate years [3,45–47].

Bone Mineral Density

BMD is a measure that helps evaluate bone health and is regulated by bone cells called osteoclasts and osteoblasts [37]. Osteoclasts are responsible for releasing calcium and phosphorus from the bone and the minerals are then reabsorbed into the blood, which can negatively influence BMD. Whereas osteoblasts are responsible for accruing calcium and phosphorus and increasing BMD [51]. Reductions in T levels have a negative impact on BMD due to the lack of activation of the androgen receptors on bone tissue [3,52]. T binds to these androgen receptors and osteoblasts are stimulated to begin bone formation. Furthermore, the binding of T to androgen receptors decreases apoptosis of osteoblasts and decreases activation of osteoclasts [52]. During reduced levels of T, fewer of the osteoblasts are stimulated for bone formation and more of the osteoclasts are activated for bone resorption. As a result, BMD is negatively influenced [52].

Men achieve their peak bone mass at approximately the age of 20 years [3]. The peak accrual rate of bone minerals is during the ages of 13-15 years [3]. It is important to accrue

bone minerals during these ages in order to optimize peak bone mass and prevent low BMD (Z-score < -1.0) and osteoporosis (Z-score < -2.0) later in life [3]. Bone loss is a natural phenomenon as one age. However, if male athletes have impaired bone health during their adolescents and college years their risk of osteoporosis increases as they age [3,13,14]. Osteoporosis in a major concern in the aging population because it is associated with hip and vertebral fractures which often results in reduced physical activity or permanent physical disability [53].

Normally, athletes exhibit greater BMD than non-athletes because sports involve high-impact and multidirectional loading that promotes bone mineralization [3]. However, endurance runners have an increased risk of low BMD which is a common symptom among those with LEA [3]. Previous research has estimated that 19-40% of elite male collegiate and post-collegiate runners are considered to have low BMD [13,54,55]. Impaired bone health during their collegiate competitive ages can inhibit their ability to train and complete. Low BMD increases the risk of bone stress injuries especially within the trabecular regions [3]. The most common bone stress injury is stress fractures [15]. Stress fractures occur when repetitive and excessive stress is placed on the bone and microfractures are produced. When the bone is not given enough time to repair these microfractures, stress fractures occur. Stress fractures are common bone injuries that inhibit athletes from training and competition [15]. According to Iwamoto and Takeda (2003), from 1991 to 2001 there were 196 cases of stress fractures that were diagnosed in their sports medicine clinic. TF and R had 20 and 5 athletes who sustained stress fractures, respectively. Out of ten sports that were represented and diagnosed at the sports medicine clinic, TF and R were ranked third and ninth with the most occurrences of incidences of stress fractures, respectively [56]. However, the prevalence of

inadequate bone health and risk of stress fractures is higher among the long-distance runners than sprinters and jumpers on track and field teams [57,58]. Therefore, low BMD during their college years can inhibit their ability to train and compete and can increase their risk of poor bone health in the future [3,13,14].

Conclusion

Even though participation in sports can have many benefits, adverse effects may also exist [3]. One of the negative results that can occur among sports that emphasize leanness is LEA [32]. LEA is a result of insufficient caloric intake to meet one's energy requirements and various body systems are then disturbed [32].

In LEA, both female and male athletes have disruption in sex hormones due to the lack of stimulation of the testis and ovaries through the hypogonadotropic releasing hormones [4]. When female athletes are in LEA, they have reduced levels of estrogen which results in menstrual dysfunction [4,7,40]. Similarly male athletes experience decreased T levels which may impair spermatogenesis, athletes' ability to recover from training, and muscular strength and function as they age [4,7,40,47]. Another parallel that has been identified between the female athlete triad and male athletes is bone health [32]. In both genders, when athletes are in LEA, they have an increased risk of low BMD, and this may occur due to reduced sex hormone levels [3,52]. In male athletes, adequate T levels stimulate osteoblast activity for bone formation and decrease osteoclast activity [52]. In most athletes, sports have a positive influence on BMD due to the high impact loading of the bone and stimulation of the osteoblasts [51]. However, during LEA, T deficiency may occur and may negatively influence BMD. Therefore, more investigations are needed to determine if

associations exist between T concentrations and BMD and assess sport differences in T levels and BMD in male collegiate athletes.

Chapter 3: Methodology

Purpose

The primary purpose of this descriptive study was to determine if there was a significant relationship between testosterone (T) and bone mineral density (BMD) in male collegiate cross-country (CC) runners, rugby (R) athletes, and track and field (TF) sprinters/jumpers. The secondary purpose was to determine if there were significant differences in T and BMD between these three sports.

Participants

A convenient sample was recruited from a nearby college, and two universities in and near the Palouse. This sample was to determine the associations between T levels and BMD in male collegiate CC runners, R athletes, and TF sprinters and jumpers from the surrounding universities and colleges. Researchers met with each team and explained the details of the study during August 2019. CC and R participants completed a recruitment form if they were interested in participating in the study and were scheduled to come to the Human Performance Lab (HPL) at the end of August/early September 2019. TF participants also completed an interest form and were scheduled to come to the HPL during the month of November 2019.

Participants (age 18 – 25 years) included male collegiate CC runners, club R athletes, and collegiate TF sprinters and jumpers. Participants were free of serious musculoskeletal injuries or other medical conditions six months prior to testing including injuries involving bones, muscle, tendons, and other soft tissues that have prevented the athletes from training six month prior to their visit to the HPL. Furthermore, participants had no known contraindications to exercises based on the American College of Sports Medicine (ACSM)

and American Heart Association (AHA) which included uncontrolled hypertension, taking blood pressure medications, or had been diagnosed with cardiovascular disease, strokes, diabetes, thyroid, or kidney dysfunction.

Procedures

Participants arrived at the HPL during the mornings, within one hour of waking-up, and wore athletic clothing or clothing that did not contain metal. Additionally, participants arrived at the HPL in a fasted state. This included no caloric drinks or food within eight of hours prior to their arrival. Furthermore, the participants did not participate in any exercise for at least 12 hours prior to testing. This included any physical activity that was above what was considered light physical activity [59]. Once the participants arrived at the HPL they read and signed an informed consent. After signing the informed consent, the athletes completed a medical history questionnaire and the eating attitudes test (EAT-26). After the completion of these questionnaires, participants used the Automated Self-Administered 24-hour dietary tool (ASA-24) to assess the athletes' dietary intake 24 hours prior to testing. Then athletes completed three Dual-Energy X-ray Absorptiometry (DXA) scans to assess BMD for the whole-body, left hip, and lumbar spine. Body composition was also assessed via the whole-body DXA scan. After the DXA scans, saliva samples were collected through SalivaBio Oral Swabs for T levels analysis.

Medical History Questionnaire

All participants completed a Medical History Questionnaire. The questionnaire obtained information on health status including history of major injuries or illnesses, including stress fractures. This questionnaire was to ensure that the participants were not smoking, had no contraindications according to the ACSM and AHA guidelines, and were

free of any serious major injury or illness within the last six months of the test. Furthermore, in combination with the dual-energy X-ray absorptiometry (DXA) scans, the participants' reports of secondary fractures were utilized to identify participants with low BMD.

Eating attitude test (EAT-26)

Participants completed a 26-item questionnaire that assesses eating disorder risk through three subdivisions: Bulimia (B), Dieting (D), and Oral control (O). The participants then completed behavioral questions that assessed extreme weight control behaviors. Participants were considered high risk for an eating disorder if they scored a 20 or higher or affirmed that they practiced disordered eating behaviors.

ASA-24 food recall

Participants completed a 24-hour diet recall through an online portal, ASA-24. This assessed the number of calories and the macronutrient proportions participants consumed within 24 hours prior to coming to the lab.

Body composition and bone mineral density

Height and mass measurements were recorded using a digital stadiometer and clinical scale (DETECTO, Apex- SH, Webb City, MO). BMD was measured non-invasively using DXA (Hologic Inc., Horizon W QDR Series, Bedford, MA, USA). One scan of the lumbar spine and left hip as well as a whole-body scan were performed by the same certified technician according to the manufacturer's instructions and specifications. Results were analyzed with APEX software, version 4.5.2.1 (Hologic Inc. Horizon W QDR Series). Lean and fat mass, appendicular skeletal muscle mass index (ASMI), visceral adipose tissue (VAT), and BMD were used for analysis.

Salivary Testosterone

Participants were given a SalivaBio Oral Swab purchased from Salimetrics. Participants placed the SalivaBio Oral Swab under their tongues for one to two minutes to allow the cotton to be saturated with the participant' saliva. After one to two minutes, participants placed the SalivaBio Oral Swab into the Swab Storage Tube. Researchers then stored the saliva collection tube in the HPL freezer at -20 Celsius (or below). Once all the saliva samples were collected, saliva samples were sent to Salimetrics Lab in Carlsbad, CA. The Salimetrics lab analyzed the saliva samples for analysis of T concentrations.

Descriptive statistics were performed on the dependent variables (T, eating disorder risk, BMD, fat mass and lean body mass) as well as analyses using measures of central tendency, variability, and frequencies. The statistical hypotheses were examined using analysis of variance (ANOVA), in order to compare the three athletic groups. A post hoc Tukey test was used to determine where the significant differences occurred. Pearson correlations were utilized to assess the associations between T and BMD at whole-body, left femur, left femoral neck, and lumbar spine among all the male athletes and for each sport. Significance was accepted with alpha set at $p \leq 0.05$. Data will be analyzed using the Statistical Package for the Social Sciences versions 24.0 (SPSS 24).

Chapter 4: Results

Description of Subjects

Table 4.1 shows participant demographics for those who completed the study (N=28, cross-country=8, rugby=10, track and field=10). The cross-country (CC), rugby (R), and track and field (TF) athletes were similar in age and height but R had significantly more body mass than CC and TF (p=0.001, p=0.035 respectively). On average, the CC and TF athletes were in the normal category for BMI (18.5-24.9 kg/m^2). However, R was categorized as overweight (25.5-29.9 kg/m^2) on average.

Table 4. 1 Participant Characteristics by Sport

	Cross-Country (*n*=8)	**Rugby** (*n*=10)	**Track and Field** (*n*=10)
Age (years)	19.88±1.36	20.50±2.37	20.20±1.23
Weight (Kg)	65.56±7.43*	99.16±27.67	78.07±7.99†
Height (cm)	180.02±9.74	182.75±6.32	184.29±6.65
BMI (kg/m^2)	20.18±1.02	29.51±7.04	22.95±1.43

Mean ± SD (all such values)
BMI, body mass index; kg, kilograms; cm, centimeters; %, percent
* Indicates significant difference ($p < 0.05$) between cross-country and rugby
† Indicates significant difference ($p < 0.05$) between rugby and track and field
‡ Indicates significant difference ($p < 0.05$) between cross-country and track and field

Eating Attitudes Test (EAT-26)

Table 4.2 shows the average eating attitudes test (EAT-26) score for all three groups. There were no differences in EAT-26 scores between all sports (p=0.55). All the participants scored <20 on the EAT-26 questionnaire, however, seven of the R athletes were identified as high risk of eating disorders based on their affirmative responses to the behavioral questions. Individual R athletes self-reported binge eating (n=1), laxative, diet pill, and/or diuretic use (n=1), and losing more than 20 pounds in the past six months (n=5).

Table 4. 2 Eating Disorder Risk by Sport

	Cross-Country (*n*=8)	**Rugby** (*n*=10)	**Track and Field** (*n*=10)
EAT-26 scores	3.6±3.8	3.2±3.7	4.9±3.3
Affirmed extreme weight control behaviors (n)	0	7	0

Mean ± SD (all such values)
BMI, body mass index; kg, kilograms; cm, centimeters; %, percent
* Indicates significant difference ($p < 0.05$) between cross-country and rugby
† Indicates significant difference ($p < 0.05$) between rugby and track and field
‡ Indicates significant difference ($p < 0.05$) between cross-country and track and field

ASA-24 Food Recall

Total caloric, carbohydrate, fat, and protein consumption are shown in table 4.3. R had significantly lower caloric intake than CC (p=0.004). R also consumed significantly less carbohydrates standardized to their body mass (g/kg) than CC and TF (p=0.00, p=0.030, respectively). There were no differences in fat and protein intake between the three groups.

Table 4. 3 Dietary Characteristics by Sport

	Cross-country (*n*=8)	**Rugby** (*n*=10)	**Track and field** (*n*=10)
Dietary Energy Intake (kcal)	3,813±1,239*	2,402±589	3,117±647
Carbohydrate (kcal)	1797±525*	850±290	1385±350†
Carbohydrate (% of total intake)	48.06±8.97*	34.64±6.40	44.71±7.11†
Carbohydrate (g/kg)	7.11±2.92*	2.26±0.92	4.53±1.46†‡
Fat (kcal)	1389±691	959±288	1178±295
Fat (% of total intake)	34.80±8.80	40.82±10.35	37.90±6.18
Protein (kcal)	685±296	569±206	595±258
Protein (% of total intake)	18.61±7.77	23.58±5.85	18.79±6.50
Protein (g/kg)	2.71±1.44	1.53±0.64	1.94±0.93

Mean ± SD (all such values)
g, gram; kcal, kilocalorie; kg, kilogram; %, percent
* Indicates significant difference ($p < 0.05$) between cross-country and rugby
† Indicates significant difference ($p < 0.05$) between rugby and track and field
‡ Indicates significant difference ($p < 0.05$) between cross-country and track and field

Body Composition

Fat mass (FM), visceral adipose tissue (VAT), lean body mass (LBM), and appendicular skeletal muscle mass index (ASMI) are shown in table 4.4. There were no differences between CC and TF in FM, VAT, LBM, or ASMI (p=0.710, p=0.901, p=0.147, p=0.200, respectively). R had a greater amount of FM than CC and TF (p=0.003; p=0.012, respectively). One R athlete had VAT above 140 cm^2 and is considered high risk for cardiovascular disease (CVD). All the rest of the athletes had less than 105 cm^2 VAT and were low risk for CVD [60]. Additionally, R had significantly more LBM than CC (p=0.002). R had a greater ASMI than CC (p=0.002). Two CC runners had an ASMI less than 7.40 kg/m^2 and were considered high risk for sarcopenia [50].

Table 4. 4 Body Composition Variables by Sport

	Cross-country (*n*=8)	**Rugby** (*n*=10)	**Track and field** (*n*=10)
FM (kg)	8.25±1.37*	24.13±14.55	11.58±2.13†
FM (%)	12.54±1.01*	23.11±7.00	14.89±1.82†
VAT (cm^2)	45.66±4.34	100.96±85.86	56.37±10.14
LBM (kg)	54.71±6.57*	70.76±12.51	62.96±5.65
LBM (%)	87.46±1.01*	76.89±7.00	85.11±1.82†
ASMI (kg/m^2)	7.81±0.70*	9.78±1.58	8.71±0.56

Mean ± SD (all such values)
ASMI, appendicular skeletal muscle mass index; cm, centimeter; FM, fat mass; g, grams; kg, kilogram; LBM, lean body mass; m, meter; VAT, visceral adipose tissue
* Indicates significant difference ($p < 0.05$) between cross-country and rugby
† Indicates significant difference ($p < 0.05$) between rugby and track and field
‡ Indicates significant difference ($p < 0.05$) between cross-country and track and field

Bone Mineral Density

Whole-body (WB), left femur (LF), left femoral neck (LFN), and Lumbar Spine (LS) bone mineral density (BMD), and Z-scores are shown in table 4.5. Athletes with a z-score less than -1.0 and exhibited a secondary fracture are considered to have low BMD. None of the

athletes had a z-score less than -1.0 in the WB or in LF. One CC athlete had a z-score less than -1.0 in the LFN, and two CC runners had a z-score less than -1.0 in the LS. However, none of the athletes exhibited a secondary fracture were not classified to have low BMD. There were no differences between CC and TF in WB, LF, LFN, and LS BMD (p=0.147, p=1.000, p=0.069, p=0.59 respectively). R exhibited a greater amount of BMD than CC in WB (p=0.007), LFN (p=0.040), and LS BMD (p=0.020). R and TF had no differences in any of the BMD categories (WB: p=0.536, LF: p=0.902, LFN: p=1.000, LS: p=1.000).

Table 4. 5 Bone Mineral Density Variables by Sport

	Cross-country (*n*=8)	**Rugby** (*n*=10)	**Track and field** (*n*=10)
WB BMD (g/cm^2)	1.21±0.03*	1.32±0.07	1.28±0.09
Total z-score	0.29±0.32*	1.30±0.63	0.86±0.86
LF BMD (g/cm^2)	1.11±0.14	1.23±0.14	1.17±0.06
LF z-score	0.68±0.68	1.31±0.90	0.90±0.61
LFN BMD (g/cm^2)	0.99±0.08*	1.12±0.14	1.11±0.06
LFN z-score	0.39±0.71*	1.36±1.07	1.30±0.48
LS BMD (g/cm^2)	0.99±0.07*	1.14±0.07	1.12±0.16
LS z-score	-0.58±0.65*	0.67±0.53	0.51±1.21‡

Mean ± SD (all such values)
BMD, Bone Mineral Density; WB, whole-body; LF, left femur; LFN, left femoral neck; LS, lumbar spine
* Indicates significant difference ($p < 0.05$) between cross-country and rugby
† Indicates significant difference ($p < 0.05$) between rugby and track and field
‡ Indicates significant difference ($p < 0.05$) between cross-country and track and field

Salivary Testosterone

Table 4.3 shows the mean salivary testosterone (T) concentrations by sport. There were no differences between all groups in salivary T levels (p=0.0795). Keevil and colleagues (2016) assessed age-specific population distribution of salivary testosterone levels in male and females [61]. The mean salivary testosterone concentration for 18 to 24-year-old

males was 204.9±72.7 pg/mL. One R and two TF athletes had salivary testosterone levels below the 25th percentile (160.3 pg/mL) from Keevil's research [61].

Table 4. 6 Salivary Testosterone Concentrations by Sport

	Cross-country (*n*=8)	**Rugby** (*n*=10)	**Track and Field** (*n*=10)
T concentration (pg/mL)	239.6±47.3	229.5±53.2	250.9±95.3

Mean ± SD (all such values)
T, testosterone, pg, picograms; mL, milliliters
* Indicates significant difference ($p < 0.05$) between cross-country and rugby
† Indicates significant difference ($p < 0.05$) between rugby and track and field
‡ Indicates significant difference ($p < 0.05$) between cross-country and track and field

Correlations between Testosterone Concentrations and Bone Mineral Density

T concentrations were not significantly related to WB (r=0.351, p=0.067, r^2=8.3%) LF (r=0.254, p=0.192, r^2=6.5), LFN (r=0.237, p=0.226, r^2=5.6%), or LS BMD (r=0.331, p=0.085, r^2=11.0%) among all three groups. T levels were significantly related to WB (r=0.635, p=0.049, r^2=40.3%), LF (r=0.671, p=0.034, r^2=45.1%), and LFN BMD (r=0.686, p=0.028, r^2=47.1%) in R athletes. Figures 4.1 to 4.4 show Pearson correlations between T concentration and WB, LF, LFN, and LS BMD respectively.

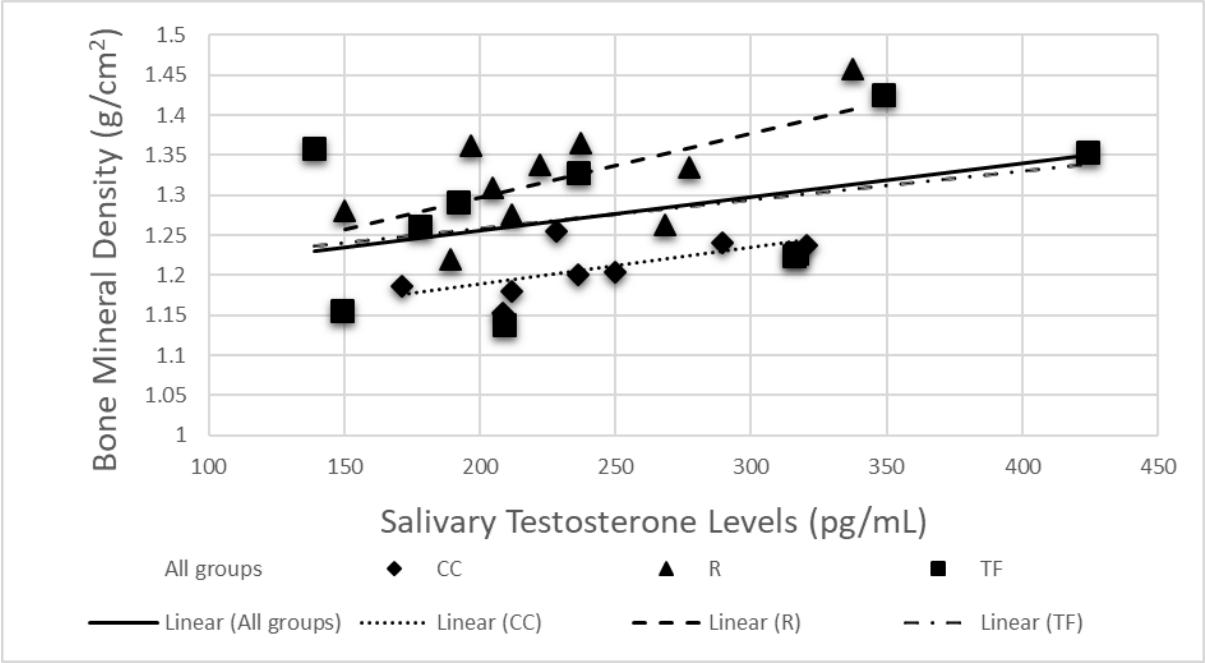

Figure 4. 1 Salivary Testosterone and Whole-body Bone Mineral Density
No significant relationship was observed between Salivary T and WB BMD among all groups (All groups: r=0.351, p=0.067, r^2=8.3%), CC (r=0.638, p=0.088, r^2=40.8%), and TF (r=0.376, p=0.285, r^2=14.1%). T had a moderately strong relationship with WB (r=0.635, p=0.049, r^2=40.3%) in R. Cm, centimeter; BMD, bone mineral density; T, testosterone; pg, picogram; mL, milliliter.

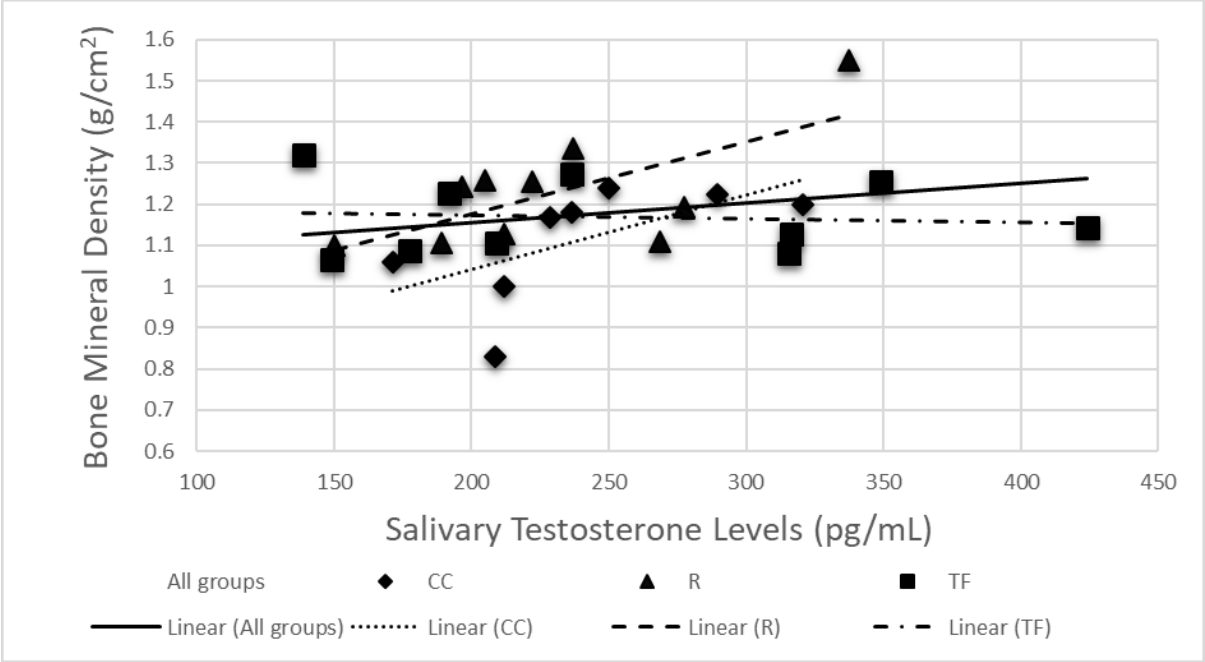

Figure 4. 2 Salivary Testosterone and Left Femur Bone Mineral Density
No significant relationship was observed between Salivary T and the LF BMD in all groups (r=0.254, p=0.192, r^2=6.5%), CC (r=0.610, p=0.108, r^2=37.3), and TF (r= -0.088, p=0.809, r^2=37.3%). There was a moderately strong relationship between T and LF BMD (r=0.671, p=0.034, r^2=45.1%) in R. Cm, centimeter; LF, left femur; BMD, bone mineral density; T, testosterone; pg, picogram; mL, milliliter.

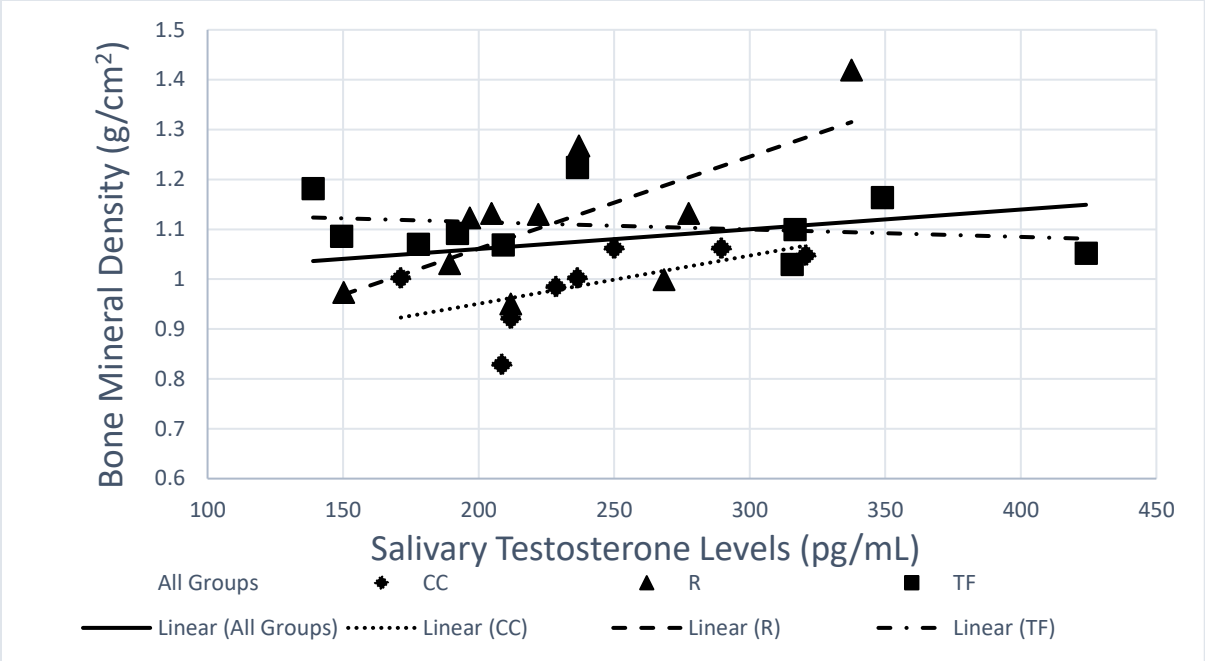

Figure 4. 3 Salivary Testosterone and Left Femoral Neck Bone Mineral Density
No significant relationship was observed between Salivary T and the LFN BMD in all groups (r=0.237, p=0.226, r^2=5.6%), CC (r=0.572, p=0.139, r^2=32.7%), and TF (r=0.572, p=0.139, r^2=5.2%). There was a moderately strong relationship between T and LFN (r=0.686, p=0.028, r^2=47.1%). Cm, centimeter; LFN, left femoral neck; BMD, bone mineral density; T, testosterone; pg, picogram; mL, milliliter.

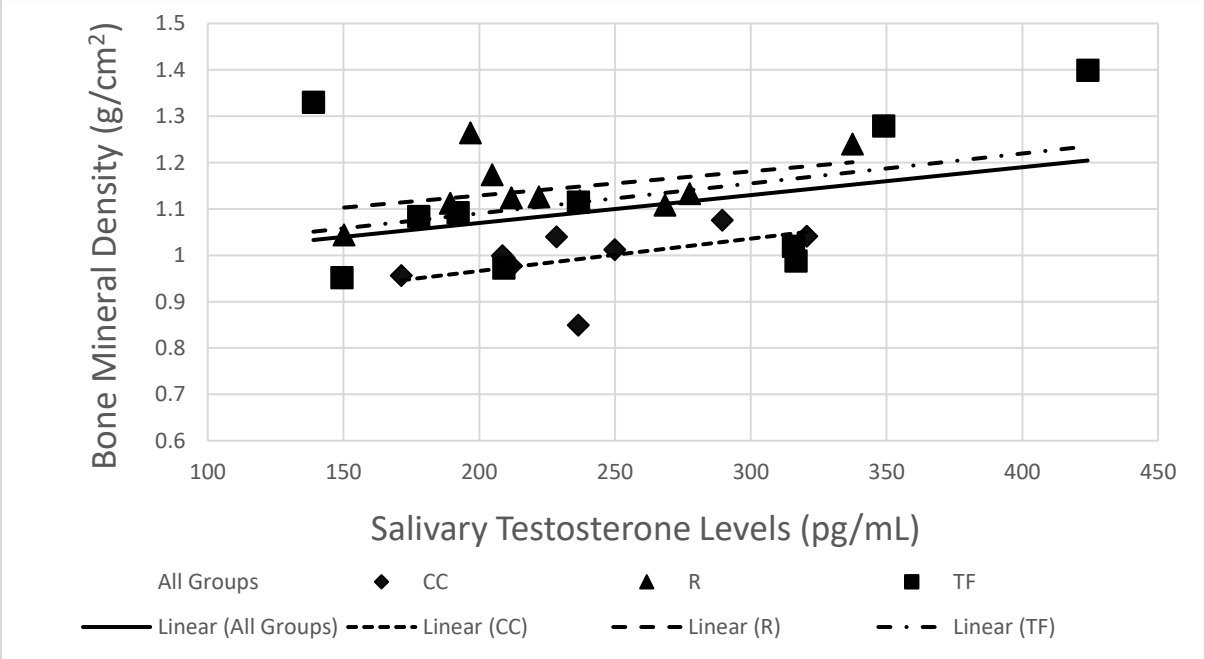

Figure 4. 4 Salivary Testosterone and Lumbar Spine Bone Mineral Density
No significant relationship was observed between Salivary T and the total LS BMD in all groups (r=0.331, p=0.085, r^2=11.0%), CC (r=0.473, p=0.237, r^2=22.4%), R (r=0.425, p=0.221, r^2=18.1%), and TF (r=0.388, p=0.268, r^2=15.1%). Cm, centimeter; LS, lumbar spine; BMD, bone mineral density; T, testosterone; pg, picogram; mL, milliliter.

Chapter 5: Discussion

In 2007, the female athlete triad was defined as a spectrum of low energy availability (LEA), menstrual dysfunction, and bone mineral density (BMD) [4]. Soon after, relative energy deficiency in sports (RED-S) was introduced because evidence suggested that LEA was negatively influencing female and male athletes and were disrupting a broader range of physiological functions [3,5,32]. The common parallels that were seen in male and female athletes were LEA, hypogonadotropic hypogonadism (HH), and low BMD [3]. Extensive research has been conducted on the associations between estrogen and menstrual dysfunction in female athletes, however, a lack of research exists that assesses the relationship between testosterone (T) and BMD in male athletes [3,4]. To our knowledge, only one other study has investigated the associations between Testosterone (T) and BMD in various male collegiate athletes [62]. Ackerman (2012) investigated the relationship between T, oestradiol, and lean body mass with BMD in male collegiate athletes and found that oestradiol and lean body mass were better predictors for bone health than T [62]. Our findings support Ackerman's study (2012) that T may not be a great predictor of bone health in male collegiate athletes.

Responses to the eating attitudes test (EAT-26) did not support previous research. Prior research detected that male athletes involved in sports that emphasize leanness including endurance, aesthetic, weight class, and anti-gravitation sports have an increased risk for eating disorders (ED) compared to sports that require greater body mass [3,63]. Seven rugby (R) athletes confirmed negatively to the behavioral questions and were considered high risk for ED meanwhile none of the cross-country (CC) or track and field (TF) athletes were considered at a high risk for ED. Five of the seven R athletes with increased risk for ED confirmed that they lost 20 pounds within the last six months. The R

team is a club sport and the level of commitment and the amount of expectations could be significantly lower compared to collegiate athletes. Collegiate athletes are expected to train throughout the year including the summer season as the majority of athlete return to their homes. Whereas club athletes have less expectations to train during the summer. The amount of weight gain during the summer and the amount of body weight loss right before their first competition might be much greater than collegiate athletes. If these Furthermore, the amount of resources and education provided to club athletes are likely to be limited compared to collegiate athletes and may increase their risk for ED in club athletes.

CC consumed significantly more calories (CC: 3,813±1,239 kcal, R: 2,402±589 kcal, TF: 3,117±647 kcal; p=0.004, p=0.159 respectively) and carbohydrates than R (CC: 1,797±525 kcal, R: 850±290 kcal, TF: 1,385±350 kcal; p=0.000, p=0.086 respectively). According to Burke and her colleagues (2001) athletes in general should be consuming 5-7 g/kg of carbohydrates per day [64]. Burke also advises endurance athletes to consume 7-10 g/kg of carbohydrates per day in order to replenish their glycogen storages [64,65]. The CC runners consumed an adequate amount of carbohydrates standardized to their body mass and significantly more than R and TF (CC: 7.11±2.92 g/kg, R: 2.26±0.92 g/kg, TF: 4.53±1.46 g/kg; p=0.000, p=0.030). On average, R and TF consumed less than 5 g/kg of carbohydrates. CC might be more educated than R and TF on the importance of carbohydrate consumption because of the necessity to replenish their glycogen storages to optimize their training and performance. This could explain why CC was the only group that met the carbohydrate recommendations.

Tarnopolski (2004) recommends that male athletes consume 1.2 to 1.7 g/kg of protein per day. Both CC and TF exceeded the protein recommendation (CC: 2.71±1.44 g/kg, TF:

1.94±0.93 g/kg). R consumed an acceptable amount of protein (R: 1.53±0.64 k/kg). There were no differences in protein and fat consumption between groups.

Tenforde and his colleagues (2016) discussed that low energy availability (LEA) may occur intentionally or advertently and may occur with or without ED [3]. However, if the R athletes were restricting caloric intake and were practicing extreme weight behaviors, they may have been considered to have reduced energy availability.

On average, both CC and TF had less than 16.7% body fat and were considered lean (CC: 12.54±1.01%, TF: 14.84±1.82%) [66]. R had significantly more percent body fat than CC and TF but were between 16.7% and 25.0% body fat and were categorized as normal on average (CC: 12.54±1.01%, R: 23.11±7.00%, TF: 14.84±1.82%; p=0.000, p=0.001 respectively) [66]. Three of the R athletes were over 25% body fat and were considered obese, but two of the R athletes were considered lean (<16.6% body fat). The range for body fat percentage was considerably small in CC and TF compared to R (CC: 10.5 – 13.7%, R: 16.0 - 36.4%, TF: 12.3 – 18.5%). The large range of body fat (%) in the R athletes could be explained by the wide range of different positions available on R teams. One R athlete was considered high risk for cardiovascular disease (CVD) due to their visceral adipose tissue (VAT) assessment. Even though the R forwards require a great amount of body mass, evidence has suggested that R athletes are considered to be low risk for CVD and usually do not exceed above 140 cm^2 VAT [19].

R had also a greater amount of LBM than CC (CC: 54.71±6.57 kg, R: 70.76±12.51kg, TF: 62.96±5.65 kg; p=0.002, p=0.146 respectively) but, R had significant less LBM percentage than CC and TF (CC: 87.46±1.01%, R: 76.89±7.00%, TF: 85.11±1.82%; p=0.000, p=0.001, respectively). This may be because the R forwards require a greater

amount of body mass which may include a greater amount of fat mass and lean body mass. There were 2 CC runners that were considered high risk for sarcopenia based on their appendicular skeletal muscle index (ASMI). To our knowledge, there is a lack of research that has investigated endurance runners and their risk for sarcopenia as they age.

The current study supports previous literature that endurance runners have significantly lower BMD than male athletes with greater body mass, especially at the trabecular region and LS [3,25,54,55]. Our findings also support the hypothesis that CC would have lower BMD than R, however our findings do not support the hypothesis that CC would have lower BMD than TF. There were no differences in CC and TF BMD at all sites. However, only three CC runners had z-scores less than -1.0. One CC runner had a z-score less than -1.0 in the left femoral neck (LFN), and two CC runners had a z-score less than -1.0 in the lumbar spine (LS). However, none of these CC runners were classified as low BMD because they did not have a secondary fracture. Therefore, none of the athletes were considered high risk for osteoporosis.

Previous literature has identified endurance runners and cyclists have exhibited decreased sex hormones compared to sedentary control groups [3,9,10,34,67]. However, in the current study, there were no differences in T concentration between the three groups. None of the CC runners were below the 25th percentile for salivary T levels, while two R (20%) and one TF (10%) athletes exhibited T below the 25th percentile from Keevil's study (2016) [61]. The hypothesis that CC would have lower T levels than R and TF was not supported by this study.

The current study provides insight on T levels in male athletes during the end of their off seasons and preparing to start their competitive seasons. However, it might have been

more beneficial to collect and analyze salivary T levels during the middle of their competitive seasons because this would be when they would be at their peak of their training. De Souza and Miller proposed a hypothesis that those who run more than 100 kilometers per week have an increased risk for T deficiency [3,43]. This CC group might have progressed their training throughout the season and a greater majority of the CC runners would be running more than 100 km per week during their competitive seasons than at the end of their off-season training. This may provide a better perspective if they would experience T deficiency while during competition.

It is unknown why the TF and R had a greater prevalence of athletes that were below Keevil's 25th percentile of testosterone levels (2016). However, no physiologic symptoms were observed even though they exhibited T below Keevil's 25th percentile. Further investigations are needed to examine TF and R athletes and their risk for T deficiency.

There were moderately strong associations between T and whole-body (WB), left femur (LF), and left femoral neck (LFN) BMD in R athletes. Our findings do not support the hypothesis that there would be a positive association with T concentrations and BMD while examining all the participants. However, this does support MacDougall's findings (1992) that there were no associations between serum T levels and BMD in endurance runners. Additionally, our findings also support Ackerman's evidence suggesting that T may not be a great predictor of bone health in male athletes. In fact, Ackerman (2012) found that oestradiol (estrogen steroid hormone) and LBM were better predictors of bone health in male athletes [62]. Oestradiol is a hormone synthesized within the adipose tissue and similar to T, oestradiol binds to androgen receptors on bone tissue. When oestradiol binds to these androgen receptors it signals for bone formation, however, it also signals for a decrease of

bone resorption [62]. Since R had a greater amount of fat mass, they may have had a greater production of oestradiol and could positively influence their BMD. LBM has been shown to positively influence BMD because it increases body mass and increases the load that is placed on the bones. This force upregulates bone formation and increases BMD [18,68]. In combination with a greater amount of fat mass and LBM, this may be one of the contributing factors why R exhibited a greater amount of BMD than the cross-country runners.

Saliva was collected and then assessed by Salimetrics for T levels. Previous research that assessed T levels in endurance runners collected serum T and this method was not plausible for this study. There is a 0.96 correlation between salivary and serum T levels [61]. However, salivary T is measured by picograms per milliliter and there are currently no cutoff points to determine T deficiency with this method.

There was a limited number of males who were sampled from the surrounding colleges. A post hoc power analysis was assessed, and 61 male athletes were needed in order to identify significance. While assessing the association between whole-body (WB) BMD and T levels, R was the only group that had a significant association between these two variables (R: r=0.635, p=0.049). However, CC had a stronger correlation between the two variables but was not considered significant (CC: r=0.638, p=0.088). With a greater number of male athletes, more significant findings may have been identified

Strengths include a validated and reliable mean to measure BMD via the dual-energy x-ray absorptiometry (DXA). DXA is the gold-standard for assessing BMD and was also used for body composition assessment. Furthermore, all the testing procedures occurred at specific times of the day (19:00-21:00 hours) to help ensure that participants came to the

lab close to the time they woke up, fasted for at least eight hours, and did not participate in any exercise for at least 12 hours for accurate basal T levels.

In conclusion, our finding does support the hypothesis that CC runners exhibit less BMD than male athletes with greater body mass. There are many variables that can influence BMD however it would be beneficial to identify risk factors to help decrease risks for low BMD. The primary aim for this study was to assess if there was a strong association between T and BMD and help determine if low T could be a risk factor for low BMD. However, our finding does not support the hypothesis that the CC runners would have a lower production of T than the TF and R athletes. It was not possible to assess T deficiency because currently there are not cut off points in salivary T measurements. This study is also hard to compare to other similar studies because other studies measured serum T to assess T deficiency in male endurance athletes. Furthermore, our findings support previous literature that testosterone levels may not be a great predictor of bone health in male athletes [62,69].

Since there are only significant associations between T levels and BMD in R, there may be other variables that are better predictors for bone health in male athletes. For example, the hormone, oestradiol, may be better than T in predicting bone health in collegiate male athletes [62]. Oestradiol is a female sex hormone that has an important role in females' menstrual cycles and bone health in female endurance runners [70,71]. Even though oestradiol is primarily a female sex hormone, oestradiol might have an important role in bone health in male athletes as well. Further investigations are needed to assess whether low energy availability (LEA) disrupts the oestradiol production and consequently negatively influences BMD in male athletes.

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Appendix A:

Informed Consent Form for Researching Involving Human Subjects

University of Idaho

Department of Movement Sciences

Informed Consent Form for Research Involving Human Subjects

Title: Prevalence of testosterone deficiency and correlation between testosterone deficiency and bone mineral density among male cross-country and rugby athletes.

Primary Investigator: Ann Brown, Ph.D., CISSN

Other Investigators: Chad Skiles

Participant's Printed Name:

_____

You are being asked to take part voluntarily in the research project described below. Please take your time in deciding. Before agreeing to take part in this research study, it is important that you read the consent form. Please ask the researcher to explain any words or information that you do not understand.

VOLUNTARY CONSENT

I voluntarily and without element of force or coercion, consent to be a participant in the research project entitled "Prevalence of testosterone deficiency and correlation between testosterone deficiency and bone mineral density among male cross-country and rugby athletes". This study is being conducted by Mr. Chad Skiles and Dr. Ann Brown, of the College of Education at the University of Idaho.

PURPOSE

The primary purpose of this study is to identify correlations between testosterone deficiencies and bone mineral density. The secondary purpose is to describe the difference in disordered eating, testosterone levels, bone mineral density, incidences of stress fractures in cross-country, rugby, and track and field athletes.

I must meet the following criteria to be included in the study: (1) current cross-country or rugby athlete between ages of 18-25, (2) have no contraindications to exercise based on the American College of Sports Medicine and American Heart Association (ACSM/AHA) risk stratification criteria including uncontrolled hypertension, currently taking blood pressure medications, or have been diagnosed with cardiovascular disease, stroke, diabetes, thyroid, or kidney dysfunction, (3) have no risk factors for cardiovascular disease as determined by ACSM guidelines, and (4) have no significant musculoskeletal injuries or other medical conditions over the past 6 months.

PROCEDURES

If you agree to take part in this study, the research team will ask you to attend 1 visits between 6:30-8:30 AM in the Human Performance Laboratory (HPL) within March and April. The 1 visit will take approximately 1 hour total and all measurements and assessments are described in detail below. You will record physical activity for 3 days (two week days & one weekend day). This will take you about 30 minutes. The total time for this study is 1 ½ hours over 1 week.
Measurements include: (1) survey information on medical history and eating disorder risk; (2) 24-hr food recall; (3) physical activity; (4) body composition; (5) Testosterone levels (Salimetrics, SalivaBio Oral Swab).

FIRST VISIT

Upon arrival to the HPL, the written informed consent and medical history questionnaire will be signed. I will then complete the Eating Attitudes Test (EAT-26) to assess eating disorder risk. I will have The Five-Day structured Training Log filled out prior or during the lab visit. I will then place a SalivaBio Oral Swab in my mouth so that the researcher can have a saliva sample. I will then be scanned by the DEXA for body composition and bone mineral density assessments. The ASA-24 login and password information will be given to me to complete the online dietary recall

Survey Information: Surveys will be used to gather information about medical history (including history of stress fractures) and eating disorder risk. This private information will be held in the utmost confidence. Surveys will not include your name and will be coded by a subject number to which only the researchers have access.

Height and weight: A measurement of your height and weight, without shoes, will be taken off.

Body composition with DXA: My body composition will be assessed via DXA scan. I will be asked to change into clothing that is free of metal and/or hard plastic (buttons, zippers, snaps, etc.) and asked to remove all metal from the body (jewelry, eyeglasses, hair accessories, etc). The body composition of my total body will be measured noninvasively via

the use of the Hologic DXA Scanner (Hologic Horizon™; Danbury, CT), with one scan; anteroposterior (AP) view of the total body lying supine. Very low doses of radiation are used; however, this test is non-invasive. Testing will be completed according to the manufacturer's instructions and specifications by a certified X-ray technician. My hands and feet will be secured in place to avoid unwanted movements during the body scan. The scan will take approximately 10 minutes to complete. From the scan, my lean mass (kg), fat free mass (kg), percent fat, and bone density will be determined.

Testosterone: My testosterone levels will be assessed via Salimetrics. I will take remove the wrapping and protective covering from the SalivaBio Oral Swab. I will then remove the SalivaBio Oral Swab from the Swab Storage Tube. I will place the SalivaBio Oral Swab in my mouth for 1-2 minutes, perferrably under the tongue. When the SalivaBio Oral Swab is soaked with saliva, I will place the SalivaBio Oral Swab back into the Swab Storage Tube and sealed with the cap of the Swab Storage Tube. The researcher will place the saliva sample in a freezer (-20 Celsius) until ready to be shipped to Salimetrics, LLC 5962 La Place Court Suite 275 Carlsbad, CA 92008.

Dietary Recall: Dietary intake will be measured using ASA-24, an online 24-hour dietary recall program for a total of 3 days (2 week days & 1 weekend day). You will be asked to maintain normal eating patterns and habits throughout the study.
I understand there is a minimal level of risk involved if I agree to participate in this study. Body composition will be evaluated by Dual-Energy X-ray Absorptiometry (DXA). This involves low exposure to radiation less than 5 mREMs per DXA scan. Doses received from DXA examinations are small in comparison to other common radiation sources and are believed to represent no significant health risk. No risk of adverse health conditions have been established for lower exposures of 5000 mREM or less. By comparison, natural background radiation is about 300 mREM/year, an x-ray of the spine is 70 mREM, a mammogram is 45 mREM, and a round trip transcontinental plane flight is 6 mREM. The measurement of body composition using DXA is non-invasive. For your safety, a research team member will be with you at all times during test procedures.
If I am identified to be at risk in any of the variables being tested I will be referred to the appropriate medical provider (i.e. campus dietician and/or counseling center).

POSSIBLE BENEFITS

You can gain knowledge of your body mass index, body composition, my bone mineral density (BMD), and testosterone levels. You may benefit by learning your disordered risk and if you should seek nutritional counseling. The benefit to society relates to a better understanding of the risk of hypogonadism and its effects on bone mineral density.

STATEMENT OF CONFIDENTIALITY

The results of this study may be published but my name or identity will not be revealed. Information obtained during the course of the study will remain confidential, to the extent allowed by law. My name will not appear on any of the results. No individual responses will be reported. Only group responses will be reported in the publications. Confidentiality will be maintained by assigning each subject a code number and recording all data by code number. The only record with my name and code number will be kept by the principal

investigator, Dr. Ann Brown, in a locked drawer in her office. Data will be kept for 10 years and then destroyed.

CONTACT INFORMATION FOR QUESTIONS OR CONCERNS

You may ask any questions you have now. If you have questions later, you may call Chad Skiles or Ann Brown at the number or email listed below.

Chad Skiles
Ph. (425) 312-8842
skil4827@vandals.uidaho.edu

Dr. Ann Brown
Ph. (208) 885-7986
afbrown@uidaho.edu

If you have questions or concerns about your participation as a research subject, please contact the University of Idaho Institutional Review Board (IRB) at (208) 885-6340.

SIGNATURE AND CONSENT TO PARTICIPATE IN RESEARCH

The nature, demands, benefits and risks of the study have been explained to me. I knowingly assume any minimal risk involved. I have read the above informed consent form. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of the benefits to which I may otherwise be entitled. In signing this consent form, I am not waiving my legal claims, rights or remedies. A copy of this consent form will be given to me.

Participant Name: _____

Participant Signature: _____ **Date:** _____ **Time:**

_____

I have discussed this research study with the subject and his or her authorized representative, using language that is understandable and appropriate. I believe I have fully informed the subject of the possible risks and benefits, and I believe the subject understands this explanation. I have given a copy of this form to the subject.

Signature of Investigator: _____ **Date:** _____ **Time:**

_____

Appendix B

Medical History Questionnaire

Human Performance Laboratory
University of Idaho
Department of Movement Sciences
Exercise Science & Health

HEALTH AND FITNESS HISTORY QUESTIONNAIRE

The following questions are designed to obtain a thorough preliminary medical history. The information you provide will help us to make the best determination about your eligibility for a particular study or other studies. Please answer all questions and provide as much information as you possibly can. This questionnaire, as well as any other medical information you provide will be kept confidential and will not be shared with any unauthorized person or organization unless you specifically request us to do so.

Name: _____

Street Address: _____

City, State, Zip code: _____

Telephone Number: H ( ) _____ W ( ) _____

Email address: _____

Date of Birth: _____ Age: _____
(mm/dd/yy)

Sex: M _____ F _____

Personal Physician's Name: _____ Phone: ( )_____

Address: _____

_____

Height _____in. _____ cm

Weight _____lb. _____ kg

Years of sport involvement: _____

Specialized event or position: _____

Race: _____

Signature: _____

Personal Health History:

Have you ever been hospitalized or had surgery? Yes____ No ____
Please list all hospitalizations and surgeries to the best of your recollection.

Hospitalized for Disease/Operation	Duration	Age when hospitalized

List any disease or illness you have had not listed above (e.g., pneumonia, strep etc.)

List any history of stress fractures (Year and location of stress fracture)

Health Concerns:

Are you currently seeing a doctor or other health care provider for any reason (psychological, hormonal, sleep apnea, acupuncture, chiropractor etc.)?

Yes_____ No_____

If yes, please explain:

Medical History:

1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

Y N High Blood Pressure

Please indicate last known reading:

Blood pressure: _____/_____

Y N High Cholesterol or High Triglycerides

Please indicate last known reading:

Cholesterol: _____

Triglycerides: _____

Y N Diabetes (Circle: Type 1 or Type 2)

Note: Type 1 diabetes is insulin-dependent diabetes mellitus. It is typically diagnosed at an early age and requires insulin shots or an insulin pump immediately upon diagnosis. Type 2 diabetes is often diagnosed at an older age (past age 20) and is usually initially treated with changes in diet and/or medication (pills).

Y N Hypoglycemia (low blood sugar)

Y N Asthma (regular or exercise induced)

2. Have you ever had a glucose tolerance test? Y N

If yes, what were the results?

3. Have you ever had a fasting blood sugar test? Y N

If yes, what were the results?

4. Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease (heart attacks, stroke, etc.)? Please explain:

5. Do you have any neurological problems including fainting, dizziness, headaches or seizures?

6. Please list all past injuries that may affect your ability to perform exercise:

7. Do you smoke or use smokeless tobacco? Y N

If yes, how many cigarettes per day? _____

8. Do you drink coffee or other caffeinated beverages? Y N

What kind, how much and how often?

9. Please list all vitamins, minerals and herbs and other nutritional (performance) supplements as well as medications you are currently taking. How long have you been taking them and how frequently?

12. What changes have you made in your diet in the last 6 months?

13. Do you exercise regularly outside of your sport? Y N

14. How often do you have required practice or training? Please be detailed in a description of your average week of training.

15. Please list the 3 most current games/tournaments that you have participated in and when they occurred:

1) _____

2) _____

3) _____

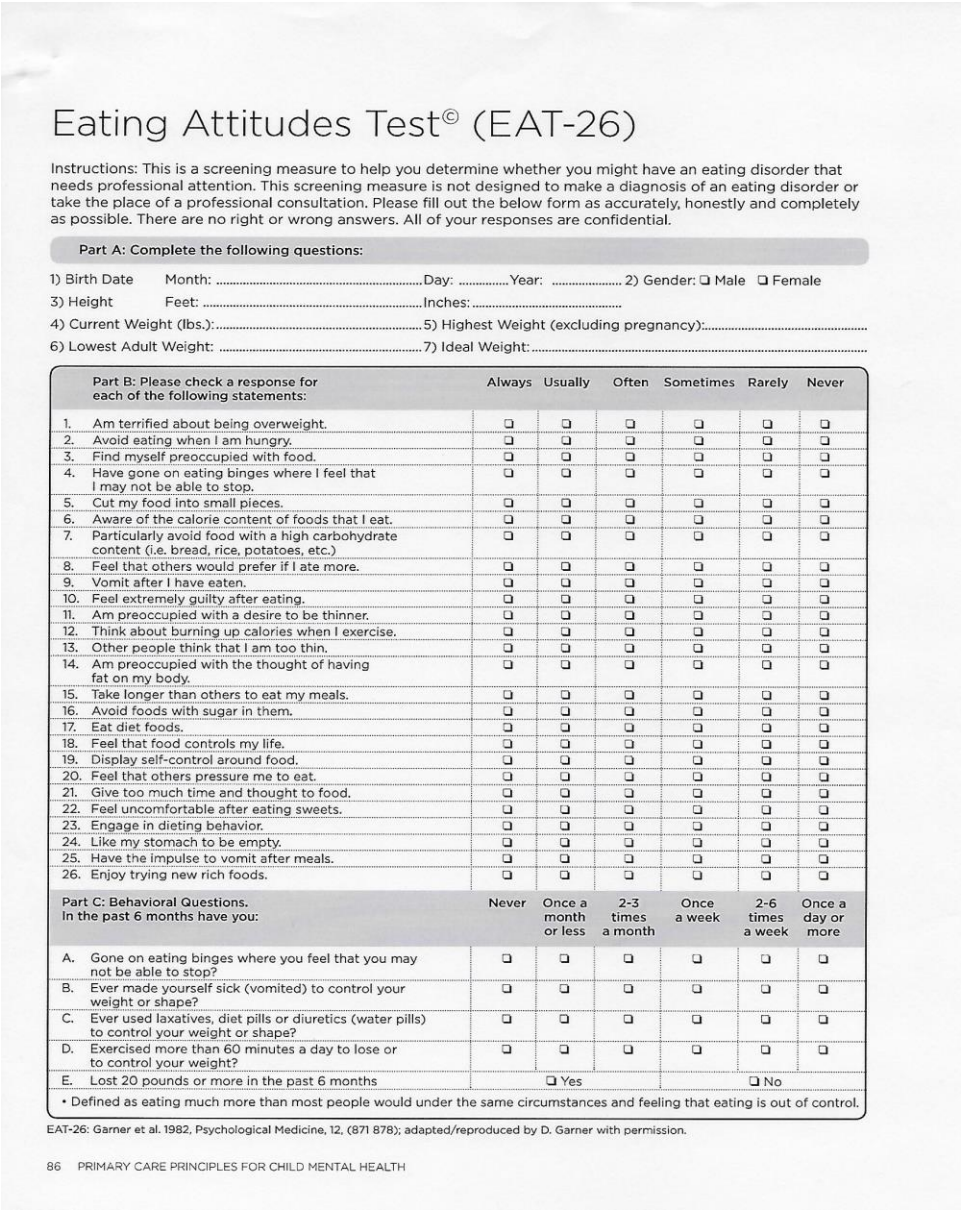
16. How does your current exercise and physical activity compare to 6 months ago? 1 year ago?

17. Have you had a physical exam in the past 2 years? Y N

Please describe your assessment of your overall health:

Appendix C

EAT-26



Eating Attitudes Test© (EAT-26)

Instructions: This is a screening measure to help you determine whether you might have an eating disorder that needs professional attention. This screening measure is not designed to make a diagnosis of an eating disorder or take the place of a professional consultation. Please fill out the below form as accurately, honestly and completely as possible. There are no right or wrong answers. All of your responses are confidential.

Part A: Complete the following questions:

1) Birth Date Month: ..... Day: ..... Year: ..... 2) Gender: ❑ Male ❑ Female

3) Height Feet: ..... Inches: .....

4) Current Weight (lbs.): ..... 5) Highest Weight (excluding pregnancy): .....

6) Lowest Adult Weight: ..... 7) Ideal Weight: .....

Part B: Please check a response for each of the following statements:	Always	Usually	Often	Sometimes	Rarely	Never
1. Am terrified about being overweight.	❑	❑	❑	❑	❑	❑
2. Avoid eating when I am hungry.	❑	❑	❑	❑	❑	❑
3. Find myself preoccupied with food.	❑	❑	❑	❑	❑	❑
4. Have gone on eating binges where I feel that I may not be able to stop.	❑	❑	❑	❑	❑	❑
5. Cut my food into small pieces.	❑	❑	❑	❑	❑	❑
6. Aware of the calorie content of foods that I eat.	❑	❑	❑	❑	❑	❑
7. Particularly avoid food with a high carbohydrate content (i.e. bread, rice, potatoes, etc.)	❑	❑	❑	❑	❑	❑
8. Feel that others would prefer if I ate more.	❑	❑	❑	❑	❑	❑
9. Vomit after I have eaten.	❑	❑	❑	❑	❑	❑
10. Feel extremely guilty after eating.	❑	❑	❑	❑	❑	❑
11. Am preoccupied with a desire to be thinner.	❑	❑	❑	❑	❑	❑
12. Think about burning up calories when I exercise.	❑	❑	❑	❑	❑	❑
13. Other people think that I am too thin.	❑	❑	❑	❑	❑	❑
14. Am preoccupied with the thought of having fat on my body.	❑	❑	❑	❑	❑	❑
15. Take longer than others to eat my meals.	❑	❑	❑	❑	❑	❑
16. Avoid foods with sugar in them.	❑	❑	❑	❑	❑	❑
17. Eat diet foods.	❑	❑	❑	❑	❑	❑
18. Feel that food controls my life.	❑	❑	❑	❑	❑	❑
19. Display self-control around food.	❑	❑	❑	❑	❑	❑
20. Feel that others pressure me to eat.	❑	❑	❑	❑	❑	❑
21. Give too much time and thought to food.	❑	❑	❑	❑	❑	❑
22. Feel uncomfortable after eating sweets.	❑	❑	❑	❑	❑	❑
23. Engage in dieting behavior.	❑	❑	❑	❑	❑	❑
24. Like my stomach to be empty.	❑	❑	❑	❑	❑	❑
25. Have the impulse to vomit after meals.	❑	❑	❑	❑	❑	❑
26. Enjoy trying new rich foods.	❑	❑	❑	❑	❑	❑

Part C: Behavioral Questions. In the past 6 months have you:	Never	Once a month or less	2-3 times a month	Once a week	2-6 times a week	Once a day or more
A. Gone on eating binges where you feel that you may not be able to stop?	❑	❑	❑	❑	❑	❑
B. Ever made yourself sick (vomited) to control your weight or shape?	❑	❑	❑	❑	❑	❑
C. Ever used laxatives, diet pills or diuretics (water pills) to control your weight or shape?	❑	❑	❑	❑	❑	❑
D. Exercised more than 60 minutes a day to lose or to control your weight?	❑	❑	❑	❑	❑	❑
E. Lost 20 pounds or more in the past 6 months		❑ Yes			❑ No	

• Defined as eating much more than most people would under the same circumstances and feeling that eating is out of control.

EAT-26: Garner et al. 1982, Psychological Medicine, 12, (871 878); adapted/reproduced by D. Garner with permission.

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