Thesis Research

Investigating Relative Energy Deficiency in Male Endurance Trained Athletes

By

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ABSTRACT

The Female Athlete Triad and Relative Energy Deficiency in Sport is a well-studied interrelationship between bone mineral density (BMD), hormone disruption and low energy availability in female athletes. Standards for identification, treatment, and prevention have been established for female athletes. Research suggests that a similar phenomenon is occurring in male athletes. However, due to the different physiologies of males and females, the vast knowledge and findings surrounding female athletes cannot be directly applied to male athletes. The purpose of this study is to act as a preliminary study investigating the relationship between percentage body fat, BMD, and hormone levels in collegiate endurance athletes. In addition, we examined these relationships by level of adiposity. Twenty subjects enrolled in the study and 19 subjects met the inclusion and exclusion criteria. Out of 19 subjects, 5 subjects had missing data and were not included in the analyses resulting in 14 subjects for analyses. No relationship was found between percentage body fat and BMD or between percentage body fat and levels of hormones. The median percentage body fat was calculated and two groups were created: (1) ≤50th percentile; and (2) >50th percentile. In subjects with a percentage body fat below the median, an inverse correlation between serum testosterone and dual femur z-score ($r = -0.76$, $p = 0.05$) was found. A borderline significant inverse correlation between serum testosterone and AP spine z-score ($r = -0.74$, $p = 0.06$) was also detected. Insulin like growth factor-1 (IGF-1) was positively correlated to AP spine z-score ($r = 0.82$, $p = 0.02$). Additionally, a strong correlation between IGF-1 and dual femur z-score ($r=0.69$, $p= 0.09$) was observed. Relationships were also detected in athletes with a percentage body fat above the median. Serum testosterone was strongly correlated to AP spine z-score ($r = 0.84$, $p = 0.19$). The relationship between serum testosterone and dual femur z-score approached significance ($r = .072$, $p = 0.07$). No

relationships between cortisol and dual femur or AP spine z-score was found in either percentage body fat group. In conclusion, no relationship was found between percentage body fat and BMD or between percentage body fat and levels of hormones. Relationships were detected when examining these correlations in athletes with a percentage body fat ≤50th percentile or >50th percentile. Further studies with a larger sample size are needed to understand these relationships.

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CHAPTER 1: JUSTIFICATION

Introduction

Physical preparedness for athletes is essential for success in their field of play. Body composition is used as a benchmark to assess an athlete's physical preparedness for competition (1, 2). Specific body composition goals are dependent upon the demand of the sport and position of the athlete. In certain sports, such as gravitation sports or weight class sports, leanness is desired. However, a fine line exists between being lean for sport and negative health outcomes due to being too lean. This phenomenon has historically been well studied in female athletes as the Female Athlete Triad (1-3).

As the number of women participating in sports increased, sports health care providers began to notice an increase in the incidence of interrelated health concerns. In 1997, the American College of Sports Medicine (ACSM) published their hallmark consensus statement on the Female Athlete Triad. It was originally defined as the interrelationship between disordered eating, amenorrhea, and osteoporosis (4). Since its publication, additional research, awareness, and knowledge has emerged surrounding the Female Athlete Triad (5, 6). In 2007, the ACSM revised their consensus statement stating that all three components of the Female Athlete Triad exist on a spectrum from a euphoric state to a clinical diagnosis. Osteoporosis was no longer a defining factor and was revised to suboptimal bone health, and disordered eating was revised to "low energy availability with or without an eating disorder." Another notable change was that bone health and amenorrhea appeared to rest upon low energy availability. The ACSM provided standards for identification, treatment, and prevention within athletes. However, these standards focused only on female athletes (7).

In 2014, the International Olympic Committee (IOC) renamed the Female Athlete Triad as Relative Energy Deficiency in Sport (RED-S). Citing a spoke and wheel concept that bone mineral density (BMD), hormone disruption, and many other physiological issues found in the traditional Female Athlete Triad were a result of underlying RED-S (Appendix A: RED-S Spoke and Wheel Concept Diagram). Until RED-S is reversed, symptoms will not subside. Additionally, the IOC alludes to the existence of RED-S in male athletes as well (8).

While the ACSM and IOC continue to have conflicting views on the Female Athlete Triad and RED-S, they both suggest that a male equivalent may exist, but more research is needed (7-9). Additional studies suggest likewise as well (10-18). A meta-analysis on current research of the Male Athlete Triad and RED-S in male athletes suggests that strong evidence has been found supporting the existence in males (6).

The type of training an athlete does elicits different responses within the body, which may contribute to the development of RED-S. Strength training elicits an anabolic response, while long term endurance training elicits a catabolic response. An early study found high volume endurance athletes experience significantly decreased testosterone levels compared to lower volume endurance athletes (10). In a study comparing the effects of short term and long-term endurance training, insulin like growth factor-1 (IGF-1) levels were observed to be increased during short-term training periods and decreased during long-term endurance training when compared to baseline levels (19, 20).

Endurance athletes were also observed to have decreased testosterone when compared to untrained and sedentary males (12, 13). In a study of Swedish male Olympic athletes, athletes participating in leanness sports were found to have decreased percentage body fat, leptin, and free testosterone levels. Percentage body fat was positively correlated to testosterone levels (14).

Another study found that male athletes who ran increased mileage per week experienced increased bone turnover and a decrease in BMD in the lumbar spine when compared to males running less mileage (15). These studies suggest a relationship between percentage body fat, hormone disruption and BMD within male athletes. Currently, there are no studies that analyze all three components of RED-S in male athletes (6). Therefore, more research is needed.

Statement of Purpose

The purpose of this study is to act as a preliminary study investigating the relationship between percentage body fat, BMD, and hormone levels in collegiate endurance athletes. This thesis project will inform the body of literature on the relationship between percentage body fat, BMD, and hormone levels. The findings of this study will increase the knowledge of the field and identify new avenues for additional research to be explored, with the ultimate goal of establishing proper identification and treatment protocols for at risk athletes.

Research Questions

1. What is the relationship between percentage body fat and BMD in male endurance trained athletes?
2. What is the relationship between percentage body fat and levels of cortisol, serum testosterone, and IGF-1?
3. What is the relationship between BMD and levels of cortisol, serum testosterone, and IGF-1 in endurance trained athletes with a percentage body fat below and above the median?

CHAPTER 2: REVIEW OF LITERATURE: THE UNDERSTANDING OF THE FEMALE ATHLETE TRIAD AND RELATIVE ENERGY DEFICIENCY IN SPORTS AND EVIDENCE FOR A MALE ATHLETE EQUIVALENT

Introduction

Physical preparedness and body composition level are essential aspects of sports that allow athletes to perform at an elite level. Body composition is used as a benchmark to assess an athlete's readiness for competition and likelihood for success (1, 2). Specific body composition goals are dependent upon the demand of the sport and position of the athlete. In certain sports, such as gravitation sports or weight class sports, leanness is desired. However, there is a fine line between optimal body composition in leanness sports and undesired health consequences (1, 2).

Athletes with low body fat levels may experience both short-term and long-term health consequences in addition to a decrease in performance. The relationship between low fat levels and negative health consequences has been most frequently studied within female athletes as the Female Athlete Triad and RED-S (1-3).

The Female Athlete Triad and RED-S are the interrelationship between low energy availability, hormone disruption, and decreased BMD (3). Studies suggest that these negative health outcomes are due to low percentage body fat and are more common in females. However, new research suggests that there may be a male equivalent (3, 6-8, 10-18, 21-24).

Currently, minimal research exists on RED-S in males. Therefore, it is important to first understand the development the Female Athlete Triad and RED-s in female athletes, in order to develop a similar understanding in the preliminary studies in males. The purpose of this literature review is to establish the progression of understanding of the Female Athlete Triad and RED-S and review suggested implications in male athletes.

In order to provide proper care of athletes more research and knowledge is needed to fully understand this phenomena occurring in males. Once an understanding of RED-S in males is completed, parameters for identification and treatment are needed just as has been established in females (3, 8).

The study purpose was to investigate the relationship between percentage body fat, BMD, serum testosterone, cortisol, and IGF-1 levels to provide preliminary results for larger studies. My hypothesis is that there will be a positive correlation between percentage body fat and BMD in the spine. I hypothesize that a positive correlation will be found between percentage body fat and testosterone and IGF-1 levels, and a negative relationship will be found between percentage body fat and cortisol levels. I hypothesize that a positive relationship will be found between BMD and serum testosterone and IGF-1, and a negative relationship in BMD and cortisol in the group of athletes with a percentage body fat below the median. I also hypothesize that in the group of athletes with a percentage body fat above the median, if the relationships between BMD and hormone levels exist, they will be weaker than the relationships observed in the group of athletes with percentage body fat below the median.

The Female Athlete Triad

Defining the Female Athlete Triad

No definition of a male athlete triad or RED-S exists for males. Therefore, I will provide background information on females where the disorder has been well characterized. As the number of females participating in sports increased, an association of disorders emerged related to inadequate energy intake, hormone disruption, and BMD issues. This has since become known as “The Female Athlete Triad” (1-4).

The Female Athlete Triad was originally defined by the ACSM in 1997 as disordered eating, amenorrhea, and osteoporosis, resulting in increased risk for injury and long-term morbidity (3, 4). Disordered eating was described as a range from restriction to a Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnosis of a clinical eating disorder. The ACSM also alluded to energy availability, describing it as the energy from consumption that is leftover after the cost of energy for exercise.

Amenorrhea could exhibit itself through primary or secondary amenorrhea. Primary amenorrhea is described as the absence of menstruation by the age of 16. Secondary amenorrhea as the absence of three or more consecutive menstrual cycles. Reversal of amenorrhea was commonly seen after injury, reduced training, or increased calorie intake. Although exact etiology is unknown, amenorrhea may be a result of low percentage body fat.

Osteoporosis is the progressive loss of mineral content in bone leading to porous bones. Osteoporosis is clinically diagnosed when BMD is more than 2.5 standard deviations below the norm for their gender. Loss of BMD is observed as a result of amenorrhea and a hypoestrogenic state. The loss of BMD increases the athletes' risk for stress fractures during training.

The conclusion of the consensus states, now that the Female Athlete Triad has been defined, research should continue in all areas related to the Triad. Additionally, psychological factors, prevalence, warning signs, and treatment should be included in further research (4).

Awareness surrounding the Triad has grown since the hallmark consensus statement first describing the Female Athlete Triad was released in 1997. Hundreds of studies have been conducted on female athletes and the associated risks of the Triad to advance the knowledge and treatment of the Female Athlete Triad.

Revision to the Female Athlete Triad

In 2007, the ASCM revised their statement based on current research regarding the Female Athlete Triad making the diagnostic criteria less exclusive. The revised statement excluded the term "disordered eating" and replaced it with "low energy availability with or without an eating disorder." The second and third elements of the original triad were revised to include a range, euphoric menstruation to amenorrhea, and optimal BMD to osteoporosis, respectively (3, 4, 7). This consensus provided screening and treatment recommendations for athletes at increased risk. In addition, instead of three separate scenarios within the triad, low energy availability was named as the underlying issue on which the other two elements, hormone disruption and BMD, rests (3, 4, 7, 8).

Major organizations involved in the treatment and care for athletes such as the ACSM, the IOC, National Athletic Training Association (NATA), and the Academy of Nutrition and Dietetics (AND) have all recognized the Female Athlete Triad and developed standards for identification, prevention, and treatment involving a multidisciplinary team. All organizations suggest that a similar triad may exist in male athletes, although with decreased frequency. However, little is known about a triad in males due to the limited research available (4, 6-8, 25, 26).

Relative Energy Deficiency in Sport

In 2014, the IOC revised their consensus statement on the Female Athlete Triad, stating that relative energy deficiency is the etiology of the Female Athlete Triad. Relative energy deficiency occurs when energy intake does not meet the demands of an individual's total energy expenditure that would allow them to maintain their health, activities of daily living, growth, and

activities related to their sport. The IOC proposed to rebrand the Female Athlete Triad as RED-S (8).

Based on the research presented by the IOC, the committee proposed that the more appropriate and more inclusive name for the Female Athlete Triad is Relative Energy Deficiency in Sport (RED-S) (4, 7, 8, 27).The IOC proposed a spoke and wheel concept where all symptoms stem from RED-S (Appendix A: RED-S Spoke and Wheel Concept Diagram). This new concept expands beyond just BMD and hormone disruption to include disturbances in metabolic rate, immune function, and cardiovascular health. This change not only broadens the scope and impact of RED-S, but poses the potential for a male athlete equivalent to RED-S, despite limited comprehensive research on the topic (8, 27).

Consistencies between the Female Athlete Triad and RED-S

The IOC proposal of RED-S is not without controversy. The ACSM wrote a rebuttal stating that the Female Athlete Triad has more research and is better known and recognized across health care providers in sports (9). The IOC responded by stating that the Female Athlete Triad was overdue for a rebrand because research suggests that the Triad extends beyond just female athletes. The IOC further explained that the name "Female Athlete Triad" excludes males, non-athletes such as recreational athletes, and limits the negative health consequences of low energy availability (27). Despite their disagreements, both the IOC and ACSM recognize the short-term and long-term negative health consequences. Both suggest that a male equivalent exists but is not fully understood (6-9, 27). It is evident that more research is needed to provide a clearer understanding of the disturbances in males.

Overall, the Female Athlete Triad and RED-S decreases an athlete's ability to perform at their highest level, can sideline athletes from competitive events, and potentially end their athletic career (1-3, 5, 7, 8, 25). The ASCM and IOC both place strong emphasis on screening, identification, and treatment of RED-S through development of protocols. The developed protocols mainly focus on identifying and treating female athletes, based on the large pool of knowledge surrounding the Female Athlete Triad (7, 8). While there is a strong history, knowledge, and awareness of the Female Athlete Triad, there is limited research on the effects of RED-S in male athletes in comparison (3, 6-8).

Relative Energy Deficiency in Males

Research on male athletes' body composition, hormone disruption, and bone density exists, but is discontinuous. In the early 1990s there were a number of studies examining aspects of RED-S in male athletes. This increase in research coincided with the increase of research in female athletes for the first ACSM consensus statement (4). After the first consensus statement there was a lull in the research conducted among male athletes for over 10 years. Research slowly resumed in the late 2000s and early 2010s. The most recent research has likely been a response to the revised ACSM statement in 2007 and the IOC Consensus Statement describing RED-S in 2014 (7, 8).

Body Composition in Male Athletes

Aspects of an athlete's physical preparedness can be determined through their body composition. Body composition assesses what constitutes an individual's weight – muscle, fat, and bone tissue. Based on the demand of the specific sport or position within the sport, the ideal

body composition changes (1, 2, 8, 28). Certain sports, such as the offensive line of a football team, value size and mass over specific muscle to fat ratios. However, in weight class sports such as wrestling or gravitational sports such as distance running, achieving the ideal body composition is essential for elite level competition. For gravitational sports, optimizing the weight on the scale to produce an ideal muscle to fat ratio, thus minimizing the nonfunctional weight, can help aid in performance (1, 2, 28).

Humans have a certain level of essential fat. Essential fat is the amount of fat needed for the body to properly function and carry out necessary physiological functions. Males have much lower essential body fat compared to females, roughly 2-5% and 10-13%, respectively. A large part of the discrepancy between the essential fat of males and females is due to the increased energy needed to maintain reproductive function in females (1, 2, 28).

Body composition can be assessed using a variety of methods. Dual Energy X-Ray Absorptiometry (DXA) scans, originally designed to assess BMD, are an effective tool at estimating body composition (1, 2). DXA scans are a valid and highly accurate method to assess athlete's body composition. Using accurate techniques are crucial since the smallest change in body composition, which initially may appear to be insignificant, could be an indication of an underlying health issue that has not yet been detected.

The typical body fat percentage range for male athletes is 6-13%, while female athletes tend to be within 14-20% body fat. As male and female athletes' body composition nears essential body fat percentage, negative health issues tend to arise as observed in the Female Athlete Triad and RED-S (8). The lower range of essential fat for males contributes to a lower incidence of RED-S in male athletes compared to female athletes (1, 2, 28). As athletes attempt to push the limit by decreasing unnecessary fat, there is a fine line between high performance

and sacrificing overall health, most notably researched through the Female Athlete Triad (1-3, 7-9, 28). Traditionally, male athletes competing in endurance sports have lower percent body fat. Studies have found that male endurance athletes have decreased immune function and increased risk for injury, as described in RED-S by the IOC, suggesting that body composition plays a key role for RED-S in both male and female athletes (10, 14, 16).

Current Literature on RED-S in Male Athletes

Recently, strong anecdotal evidence suggests that although, not as prominent in males as females, a male equivalent to the Female Athlete Triad exists (3, 8). Males participating in sports that emphasize leanness have been observed to have similar manifestations as female athletes with stress fractures and hormone disruption. Most studies conducted on male athletes have been relatively small and only include one to two elements of RED-S (3, 6, 7, 25, 26).

Based on studies of the Female Athlete Triad, low energy availability has a myriad of effects on an individual's metabolism. The IOC states that RED-S impacts many physiological functions including an individual's hormone production, BMD, metabolic rate, protein synthesis, cardiovascular, and psychological health (8).

Male Hormones

When applying concepts of the Female Athlete Triad and RED-S to the male population, one major complexity faced is the differing physiologies between the two genders. Major hormonal differences exist between males and females, therefore the understanding of the disruption of female hormones cannot be directly applied to males (8, 9). Rather, the functions of

male hormones must be understood in order to adequately understand the Female Athlete Triad and RED-S when applied to males.

Testosterone

Testosterone is the primary androgen hormone responsible for the development of male sex characteristics (1, 28). In males, it is primarily produced in the testicles and is regulated by the hypothalamus and pituitary gland. The hypothalamus stimulates the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the pituitary gland. LH stimulates the production of testosterone in the testes. If the body senses that testosterone levels are too high, the pituitary gland decreases the amount of LH released, thereby decreasing the production of testosterone (28).

In addition to its key role in development of male sex characteristics and sperm production, testosterone is an anabolic hormone that is primarily responsible for the development of muscle tissue and maintenance of BMD (1, 28). Testosterone is a precursor to the hormone estradiol in males. Estradiol regulates BMD in males in a similar manner to females. Testosterone promotes muscle tissue development by increasing intracellular amino acid utilization, thus decreasing protein degradation. Even without resistance training, testosterone increases lean body mass.

Testosterone with resistance training only amplifies the effect of lean body mass development (1, 28). Resistance training also increases serum testosterone concentration. Testosterone levels may be decreased in males due to disruptions with the pituitary gland, typically in the form of a tumor, aging, medications, and testes-based conditions such as severe injury, chemotherapy, or radiation (1, 28). Studies suggest that subclinical decreases in serum

testosterone may be a result of decreased percentage body fat (10, 12-15). In testosterone deficient males, effects of testosterone supplements can be observed within 3 months of administration (1, 2).

Cortisol

Cortisol is a glucocorticoid, a stress hormone. When the body undergoes stress from a physical activity, the hypothalamus releases corticotropin relapsing factor, which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) causing the adrenal cortex to produce cortisol (28). The main effect of cortisol is to increase the energy available to the body. Cortisol has a catabolic effect, supporting gluconeogenesis by stimulating lipolysis and proteolysis, increasing the release of glucagon and decreasing the potency of insulin. Cortisol also decreases immune function and places the body into negative calcium balance (28).

Cortisol levels vary greatly throughout the day. Cortisol levels are higher in the morning and gradually decrease throughout the day (1, 2). Exercise increases cortisol production (1, 2, 28).

Prolonged physical activity increases cortisol production over time, and some endurance athletes experience “hypercortisolism”. Chronically elevated cortisol levels in individual increase mobilization of protein and fat for energy, decreasing individuals lean body mass and adiposity (28).

IGF-1

IGF-1 is similar in structure to insulin and is a member of the Growth Hormone (GH) family. GH is a group of hormones whose primary physiological function is to increase cellular

proliferation and protein synthesis. As GH is released, the liver increases production of IGF-1. IGF-1 decreases carbohydrate metabolism and increases lipolysis. IGF-1 increases with age reaching its peak in puberty and plateauing in adulthood.

Physical activity increases IGF-1, while increased levels of cortisol has been found to suppress the production of IGF-1 (28). In IGF-1 deficient individuals, effects of administered IGF-1 can be observed as quickly as in one to two months, up to 6 months (1, 2).

Hormonal Response to Resistance Training

Hormonal response within the body differs based on the type of training performed. Resistance or strength training, training designed to increase muscle mass by utilizing resistance, produces an anabolic effect (1, 2, 28). Resistance training increases testosterone, GH, and IGF-1 levels (1, 28). Cortisol is increased for the force production needed during exercise, but declines with recovery (28).

Hormonal Response to Endurance Training

Endurance training is designed to increase cardiovascular and respiratory capacity in aerobic and anaerobic activity. Endurance training decreases testosterone and IGF-1 and increases cortisol levels (28). The decrease in anabolic hormones and increase in cortisol places the body into a catabolic state, mobilizing protein and adipose tissue for energy and stimulates bone resorption (1, 28).

The increase in cortisol also suppresses the immune system. The catabolic state, bone resorption, and immune suppression are all outcomes of RED-S. Suggesting that endurance training may increase an individual's risk for development of RED-S (28).

Early studies proposed a "Volume Threshold Hypothesis" to explain the hormonal response to endurance training. The research found that as the volume of training in male runners is increased, male athletes experience subclinical alterations to their reproductive system. Serum testosterone is significantly decreased with high volume training compared to lower volume groups. Despite a statistically significant decrease in serum testosterone, laboratory values did not fall below normal limits. The study found no difference in cortisol levels of high volume trained males (10). The observed decrease in hormone levels of male distance runners in the volume threshold hypothesis study, parallels the decrease in hormone levels seen in female distance runners (10, 24). Although the exact mechanism has not been identified, decreased testosterone levels were associated with decreased percentage body fat. A possible explanation is that these athletes may have been in negative energy balance with increased training, and experiencing RED-S (10).

A study examining the effects of growth hormones in response to both short-term and long-term endurance training found consistent results with the Volume Threshold Hypothesis study. Decreased levels of IGF-1 and insulin like growth factor binding protein 5 (IGFBP-5) was observed with long term endurance training in females (20). A study on professional cyclists found similar results with the response to IGF-1 and extensive training. After 1 week of intense endurance training IGF-1 levels were increased compared to baseline, but at week 3 of intense training IGF-1 levels were decreased, further supporting the Volume Threshold Hypothesis study (19).

Hackney, Fahrner, and Gulledge (1998) compared endurance-trained males to sedentary males, and found decreased serum testosterone and free testosterone in endurance-trained males compared to sedentary males. However, no differences were observed in Sex Hormone Binding

Globulin (SHBG), LH, or cortisol levels (12). A similar study by Hackney, Fahrner, and Stupnicki (1997) compared reproductive hormone profiles of untrained males to endurance-trained males. Comparable results were seen exhibiting decreased serum testosterone levels and increased LH in endurance athletes compared to untrained males. These findings suggest that long-term endurance training may lower testosterone levels (13).

A study of Swedish male Olympic athletes was conducted to assess the difference between endocrine profiles, body composition, and moods of male athletes participating in leanness and non-leanness sports. The study included 44 athletes from 26 disciplines, and divided them into non-lean or leanness sports. Subjects underwent endocrine analysis, the Profile of Mood States test, and body composition and BMD analysis via a DXA scan (14).

Hagmar et al. found that leptin levels, a key hormone involved in satiation signals within the body, were significantly lower in the group of athletes participating in leanness sports.

Decreased leptin levels associated with lower body fat suggests metabolic changes as a result of low energy availability (14). This may imply that decreased leptin levels may further exacerbate the problem of RED-S by not stimulating proper hunger and satiation signals.

Endocrine profiles were within normal limits for both groups, except for free testosterone and leptin. Both were significantly lower in the leanness athletes. Percentage body fat was positively associated with increased leptin and IGF-I:IGFBP-1 ratio. Testosterone was also positively associated with higher levels of body fat. However, this study found that those involved in leanness sports had higher z-scores and BMD, than non-lean sports.

The study did not differentiate between the type of training of the athletes, endurance training versus resistance training. Additionally, no subjects were considered unusually lean,

therefore may not be truly representative of an individual in a RED-S state. While the sample size of this study was relatively small, it allowed for research to be done on elite athletes (14).

More recent studies further support that this alteration in reproductive hormones in males may be a result of undernutrition in athletes. Additional research indicates that it is not uncommon for endurance athletes to experience low energy availability. Undernutrition in endurance athletes can be the result of desired changes in body composition, disordered eating or eating disorders, or may be unintentional due to the high volume of training. Estimates for adequate energy availability is the consumption of 45 kilocalories (kcal) / kilogram (kg) fat free mass (FFM) / day. A consumption of 30 kcal / kg FFM / day has been associated with subclinical reproductive issues in females (16). Smaller studies among male endurance athletes have reported energy intake below 30 kcal / kg FFM / day. Jockeys also have been found to have low energy availability as a means of maintaining desired weight (29). The low reported energy intake by some male athletes, indicates that male athletes can and do experience RED-S states throughout their athletic career. .

Bone Mineral Density

Calcium is the body's most abundant mineral, and is found in the plasma and stored in the bone. Cortical bone is the dense outer layer of the bone prominently found in shafts of long bones. Trabecular bone is the spongy part of the bone commonly found in vertebrae and the femoral head. BMD is a measure of the average bone mineral content of the specific region of the body. Roughly 60-80% is determined by genetics while 20-40% is impacted by lifestyle. BMD is most commonly analyzed through DXA scans. BMD scores are based on the standard deviation of BMD in the individual compared to other individuals of the same sex and age. T –

scores represent the standard deviation of BMD compared to other healthy individuals of the same sex. z-scores represent the standard deviation of BMD compared to the same age, sex, weight, and ethnicity. Positive T or z-scores represent above average BMD compared to the standard, and negative T or z- scores represent less than average BMD. Osteoporosis is diagnosed at a -2.5 T – score. The International Society for Clinical Densitometry recommends annual screening to monitor changes in BMD post intervention (30).

Although the largest changes observed in BMD occur during puberty, the bone is a dynamic matrix constantly undergoing remodeling even as an adult. The primary mechanism to increasing or maintaining ideal BMD is through adequate calcium. The body strives to keep calcium balance through intake, absorption, and excretion of calcium. Calcium balance is regulated by the parathyroid hormone (PTH). When intake and/or absorption of calcium is inadequate to meet the demands of calcium balance, PTH is released. PTH stimulates bone resorption to increase serum calcium levels. Chronic bone resorption can lead to decreases in BMD, commonly observed in the trabecular bone, such as the spine or femoral head.

Resistance training, in which the bone experiences muscle pull and mechanical load, also increases BMD. Due to the strong influence of genetics on BMD, strength training will only increase BMD up to an individual's genetic potential and then levels of BMD will plateau. If resistance training stops, resorption can occur and levels of BMD will decline. Estrogen and testosterone both provide protective effects to BMD. As BMD decreases, the strength and integrity of the bone decreases, thus increasing risk for injury (1, 2, 28).

Lower levels of testosterone, although still within normal range, may contribute to lower BMD in athletes (31). The Swedish Olympic athlete study found significantly lower percentage of body fat in individuals involved in leanness sports, however, BMD was significantly higher in

this group as well. The observed differences in BMD may be due to type of training – endurance versus strength, and due to testosterones role in estradiol production in males. Findings suggest that more research needs to be conducted to fully understand the effect of percentage body fat on testosterone and BMD within male athletes (14).

In a study by Smith and Rutherford (1998), the researchers compared male triathletes and rowers to the general male population assessing testosterone levels and BMD. For this study, BMD and percentage body fat were assessed via DXA scans. Testosterone levels were analyzed from blood samples. Researchers recorded the activity levels and calcium intake of participants. Triathletes and rowers had significantly lower percentage body fat compared to the control group. Rowers had significantly higher total body BMD compared to the control participants and triathletes. However, there was no difference in BMD between the control participants and triathletes. Triathletes had significantly lower testosterone than the control group. No difference in testosterone levels was observed between the control group and the rowers. Additionally, no groups testosterone levels fell below the normal range. Overall, the study found no relationship between spine BMD and body fat (18).

While the study provided compelling evidence, it was limited in sample size and the competitive level of athletes included in the study was unclear. The type of training performed by the rowers, triathletes, and control participants was also not specified. Rowers may have performed more strength training, eliciting an increased response in BMD or increasing lean body mass. It would be expected that both sets of athletes would have increased BMD compared to the controls due to their increased levels of training.

The results of this study indicates that the endurance training performed by the triathletes may have had negative effects on the BMD of the triathletes. Further research could involve elite

level athletes and a longer period of study to assess trends over periods of training. However, this study did provide information relating serum testosterone levels and percentage body fat, suggesting the need for more studies to be conducted assessing testosterone levels, leanness, and BMD (18).

In a study conducted by Hetland, Haarbo, and Christiansen (1993) comparing the results of physically active men running 0 – 160 kilometers / week, the researchers found an inverse correlation between kilometers ran per week and BMD in the lumbar spine. Increased markers of bone turnover, specifically, urinary pyridinium cross-links, plasma osteocalcin, and serum alkaline phosphatase, were observed in elite runners compared to controls (15). The increased markers of bone turnover suggest subjects were undergoing bone remodeling and calcium store mobilization. Other studies have found similar results – elite endurance athletes were found to have significantly decreased lower body BMD when compared to other highly active males (18, 22). The training of the highly active males was not specified in this study. The highly active males may be participating in more strength training than the elite endurance athletes, who primarily train using endurance exercises.

Weight class sports also appear to be at risk for negative impacts on BMD when attempting to make weight cuts. Judoists who cut roughly 4% of their weight stimulated bone resorption. However, resorption was observed to be halted as weight was regained. This study could have provided more insight into RED-S in male athletes if percentage body fat had been analyzed in addition to BMD (17). A study comparing hormone profiles and BMD of jockeys found decreased BMD and increased bone turnover within jockeys compared to controls. Notably, lean mass and height were the primary indicators of observed low BMD (11). Both studies suggest that nutritional deficit may contribute to fluctuations in BMD.

Limitations to Current Studies

The meta-analysis, *Parallels the(7-9) Female Athlete Triad in Male Athletes* by Tenforde et al., provides the broadest look at the limited and discontinuous research available on RED-S in male athletes. This meta-analysis suggests that studies exist analyzing snippets of the proposed Male Athlete Triad or RED-S in male athletes. However, there are no studies analyzing all three elements –percentage body fat, hormonal dysfunction, and BMD. Studies analyzing two of the three elements of the proposed triad provide promising results that a correlation exists, and warrants further and broader research (6).

While RED-S in male athletes may have many parallels with the Female Athlete Triad and RED-S in female athletes, the physiology of males and females remain different and more research must be conducted to fully understand the impact of male hormones and RED-S on the male physiology. Overall, there is limited understanding of RED-S in male athletes, but the limited evidence does strongly suggest that the condition does exist (7-18, 21, 23, 27).

Moreover, no studies conducted have included athletes with unusually low levels of body fat percentage (<7%) and may not be representative of an individual experiencing true RED-S (6). Most studies utilize a relatively small sample size, which may be due to the limited prevalence of RED-S in male athletes. Studies conducted on components of RED-S have used males with varying levels of training from elite Olympians to untrained or sedentary males. By increasing the awareness of the RED-S in male athletes, more researchers will be drawn to this subject. The aim of future research should be to assess and provide a greater understanding of the most susceptible population at risk for the negative health outcomes and impairment on performance.

Conclusion

The vast knowledge of the Female Athlete Triad and RED-S in female athletes has improved the level of care and treatment for female athletes. Based on the understanding of the Female Athlete Triad and RED-S in females, there is a need for increased knowledge surrounding RED-S in male athletes. General background and understanding of the physiological process occurring in male athletes needs further research. Once fully understood, parameters for identification and protocols to treat and prevent injury can be established. Return to play protocols and referral to proper care will allow for the best recovery of the athletes. In addition to optimizing the male athlete's performance, this knowledge will also benefit athletes after their athletic career concludes by helping prevent the negative long-term health consequences seen from RED-S (8).

The purpose of this thesis project was to investigate the relationship between low body fat percentage, BMD, RED-S in male athletes and the relationship with testosterone, IGF:1, and cortisol levels, to increase the literature surrounding RED-S in male athletes.

CHAPTER 3: METHODS

Overview

The purpose of this study was to investigate the relationship between percentage body fat, BMD, serum testosterone, cortisol, and IGF-1 levels in endurance trained male athletes.

Secondary questions were to describe the relationship between BMD and serum testosterone, cortisol, and IGF-1 levels in athletes above and below the median percentage body fat. Male athletes participating in Division I endurance sports were recruited for the study and asked to sign written consent (Appendix B: Consent). Data was collected from their routine clinical care, including results from DXA scans and blood tests.

Sample

The research population was male athletes over 18 years of age participating in Division I Cross Country at the University of Colorado Boulder enrolled in the 2017 – 2018 academic year. Given that the data used for the study was part of the athlete's routine clinical care, I anticipated that all subjects enrolled would complete the study.

Inclusion criteria for this study was males enrolled as a Division I athlete in Cross Country at the University of Colorado Boulder for the 2017 – 2018 academic year. Exclusion factors was males with a history of steroid use, hypogonadism, hypothalamic disorders, injury to the testes, and percentage body fat over 15%. The upper limit for percentage body fat was set *a priori* at <15% to capture a lean population.

Setting

Pre-participation Physical Examinations (PPEs) for Cross Country and Nordic athletes took place at the University of Colorado Sports Medicine Center throughout August 2017. Written consent was collected during PPEs.

Blood collection and body composition was performed at the University of Colorado Wardenburg Health Center. Blood samples were transported to the University of Colorado Wardenburg Health Center Laboratory for analysis. Free and total testosterone, IGF-1, and cortisol were processed through Quest Diagnostics Lenexa. Quest Diagnostics is licensed/certified under the Clinical Laboratory Improvement Amendments.

DXA scans occurred at University of Colorado Boulder Wardenburg Health Center on a GE Medical Systems Lunar Prodigy for a total body, spine, dual femur bone density, and percentage body fat according to GE Medical Systems Standard Operating Procedures (32).

Ethics

Ethics approval was obtained from the Human Subjects Committee (HSC) at the University of Colorado Boulder, study IRB 17-0239. The University of Kansas HSC requested to rely on the HSC from University of Colorado Boulder. Subjects did not receive any incentives for participating in this study.

Procedures

Recruitment

At PPEs for Cross Country and Nordic Skiing, prospective subjects were informed of the study and asked to sign written consent. Subjects were informed that the study was voluntary, and subjects could withdraw at any time. Any questions posed about the study were answered.

The written consent listed the inclusion and exclusion criteria for the study. If subjects met the inclusion and exclusion criteria, and were still willing to participate, they were asked to sign the written consent prior to participation in the study (Appendix B: Written Consent Form).

Data Collection

Upon recruitment subjects answered a brief questionnaire collecting demographic information and brief medical history (Appendix C: Questionnaire). Body composition, blood parameters, and DXA scan results were collected.

Body Composition and Bone Mineral Density Assessment

DXA scan was conducted at the University of Colorado Boulder Wardenburg Health Center on a GE Medical Systems Lunar Prodigy for a total body, spine, dual femur bone density, and body fat percentage according to GE Medical Systems Standard Operating Procedures. DXA scans were scheduled and completed within 1 week of blood. Subjects with a percentage body fat greater than 15% were removed from the study. Age adjusted z-scores were estimated on total BMD values and standard deviations were supplied by the manufacturer (32).

Blood Collection

Subjects underwent a routine blood draw performed at the University of Colorado Boulder Wardenburg Health Center by a Medical Assistant or Nurse Practitioner. A total of 21 mL (1 – 4 mL vile and 2 – 8.5 mL vile) of blood was collected via a 21 gauge needle and was

transported to the Wardenburg Health Center Laboratory for storage until being shipped to Quest Diagnostics Lenexa, KS for analysis of serum testosterone, cortisol and IGF-1.

Data Management

DXA scan, body composition, and lab results were provided to both the Sports Medicine Provider and the Research Assistant. The Research Assistant was responsible for coding the data by providing unique identification (ID) numbers to each subject.

The Research Assistant entered all results from the DXA scan and laboratory values into the electronic spreadsheets associated to their assigned ID (Appendix D: Data Collection Form). Electronic files were saved to the University of Colorado–Boulder Server for Athletics & Sports Medicine and all data were password protected. The Research Assistant was responsible for safeguarding the key to the coded data until the study is complete, December 2017.

Analysis of Data

Statistical analyses were performed using SPSS (IBM, Version 24) in conjunction with working with the faculty mentor. Statistical significance was set at $p \leq 0.05$.

1. What is the relationship between percentage body fat and BMD in male endurance trained athletes?

Data analysis: A Pearson correlation coefficient was calculated to determine the relationship between body fat percentage and BMD.

2. What is the relationship between percentage body fat and levels of cortisol, serum testosterone, and IGF-1?

Data analysis: A Pearson correlation coefficient was calculated to determine the relationship between percentage body fat and levels of cortisol, serum testosterone, and IGF-1.

3. What is the relationship between bone mineral density and levels of cortisol, serum testosterone, and IGF-1 in endurance trained athletes with a body fat percentage below the median and above the median?

Data analysis: A Pearson correlation coefficient was calculated to determine the relationship between bone mineral density and levels of cortisol, serum testosterone, and IGF-1 in two groups of endurance athletes: those that have a percentage body fat below the median and for endurance athletes that have a percentage body fat above the median.

CHAPTER 4: RESULTS

The purpose of this study was to act as a preliminary study investigating the relationship between percentage body fat and BMD, and percentage body fat and hormone levels in collegiate endurance athletes. Additionally, the study investigated the relationship between BMD and hormone levels in endurance trained athletes with a percentage body fat below and above the median.

Subjects:

20 subjects enrolled in the study. One subject was excluded due to percentage body fat above 15%. Out of the 19 subjects, 5 subjects had missing data: 3 subjects did not complete their DXA and bloodwork, one subject did not complete their DXA, and 1 subject did not complete their bloodwork. Therefore, 14 subjects were included in the analyses.

The descriptive characteristics of the sample are included in Appendix E: Table 1. On average, the subjects were 20.5 years old and average BMI was 20.4 kg/m^2 and the average percentage body fat was 8.6 %. The majority of subjects (64%), were running 80-100 miles per week. One subject was running greater than 100 miles per week, which was the maximum mileage, while one subject was running 30-40 miles per week, which was the minimum.

Percentage Body Fat and BMD:

Listed in Appendix F: Table 2 is a summary of the relationships between percentage body fat and BMD. No relationship was found between total body fat percentage and spine ($r = 0.88$, $p = 0.76$) or dual femur z-score ($r = 0.85$, $p = 0.77$).

BMD and Hormone Levels:

Listed in Appendix G: Table 3 is a summary of the relationships between body fat percentage and levels of cortisol, serum testosterone, and IGF-1. No relationships were found between total body fat percentage and levels of these hormones (testosterone: $r = 0.01$, $p = 0.74$; cortisol: $r = 0.09$, $p = 0.78$; or IGF-1: $r = -0.33$, $p = 0.27$).

BMD and Hormone Levels in Athletes with Percentage Body Fat Below the Median

A median was calculated for percentage body fat, 8.2% percent. A variable was created to categorize those that fell below the median (<50th percentile) and those that fell above the median (≥50th percentile). Listed in Appendix H: Table 4 is a summary of the relationships between BMD and levels of cortisol, serum testosterone, and IGF-1 for endurance trained athletes with a percentage body fat below the median. An inverse relationship was found between serum testosterone and dual femur z-score ($r = -0.76$, $p = 0.05$). Additionally, a borderline significant inverse correlation was found between serum testosterone and AP spine z-score ($r = -0.74$, $p = 0.06$). IGF-1 was positively correlated to AP spine z-score ($r = 0.82$, $p = 0.02$) and a strong correlation was found between IGF-1 and dual femur z-score ($r = 0.69$, $p = 0.09$). Cortisol was not related to AP spine or dual femur z-score.

BMD and Hormone Levels in Athletes with Percentage Body Fat Above the Median

Listed in Appendix I: Table 5 is a summary of the Pearson Correlation analysis regarding the relationships between BMD and levels of cortisol, serum testosterone, and IGF-1 for endurance trained athletes with a percentage body fat above the median. Serum testosterone was positively correlated to the AP spine z-score ($r = 0.84$, $p = 0.02$). A borderline significant correlation was detected between serum testosterone and the dual femur z-score ($r = 0.72$, $p = 0.07$). Cortisol was not related to AP spine or dual femur z-score.

Summary of Results for Research Questions

1. What is the relationship between percentage body fat and BMD in male endurance trained athletes?

No relationship was found between total body fat percentage and spine ($r = 0.88$, $p = 0.76$) or dual femur z-score ($r = 0.85$, $p = 0.77$).

2. What is the relationship between percentage body fat and levels of cortisol, serum testosterone, and IGF-1?

No relationship was found between total percentage body fat and levels of these hormones (testosterone: $r = 0.01$, $p = 0.74$; cortisol $r = 0.086$, $p = 0.780$; IGF-1 $r = -0.33$, $p = 0.27$.

3. What is the relationship between BMD and levels of cortisol, serum testosterone, and IGF-1 in endurance trained athletes with a percentage body fat below and above the median?

In endurance trained athletes with a percentage body fat below the median, an inverse relationship was found between serum testosterone and dual femur z-score ($r = -0.76$, $p = 0.05$). A borderline significant inverse correlation was found between serum testosterone and AP spine z-score ($r = -0.74$, $p = 0.06$). IGF-1 was positively correlated to AP spine z-score ($r = 0.82$, $p = 0.02$) and a strong correlation was found between IGF-1and dual femur z-score ($r = 0.69$, $p = 0.09$). Cortisol was not related to AP spine or dual femur z-score.

In endurance trained athletes with a percentage body fat above the median serum testosterone was positively correlated to the AP spine z-score ($r = 0.84$, $p = 0.02$). A borderline significant correlation was detected between serum testosterone and the dual femur z-score ($r = 0.72$, $p = 0.07$). Cortisol was not related to AP spine or dual femur z-score.

CHAPTER 5: DISCUSSION

The purpose of this study was to investigate the relationships between percentage body fat and BMD, and percentage body fat and levels serum testosterone, cortisol, and IGF-1. In addition, this study explored the relationships between BMD and levels of cortisol, serum testosterone, and IGF-1 in endurance trained athletes with a percentage body fat above and below the median.

Percentage Body Fat and BMD

No relationship was found between percentage body fat and BMD in this study. The lack of a relationship between BMD and percentage body fat in male endurance athletes conflicts with the findings among female athletes. In females, low percentage body fat is considered a risk factor for low BMD (1-4, 6-8, 33). One study in males more sensitive biomarkers of bone turnover than BMD. They found a positive relationship between percentage body fat and bone turnover (11). The measurement of BMD is not a highly sensitive measure of the bone remodeling process, as changes in BMD will only be seen in as early as 6 months to 1 year (30). Since this study is a cross-sectional design and not longitudinal, we are unable to understand relationships for changes in BMD.

Other studies that collected data on both BMD and percentage body fat did not assess the relationship between the two (10, 15, 18, 22). These studies compared BMD and percentage body fat as independent variables between groups of endurance athletes and controls. Smith and Rutherford (18) compared percentage body fat and BMD between groups of male rowers, triathletes, and a control group. Although both rowers and triathletes had lower percentage body fat compared to the control group, rowers had an increased BMD compared to the control group

and triathletes. Other studies (18, 22) found decreased BMD in elite endurance athletes when compared to active males. Substantial evidence (15, 18, 22) suggests differences in BMD in endurance athletes, when compared to resistance trained athletes, or a control group. However, comparing BMD among various groups of athletes was not within the scope of this study.

Percentage Body Fat and Hormone Levels

No correlation was found between percentage body fat and hormone levels of serum testosterone, cortisol, and IGF-1. It was hypothesized that a positive correlation would be found between percentage body fat and testosterone and IGF-1 levels, and a negative relationship would be found between percentage body fat and cortisol levels.

The lack of relationship between cortisol and percentage body fat was supported by other studies (10, 12, 13, 15). However, the lack of relationship between testosterone and IGF-1 and percentage body fat conflicts with findings from other research studies (10-15, 18, 19, 23, 24, 29). Hormone levels are more reactive to stressors than the measurement of BMD (1, 2, 28, 30). Hormone levels can change within a few weeks to a few months (1, 2, 28). To observe changes in BMD a minimum of 6 months to 1 year is recommended (30). Given changes in BMD and mobilization of calcium stores rest upon hormone regulation, the lack of relationship between percentage body fat and hormones could contribute to the explanation for the lack of relationship between percentage body fat and BMD (1, 2, 14, 17, 18, 28, 29).

Cortisol

Cortisol increases the mobilization and utilization of body stores of calcium, protein, and fat (1, 2). Endurance training also increases cortisol production (1, 2). Cortisol levels vary

throughout the day. Levels are observed to be at their highest in the morning, and decline throughout the day (1, 2). Cortisol levels were collected first thing in the morning for this study. Overall, no relationship between cortisol levels and percentage body fat was observed. De Souza and Miller (10) found no relationship between cortisol and mileage per week or percentage body fat. Studies conducted by Hackney et al. (12, 13) found no difference in cortisol levels of endurance athletes when compared to sedentary and untrained male adults. Though endurance training increases cortisol, a lack of relationship may indicate appropriate recovery between training sessions and the athlete is physiologically adapting to the training prescribed (1, 2). Additionally, this study was conducted prior to the start of the school year. Despite the high training volume of mileage run per week, external stressors, such as college course load and homework, had not been experienced by the subjects.

Testosterone

Although the literature has not found clinically low levels of serum testosterone in endurance athletes, consistent findings of subclinical low levels of testosterone are prevalent. Two separate studies (10, 14) found a positive relationship between percentage body fat and serum testosterone. Other studies found endurance athletes had significantly lower levels of serum testosterone when compared to sedentary and untrained males (12, 13). While clinical manifestations of altered testosterone levels have not consistently been found subclinical differences have been observed when comparing endurance athletes to controls. Perhaps these subclinical findings may indicate that specific ranges for athletes that differ from the standards may be necessary for athletes to maintain ideal health at high levels of training.

Testosterone levels are lowest during childhood years and peak during puberty (1, 2). The lack of relationship between percentage body fat and serum testosterone in this study may be attributed to the young adult population and increased levels of testosterone for development.

IGF-1

IGF-1 production can be suppressed by elevated cortisol levels (1, 2). The lack of relationship between percentage body fat and IGF-1 may be attributed to appropriate cortisol levels. IGF-1 decreases with endurance training long-term but not short-term (19, 20). While this study was conducted when athletes returned to start their season, athletes were running their highest mileage per week. The early season assessments may have contributed to a lack of relationship between percentage body fat and IGF-1 as the body may not have been experiencing the stress of endurance training for an extended period of time.

Relationship of BMD and Hormone Levels Based on Percentage Body Fat Distribution

BMD and Testosterone

The study found differing results for the relationship between BMD and testosterone, when assessing groups above and below the median percentage body fat. These findings may indicate a disruption in testosterone production at a certain percentage body fat levels.

In athletes with percentage body fat above the median, a positive correlation was found between serum testosterone with BMD. Although limited studies exists on the relationship between testosterone and BMD, this finding was expected. Testosterone in males is a precursor to estradiol production. In males, estradiol helps regulate BMD (28). In a study examining the impact of running mileage per week on hormones (10), results indicated that with increased

running mileage per week, testosterone decreased. One study (15) found that with increased mileage per week, there was an increase in markers of bone turnover. An additional study (18) found that elite triathletes had both lower testosterone and BMD, than rowers.

In athletes with a percentage body fat below the median, serum testosterone was inversely correlated with BMD. This finding was not expected. Based on prior published data (10, 15, 18), we would have expected to find a similar or even stronger relationships as was found in athletes with a higher percentage body fat. Further research is needed to understand these unexpected findings.

IGF-1 and BMD

The study found a positive correlation between IGF-1 and BMD in the athletes with percentage body fat below the median and a negative relationship with the athletes above the median. While the positive relationship in the athletes below the median was expected, the negative relationship for those above the median was not (14, 19, 20). Two studies (19, 20) found decreased IGF-1 levels with long term endurance training. Another study (20) found IGF-1 levels had a negative correlation with mileage ran per week. Additionally, markers of bone turnover were negatively correlated to mileage ran per week (19). However, Hagmar et al. (14) observed both IGF-1 and BMD within the same study and observed different results in terms of BMD. The study found a positive correlation with percentage body fat and IGF-1:IGFBP-1 ratio in lean sport athletes. When BMD was compared between lean and non-lean athletes, lean sports had an increased BMD compared to non-lean athletes (14). The different literature and results from this study suggest that the relationship between IGF-1, percentage body fat, and BMD

should continue to be explored. The findings from this study may indicate an alteration in production of IGF-1 levels at a certain percentage body fat level that is not fully understood.

Cortisol and BMD

No relationship was found in either group for cortisol and BMD. Given the role of cortisol in the mobilization of calcium stores (1, 2), it was expected that both groups would have a negative correlation between BMD and cortisol, with the percentage body fat below the median group having a stronger relationship. The Volume Threshold Hypothesis (10) found no relationship between cortisol and mileage per week or percentage body fat. Studies conducted by Hackney et al. (12, 13) have found no difference in cortisol levels of endurance athletes when compared to sedentary and untrained male adults. Despite endurance training increasing cortisol levels, a lack of relationship may indicate appropriate recovery between training session and athlete's physiologically adapting to the training prescribed.

Limitations

There are limitations of this study. First, the number of subjects examined in this study was small. Only 14 athletes completed BMD and cortisol, and only 13 athletes had testosterone and IGF-1 measurements. Second, data were collected at a snapshot in time. While hormone levels are more responsive, and fluctuations can be observed in as short as a few weeks to months, BMD is much more stagnant (1, 2, 28, 30). BMD does not provide insight as to whether an individual is in the bone remodeling process, or where along the bone remodeling process they may be (1, 2, 28, 30). Therefore, longitudinal studies are needed to assess the impact of

training at this intensity over time and to provide more insight on the impact to body functions with a slower response rate such as BMD.

This study examined a limited number of hormones. Other evidence suggests additional hormones, such as FSH, LH, estradiol, leptin, and grehlin, may contribute to RED-S in males (14, 28). While limited research exists on males, there may be additional hormones that may contribute to RED-S in males that has yet to be identified (6).

Dietary intake was not considered in this study. Percentage body fat was used and an indirect indicator of nutritional status and RED-S. Studies have indicated that energy availability plays a key role in RED-S, and endurance athletes are more likely to have low energy availability with increased volume of training (16, 29). Overall calorie consumption and macronutrient makeup could affect body composition and hormone production (4, 6, 8). Additionally, micronutrient intake, specifically calcium and vitamin D, could have a substantial effect on BMD (1, 2, 30).

Though not a purpose of this study, future studies should include more incoming freshman athletes to evaluate the response and impact of training at a high intensity over time. As discussed, different intensities of training and the duration of training at specific intensities, may have a profound impact on recovery and hormonal response (10, 18-20, 22).

Future Studies

Future studies should include a larger subject pool to assess whether similar results are found with a greater sample size of subjects. As discussed, this study assessed a snapshot in time. Further, longitudinal data collection to capture the change of percentage body fat, hormone levels, and BMD is needed. Other biomarkers may also be considered, such as biomarkers of

bone turnover, which are more indicative of the bone remodeling process rather than BMD. Additional time periods of data capture, including at baseline, during the training period with the most intensity, off season, and immediately following injury should be conducted.

If significant findings hold with a larger subject pool, additional studies in male athletes with similar body composition participating in a strength based sport may be warranted to examine the impact of strength training on the anabolic hormones and impact on bone mineral density.

Implications

Currently, there is not a large body of research on the relationship between percentage body fat, hormone levels, and BMD in male athletes. Our study found a change in the direction for the relationship between BMD and testosterone and IGF-1 among athletes with a percentage body fat above and below the median. Our research suggests that the relationship may be worth exploring to understand the relationship between percentage body fat, BMD, and hormones in male endurance athletes and how this relates to RED-S.

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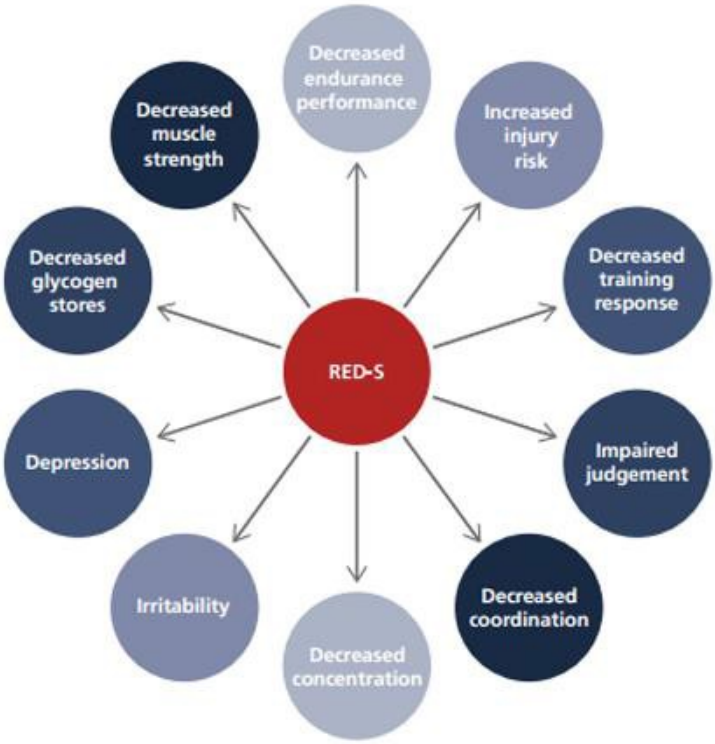
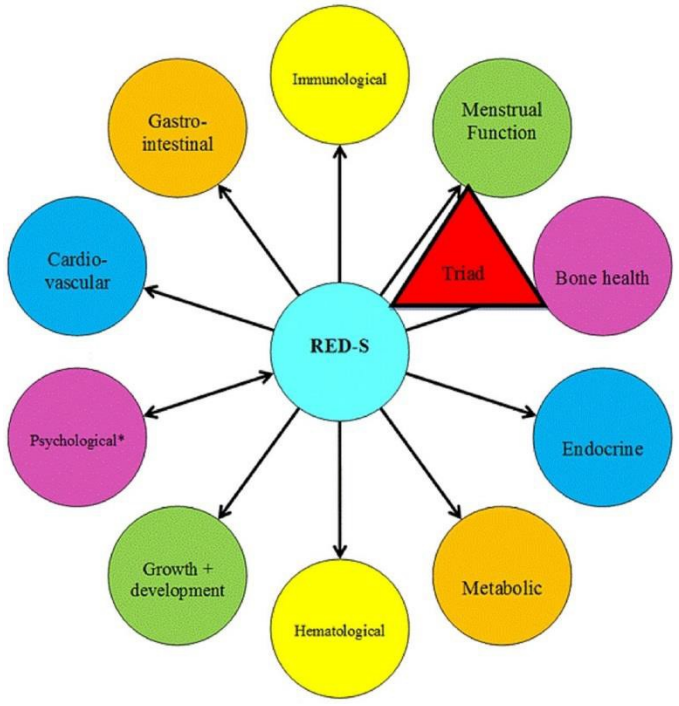
APPENDIX A: RED-S SPOKE AND WHEEL CONCEPT DIAGRAM





APPENDIX B: CONSENT

Permission to Take Part in a Human Research Study

Title of research study: ***Investigating Relative Energy Deficiency in Sport in Endurance Trained Athletes***

Investigator: ***Dana Bielinski***

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 athlete over 18 years of age participating in a Division I endurance based sport for the 2017-2018 Academic year at the University of Colorado Boulder.

Inclusion Criteria for this study include male athletes participating in varsity endurance based sports at the University of Colorado Boulder and over 18 years of age.

Exclusion Criteria of this study include history of steroid use, hypogonadism, and/or endocrine disorders. If you meet the inclusion and exclusion criteria for the study and would like to participate, please proceed with the consent process and sign below.

What should I know about 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.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at Dana.Bielinski@colorado.edu (815) 274-3248.

This research has been reviewed and approved by an Institutional Review Board ("IRB"). You may talk to them at (303) 735-3702 or irbadmin@colorado.edu if:

- 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.
- You have questions about your rights as a research subject.
- You want to get information or provide input about this research.

Why is this research being done?

The purpose of this study is to investigate the relationship of body fat percentage, hormone disruption and bone mineral density in male endurance trained athletes. This phenomenon historically has been well studied in female athletes as the Female Athlete Tria, however limited research exists on male athletes. Potential benefits of this research would serve as a preliminary study to larger research. This study has the potential to indicate greater need for research on RED-S in males, increased monitoring of identified parameters, and increased funding for these studies.

How long will the research last?

We expect that you will be in this research study for 1 day, with total active time of involvement of 45 minutes.

How many people will be studied?

We expect about 40 people will be in this research study.

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

- For this study we would like to access the following data from your Pre Participation Physical Exam:
    - Laboratory Values: Cortisol, Testosterone, and Insulin Like Growth Factor – 1
    - DXA Scan Results: Total body, spine, dual femur bone mineral density, and body fat percentage.
- Subjects will complete a short survey on demographic information, medical history and training at baseline. Expected duration: 5-105 minutes

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

You can leave the research at any time and it will not be held against you.

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

You can leave the research at any time it will not be held against you.

If you stop being in the research, already collected data may not be removed from the study database.

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

Subjects medical information will be managed under HIPAA Standards, and data will be coded before being provided to the Principal Investigator. All electronic files containing medical information will be password protected on the CU Boulder Athletics Sports Medicine Server.

What happens to the information collected for the research?

Efforts will be made to limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this

information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

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

What else do I need to know?

This research is being funded by the University of Colorado Boulder Sports Medicine Department.

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

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

APPENDIX C: QUESTIONNAIRE

Intake Survey

Name: _____

Age: _____

Academic Year: _____

Sport: _____

Do you currently have an injury prohibiting you from training for more than 3 weeks?

Yes No

Please list all medications and supplements you are currently taking:

_____

Current Running Mileage per week:

30-40 40-50 50-60 60-70 70-80 80-100 100+

Total Hours spent training per week:

< 10 10-12 12-14 14-16 16-18 18-20 20+

APPENDIX D: DATA COLLECTION

Data Collection table appears on the following page. This page is intentionally left blank.

ID	Aca-demic Year	Injury	Mileage Per Week	Hours Per Week	AP Spine Z Score	Dual Femur Z Score	Total Body Z Score	Body Fat	Serum Testos-terone	Cortisol	IG F-1	Age (Years)	Height (m)	Weight (km)

APPENDIX E: TABLE 1

Table 1. Descriptive characteristics of study participants

Variables	n	Mean	SD	Min	Max
Age (years)	14	20.5	1.09	19	22.5
Height (cm)	14	180	6.08	167	189
Weight (kg)	14	65.91	5.22	55.1	75.7
Body fat percentage (%)	14	8.64	2.01	6.5	12.2
BMI (kg / m^2)	14	20.4	1.15	18.6	22.4
AP spine z-score	14	-0.24	1.17	-2.4	1.8
Dual femur z-score	14	0.86	1.21	-0.60	3.00
Total body z-score	14	0.81	1.09	-0.80	2.90
Serum testosterone (ng / dL)	14	776	254.41	240	1106
Cortisol (μg / dL)	13	16.42	3.76	9.70	20.70
IGF-1 (ng / mL)	13	264.15	59.50	154.00	356.00

APPENDIX F: TABLE 2

Table 2. Relationships between percentage body fat and bone mineral density (n = 14)		
	AP spine z-score	Dual femur z-score
Percentage body fat	r = 0.88 p = 0.76	r = 0.85 p = 0.77

APPENDIX G: TABLE 3

Table 3:.Relationships between percentage of body fat and hormone levels (n =13)			
	Serum testosterone	Cortisol	IGF-1
Percentage body fat	r = 0.01 p = 0.74	r = 0. 86 p = 0.78	r =- 0.33 p = 0.27

APPENDIX H: TABLE 4

Table 4. Relationships between bone mineral density and hormone levels in athletes with a percentage body fat below the median (n = 7)			
	Serum Testosterone	Cortisol	IGF-1
AP spine z-score	r = -0.74 p = 0.06	r = -0.25 p = 0.58	r = 0.82* p = 0.02
Dual femur z-score	r = -0.76* p = 0.05	r = -0.10 p = 0.83	r = 0.69 p = 0.09

**Correlation is significant at the 0.05 level (2-tailed)*

APENDIX I: TABLE 5

Table 5. Relationships between bone mineral density and hormone levels in athletes with a percentage body fat above the median (serum testosterone n = 7, cortisol and IGF-1 n = 6)			
	Serum Testosterone	Cortisol	IGF-1
AP spine z-score	r = 0.84* p = 0.02	r = -0.34 p = 0.51	r = -0.56 p = 0.27
Dual femur z-score	r = 0.72 p = 0.07	r = -0.33 p = 0.53	r = -0.54 p = 0.27

**Correlation is significant at the 0.05 level (2-tailed)*