Associations Between Collegiate Female Athletes' Energy Availability and Various Female Athlete Triad Components/Risk Factors When Using Three Different Criteria to Calculate Exercise Energy Expenditure

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by

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Authorization to Submit Thesis

This thesis of Jenna Dyckman, submitted for the degree of Master of Science with a major in Family and Consumer Sciences and titled "Associations Between Collegiate Female Athletes' Energy Availability and Various Female Athlete Triad Components/Risk Factors When Using Three Different Criteria to Calculate Exercise Energy Expenditure" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

The Female Athlete Triad is a disorder that includes energy availability, menstrual function, and bone mineral density (BMD). Energy availability (EA) has been designated as the root cause for female athletes for athletes developing menstrual dysfunction (MD) and/or low BMD. Energy availability is defined as energy intake minus exercise energy expenditure (EEE) divided by lean body mass (LBM). For research conducted in laboratory setting, indirect calorimetry is the standardized method for measuring EEE. But for free-living studies, researchers have calculated EA using a variety of different criteria for quantifying EEE. In the present study, 19 participants wore triaxial accelerometers to collect EEE data, kept exercise logs, and recorded food intake for four days (3 weekdays and 1 weekend day). To compare methods for quantifying EEE, three EEE criteria were used to calculate the participants' EA. The three EEE criteria included total activity EEE, structured planned EEE, and METs ≥ 3 EEE. Participants also completed the Low Energy Availability in Females Questionnaire (LEAF-Q), body composition assessment using the Bod-Pod, and a dual energy x-ray absorptiometry (DXA) scan to measure BMD. Data was summarized and analyzed to determine prevalence of Triad components in the participants, agreement between the three EEE criteria, associations between EA (calculated using the three EEE criteria) and Triad components/risk factors, and associations between the participants' actual Triad component results to the LEAF-Q's prediction. Statistical tests used to test agreement included kappa statistic, while association was tested using ANOVA, odds ratio, Spearman correlation, and logistic regression. Sensitivity and specificity tests were calculated to examine the predictive ability of the LEAF-Q. Using the three EEE criteria, low EA was the most prevalent Triad component among the participants (26-53%). Prevalence of MD was

26% (n=5) and low BMD prevalence was 15% (n=3). Prevalence of at least one Triad component ranged from 53% to 68% and prevalence of at least two Triad components ranged from 11-21%. No participants presented with all three components of the Triad. LEAF-Q results showed that this questionnaire had the ability to predict if an athlete is at risk for at least one Triad component, however, the LEAF-Q was not able to identify which Triad component an athlete is at risk for. Results of the EEE criteria comparison indicate that the total activity EEE and structured planned EEE criteria have low agreement, whereas the total activity EEE and METs ≥ 3 EEE criteria have moderate agreement. Overall, the METs ≥ 3 EEE criteria may better capture an athlete's daily EEE not only from their planned exercise related to their sport, but also other moderate to vigorous intensity leisure exercise sessions they participate in throughout the day that add to their daily EEE.

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Dedication

This thesis is dedicated in memory of Dr. Samantha Ramsay. It was a privilege and honor to be your student and learn from you both in and out of the classroom. Your passion for nutrition, research, and life inspired me to live life to the fullest and never give up on my dreams.

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List of Abbreviations

ACSM	American College of Sports Medicine
BMD	Bone Mineral Density
DXA	Dual-energy X-ray Absorptiometry
EA	Energy Availability
EAT-26	Eating Attitudes Test 26
ED	Eating Disorder
GnRH	Gonadotropin Releasing Hormone
ISCD	International Society of Clinical Densitometry
LEAF-Q	Low Energy Availability in Females Questionnaire
LH	Luteinizing Hormone
MD	Menstrual Dysfunction
MET	Metabolic Equivalent
Triad	Female Athlete Triad

Chapter One

Introduction

As female participation in sports and athletic events steadily increased over the past several decades, emphasis has been placed on research conducted on female athletes (Otis et al., 1997). Although it is common knowledge that exercise is beneficial for health, adverse effects were seen in female athletes when there was not a balance between exercise energy expenditure and dietary energy intake (Slater, Brown, McLay-Cooke, & Black, 2017). Cumulative research on female athletes led to the identification of the condition known as the Female Athlete Triad which is made up of three main components including energy availability, menstrual function, and bone health (Otis et al., 1997; Loucks et al., 1998; Nattiv et al., 2007). The energy availability (EA) of a female athlete is the main Triad component that is associated with determining an athlete's risk of developing menstrual dysfunction (MD) and low bone mineral density (BMD) (Nattiv et al., 2007). The lower the EA of a female athlete, the greater the risk of developing MD or low BMD (Nattiv et al., 2007). The standardized equation for quantifying an athlete's EA is dietary energy intake minus exercise energy expenditure (EEE) divided by lean body mass (LBM) (Nattiv et al., 2007). A main component influencing an athlete's EA is their daily EEE. While there is a standardized equation used for calculating EA, there is not a standardized method for quantifying EEE in athletes. As Triad research progresses, discovering the best way to quantify EEE would be beneficial and allow for increased generalizability of study results.

Problem Statement

The purpose of this study was to examine associations between University of Idaho collegiate female athletes' energy availability (EA) and various Triad components/risk factors when using three different criteria to calculate exercise energy expenditure (EEE).

Research Objectives

1. Determine the prevalence of the Triad components among University of Idaho female athletes.
2. Compare agreement between three EEE criteria used to calculate the female athletes' energy availability (1. Total activity EEE, 2. Structured planned EEE, and 3. METs ≥ 3 EEE).
3. Test associations between University of Idaho collegiate female athletes' energy availability and various Triad components/risk factors when using three different criteria to calculate EEE.
4. Examine the sensitivity and specificity of the Low Energy Availability in Females Questionnaire (LEAF-Q) when predicting the prevalence of (sensitivity), and absence of (specificity) Triad risk factors among female athletes at the University of Idaho.

Hypotheses

1. University of Idaho female athletes will exhibit one or more components of the Triad.
2. There will be no agreement between the three EEE criteria.
3. There will be an association between the actual Triad components the participants have and the LEAF-Q prediction of Triad risk.
4. The LEAF-Q will be able to predict when an athlete has one or more components of the Triad and when they do not.

Significance of Study

Existing research indicates that low EA is the driving force for female athletes to develop one or more Triad components (Beals & Meyer, 2007). While many factors can affect menstrual function and bone health, including genetics, diseases, and environmental factors, for athletes who develop one or more triad components, in most cases it can be traced back to low EA (Nattiv et al., 2007). The connection between low EA and female athletes' health allows researchers to focus attention on developing interventions to decrease the prevalence of low EA among female athletes. Since the first official American College of Sports Medicine (ACSM) committee that defined the Triad and it components in 1992, significant strides have been made in Triad research (Nattiv et al., 2007; Slater, Brown, McLay-Cooke, & Black, 2017; Beals & Meyer, 2007). Despite this, there is no standardized method for calculating EA in female athletes. Yet, there are standardized methods for all other aspects of Triad components including calculating bone mineral density (BMD) using dual energy x-ray absorptiometry (DXA), identifying eating disorders or disordered eating using a standardized screening tool known as Eating Disorder Examination Questionnaire (EDE-Q), and a gynecological assessment is the standardized way for identifying if an athlete has menstrual dysfunction (MD) (Beals & Meyer, 2007).

Previous Triad research studies have used a variety of methods for collecting exercise energy expenditure (EEE) data for use in calculating EA (Guebels, Kam, Maddalozzo, & Manore, 2014; Hoch et al., 2009; Ihle & Loucks, 2004; Melin et al., 2014; Day, Wengreen, Heath & Brown, 2015). Because of the inconsistency there is a need for developing a standardized method for collecting EEE data to help identify low EA in athletes. Due to the key role that EA plays in an athlete's health, developing a standardized method for calculating EEE in athletes will benefit Triad researchers. It will increase the validity, reliability, and generalizability of Triad research.

Delimitations

1. Female student-athletes from the University of Idaho were recruited in this study. Male student-athletes and female and male non-athletes were not used due to the specific population studied in Triad research.

Limitations

1. Sample size of the study was small (N= 19).
2. All data collected from the LEAF-Q was self-reported.
3. The University of Idaho female student-athletes' training volume, duration, and intensity could vary if the athlete was currently in season versus off season.
4. Dietary intake was self-reported using the ASA-24.
5. This study did not control for polycystic ovarian syndrome and other menstrual dysfunction disorders not related to exercise.
6. Accelerometers are not well suited for capturing energy expended during biking and upper body exercises.

Chapter Two

Literature Review

Female Athlete Triad

The participation of females in sport has steadily increased since 1972 after the passage of Title IX, which prohibited the discrimination against the participation of sport or receiving federal funding, at an educational institution, based on gender (Beals & Meyer, 2007). In general, females receive significant benefits when participating in sport and exercise which include, improved physical fitness, enhanced self-esteem, and decreased risk for chronic diseases (Nattiv, Agostini, & Drinkwater, 1994). Despite the benefits, there are accompanying risks as well. As female participation in sport has steadily increased over the past few decades, these risks became the focus of female athlete research. Initially, research focused on disorders female athletes experienced that exhibited visible and measurable symptoms, such as menstrual dysfunction (Slater, Brown, McLay-Cooke, & Black, 2017). As research on female athletes progressed, more exercise-related disorders were discovered which led to a 1992 conference hosted by the American College of Sports Medicine (ACSM) Task Force on Women's Issues. The conference focused on the topic that would eventually be known as the female athlete triad (Triad) (Otis, Drinkwater, Johnson, Loucks, & Wilmore, 1997). The components of the Triad (disordered eating, amenorrhea, and osteoporosis) were identified and later expounded upon in the 1997 Position Stand of the ACSM. At the time, the Triad and its components were often not recognized, under reported, or even denied (Otis et al., 1997). The ACSM position stand aimed to increase awareness of the Triad and its implications for health to call for more Triad research, including the etiology, prevalence, prevention, and treatment.

A decade after the publication of the ACSM's first position stand, a revised Triad position stand was published. Within the span of time between the first and second Triad position stands, a significant amount of research as completed on the Triad (Otis et al., 1997; Nattiv et al., 2007). While the foundational concepts of the Triad remained the same, many revisions were introduced in the new position stand that correlated with the new knowledge gained from Triad related research (Nattiv et al., 2007). A major change in the new position stand was the definition of the three components of the Triad. The original Triad components were reevaluated and deemed restrictive classifications for female athletes. In the revised position stand, the new components were defined as energy availability (EA), menstrual function, and bone mineral density (BMD) (Nattiv et al., 2007). Along with the development of new component definitions, the ACSM also released the spectrum of EA, menstrual function, and BMD. This spectrum of the three Triad components goes into greater depth on how they are interrelated and how female athletes fall within the spectrum of health depending on their exercise and dietary habits (Nattiv et al., 2007).

In response to the call for further research into etiology, prevalence, prevention, and treatment of the triad, the updated position stand provided additional information on each of these topics. Originally, low body fat, the stress of exercise, or eating disorders were thought to be a possible cause for female athletes to develop one or more of the Triad components (Slater et al., 2017). Further research disproved the theory that low body fat or the stress of exercise increased a female athlete's risk for developing the Triad (Slater et al., 2017). It was also discovered that athletes who do not have an eating disorder can still be diagnosed with the Triad (Slater et al., 2017). Despite these theories being rejected, evidence shows a correlation between nutrition status and the Triad (Nattiv et al., 2007). It is now known that the root cause of the Triad is low EA in athletes and sustained low EA can impair the health and performance of female athletes (Nattiv et al., 2007).

When the Triad was originally introduced and defined, prevalence studies had not been completed. What had been identified was the type of female athletes that were at risk for developing the Triad or a component of the triad. All physically active females are potentially at risk for developing one or more of the Triad components, but there are specific criteria identified that could increase an athlete's risk for the Triad. Risk factors include, female athletes who participate in a sport that emphasizes low body weight (distance running, cycling, and cross country skiing), begin training at a young age, are required to wear contour-revealing clothing in their sport (volleyball, swimming, diving, and distance running), participate in a sport that weight categories are used (wrestling, horse racing, martial arts, and rowing) or when performance is subjectively scored (dance, figure skating, and gymnastics) (Brunet, 2005; Manore, Kam, & Loucks, 2007; Nattiv et al., 2007; Otis et al., 1997).

Prevalence of the Triad is an important topic in Triad research. Knowing how many female athletes have one or more components of the Triad and what type of athletes are developing Triad help researchers develop interventions to educate athletes on the Triad. Currently in Triad research, the prevalence of female athletes with one or more components of the Triad is unknown. This is due to variation in research methods between Triad studies. Other factors, identified by the ACSM's Triad Position Stand (2007), that affect the difficulty of classifying the prevalence percentage of athletes with the Triad include lack of a control group, variation in assessment criteria, and heterogeneity in the athletic populations studied (Nattiv et al., 2007).

In a meta-analysis conducted by Gibbs, Williams, and De Souza (2013), a varying amount of Triad prevalence in female athletes was observed. Prevalence of all three Triad components in athletes ranged from 0% up to 4.3% (Gibbs, Williams, & De Souza, 2013). One study conducted in 2010 by Pollock et al. on 44 elite female endurance athletes, produced a 15.9% prevalence of all three Triad components. When identifying if athletes had at least two components of the Triad, prevalence ranged from 2.7-26.9%. And studies that analyzed the prevalence of athletes having at least one Triad component ranged from 16-60%. The prevalence of low EA, menstrual dysfunction, and low BMD varies in athletes and will be discussed further in their respective sections.

Educating female athletes on the Triad and implementation of screening female young athletes for the Triad can help to prevent the development of the Triad in adolescent and collegiate female athletes. The ideal time to screen an athlete for the Triad is during their preparticipation health exam early on in the female athletes' sports career and the athlete should be annually screened for the Triad (Johnson, 1992; Nattiv et al., 2007; Rumball & Lebrun, 2004). During the exam the physician specific information to inquire about when collecting the athlete's health history include energy intake, dietary practices, weight fluctuations, eating behaviors, and EEE. If their history suggests that the athlete may have one or more components of the triad, the physical exam can be used to investigate for further signs and symptoms. Other signs and symptoms of the Triad components are bradycardia, cold/discolored hands and feet, hypercarotenemia, lanugo hair, and parotid gland enlargement (Nattiv et al., 2007). Laboratory tests supplement the physical exam to provide further evidence to the athlete's health status (Nattiv et al., 2007). If an athlete has a history of fractures or stress fractures, it would be beneficial to have the athlete's BMD assessed using a dual-energy X-ray absorptiometry (DXA).

Disordered Eating

In the first position stand released by the ACSM in 1997, one of the three components of the triad identified was disordered eating. Disordered eating can be defined as a spectrum of harmful and/or ineffective eating behaviors the individuals use to lose weight or attain a lean appearance (Otis et al., 1997). Disordered eating behaviors influence the athlete's dietary intake, which affects their performance in their sport and ultimately their overall health (Otis et al., 1997). Disordered eating behaviors that have been observed in female athletes include fasting, diet pills, excessive exercise, laxatives, diuretics, vomiting, inadvertently failing to match their energy intake with their energy expended, episodic fasting, and chronic voluntary starvation (Otis et al., 1997). Restrictive eating behaviors can lead to the development of anorexia nervosa and bulimia nervosa. Anorexia nervosa (AN) is "an eating disorder characterized by restrictive eating in which the individual views herself as overweight and is afraid of gaining weight even though she is at least 15% below the expected weight for age and height" (Nattiv et al., 2007). Bulimia nervosa (BN) is "an eating disorder in which affected individuals, usually in the normal weight range, repeat a cycle of overeating or binge-eating and then purging or other compensatory behaviors such as fasting or excessive exercise" (Nattiv et al., 2007).

Female athletes are at a greater risk for developing disordered eating habits or an eating disorder (Nattiv et al., 2007). During adolescence, a female is most vulnerable to the numerous sport specific pressures including changes that occur during puberty, pressure from fellow athletes, a focus on body image, and society's preoccupation with thinness (Otis et al., 1997). If a female athlete does not have proper support from her coaches, teammates, and parents, her desire for success in sport can lead to unhealthy dietary or exercise habits. If unhealthy habits are formed, the female athlete can fall into a progressive pattern, which if left untreated, can lead to the development of the Triad. Due to social pressures and misinformation, the female athlete may begin dieting to achieve a lower body weight in order to improve athletic performance. The diet of the athlete may become increasingly restrictive over time and more unhealthful eating behaviors may form. This could lead to the use of pathogenic weight control behaviors including fasting, diet pills, laxatives, vomiting, or excessive exercise (Beals & Meyer, 2007). The resulting energy restriction related to either low dietary intake, excessive exercise, or a combination of the two, may increase female athlete's susceptibility to MD and low BMD. In order to prevent athletes from entering the progressive pattern leading to restrictive eating behaviors, education on the importance of fueling the body properly should be given to adolescent female athletes. Triad education interventions could help decrease the prevalence of the Triad in adolescent, high school, collegiate, and even elite female athletes.

A false, but prevalent notion among some female athletes and coaches is that lower body weight will improve performance. Brownell et al. (1992) described a list of personality traits associated with disordered eating problems which include competitiveness, concern with performance, compulsive concern with body shape, and perfectionism. The prevalence of disordered eating habits among collegiate female athletes is equal to or greater than the general female population (Otis et al., 1997). Smolak, Muren, and Rubble (1999) conducted a meta-analysis and found that among college students, collegiate athletes were at a significantly higher risk for eating issues compared to their non-athlete classmates. As collegiate female athletes participate in restrictive eating habits to lose weight the consequence of the subsequent decrease in energy intake is that the athlete is at a higher risk for developing the other two components of the Triad, MD and low BMD (Beals & Meyer, 2007).

As Triad research has progressed, more studies have examined more than one component of the Triad at a time (Slater, Brown, McLay-Cooke, & Black, 2017). This has led to the discovery that female athletes could develop MD and decreased BMD without disordered eating behaviors or an eating disorder (Beals & Manore, 2002). Loucks and colleagues developed and carried out two different studies to further investigate the correlation of dietary intake and EEE and how it relates to MD (Loucks & Heath, 1994; Loucks, Verdun, & Heath, 1998). Based on the results of their studies, they proposed that EA is a key factor in menstrual issues associated with the Triad (Slater et al., 2017).

Energy Availability

In 2007, the ACSM published a new position stand on the Triad which defined the new terms of the component EA. Instead of limiting the classification to disordered eating or eating disorders, the ACSM broadened their definition and established the new classification as EA (Nattiv et al., 2007). While there are female athletes who still battle with eating disorders and disordered eating, the component was reclassified because the root cause of the Triad was identified as low EA.

EA is defined as energy intake minus exercise energy expenditure per kg of lean body mass (LBM) (Nattiv et al., 2007). LBM is used in the calculation because LBM is more metabolically active than fat mass (Manore, Kam, & Loucks, 2007). Optimal EA in a female athlete is defined as > 45 kcal/kg LBM/day. (Manore et al., 2007). If an athlete’s EA is lower than 30 kcal/kg LBM/day, menstrual function and bone health can be negatively affected (Loucks, Kiens, Wright, 2011; Loucks, 2017; Melin et al., 2014). This is because energy consumed in the diet is used by the body to fuel basic physiological processes including cell maintenance, immunity, growth and development, reproduction, thermogenesis, and physical activity (Manore et al., 2007). When the body uses energy for one of these processes, it is no longer available to be used in another process. As an athlete participates in their sport's training, practices and games, they will expend energy that is no longer available for other physiological processes. Athletes can adjust EA through increasing dietary intake or by decreasing EEE (Nattiv et al., 2017). If an athlete does not consume the daily recommended number of calories, low EA can occur. If an athlete consumes the daily recommended calories, but then participates in excessive exercise which exceeds the number of calories they consumed, low EA can occur. The goal of every female athlete should be to adjust daily energy intake to match the daily EEE, allowing the female athlete's EA to be greater than 45 kcal/kg LBM/day (Nattiv et al., 2007). Achieving this goal will cause the female athlete to maintain eumenorrhea and optimal BMD, not accounting for any unseen disorders unrelated to sport or exercise. Chronic low EA can result in health complications that involve the body's cardiovascular, endocrine, reproductive, skeletal, gastrointestinal, renal, and central nervous systems (Thein-Nissenbaum, 2013).

As mentioned previously, some athletes have an eating disorder or practice disordered eating behaviors may lead to low EA. While other athletes are unintentionally in a state of low EA (Nattiv et al., 2007). Triad education could be beneficial to either type of athlete. Educating the female athletes to fuel themselves properly is an important step in preventing adolescent and collegiate athletes from developing symptoms of the Triad. The first goal of prevention and treatment should be to help athletes increase EA because the root cause of the Triad is low EA (Nattiv et al., 2007). Athletes can increase EA by modifying their training level or dietary intake. If the athlete has no desire to decrease the duration or intensity of training, they will need to increase their total dietary intake for the day. Further treatment with a team of health professionals may be needed if the athlete displays behavioral characteristics of disordered eating or is diagnosed with an eating disorder.

Energy Availability Prevalence

The prevalence of low EA varies among athletes, ranging from as low as 26% to as high as 63% (Melin et al., 2014; Slater et al., 2016; Reed et al., 2013). Factors that affect low EA prevalence include the type of sport an athlete plays, if an athlete is in season or in off-season, total training hours in a week, and daily dietary intake. Reed, De Souza, and Williams (2013) conducted a study on Division 1 female soccer players (n= 19) to determine the change in EA across the season. The prevalence of low EA across the athletes ranged from 12-33% throughout the season, with low EA being the highest during mid-season. Reed and colleagues discovered that among these athletes there was a lack of an increase in the athlete’s energy intake from pre to mid-season. To prevent an increase in prevalence of low EA, athletes need to increase their dietary intake as their sport training intensity and duration increases.

Identifying an athlete’s risk for developing low EA is an important factor in the prevention of Triad components. Slater, McLay-Cooke, Brown, and Black (2016) estimated the prevalence of risk of low EA in recreational athletes. The prevalence of risk of low EA was 45% (n= 49) and 55% (n= 60) of athletes were not at risk for low EA. Based on the data collected on the recreational athlete’s average amount of exercise a week, it was concluded that for every extra hour of exercise per week, risk for low EA increased by 1.13 times. As triad interventions are developed, educating athletes on the relationship between excessive exercise and risk for the Triad is an important topic that should be addressed in order to help athletes prevent the development of the Triad.

Menstrual Function

Physiology of the Menstrual Cycle

The menstrual cycle is a process associated with the reproductive system in the female body that involves specific organs and regulating hormones which control three phases (follicular, ovulation, and luteal phase). The main organs and glands involved in a female's reproductive system are the ovaries, uterus, hypothalamus, and the anterior pituitary gland (Tingley, 2005). For most women, the length of a normal menstrual cycle is 28-30 days (Nattiv et al., 2007). The follicular phase lasts about 14 days and the luteal phase lasts about 12-15 days (DeSouza, 2003).

The four core hormones in the cycle are estrogen, progesterone, luteinizing hormone (LH), and the follicle stimulating hormone (FSH) (Mosavat, Mohamed, & Mirsanjari, 2013). Another important hormone, but not one of the core hormones is the gonadotropin-releasing hormone (GnRH). The hypothalamus releases GnRH which stimulates the anterior pituitary gland to release FSH and LH (Mosavat, Mohamed, & Mirsanjari, 2013). As FSH increases in concentration its primary action affects the primary follicle found in the ovary. FSH stimulates the primary follicle, containing the primary oocyte, to develop into the secondary follicle and secondary oocyte. GnRH also stimulated the release of LH, which acts on the secondary follicle. The structure of the secondary follicle has granulosa cells and theca cells. The LH stimulates the theca cells to form androgens. The newly made androgens will find their way to the granulosa cells, which will absorb the androgens to produce estrogen. Without a steady secretion of LH, the production of estrogen could be affected. Estrogen has two important roles in the menstrual cycle. First, estrogen causes an increase or thickening of the endometrium in the uterus, preparing the uterus for fertilization and a possible pregnancy. Second, estrogen produces a positive feedback cycle stimulating the hypothalamus to release more GnRH, consequently increasing the amount of LH released (DeSouza et al., 1998). Eventually, towards the end of the follicular phase because of the increased amount of LH being secreted, also known as the LH surge. The LH surge signifies the beginning of ovulation (Loucks et al., 1998; Loucks & Verdun, 1998). During ovulation, the secondary follicle bursts and the secondary oocyte is released into the fallopian tubes and travels to the uterus. LH will then cause the remaining structure of the secondary follicle to form the corpus luteum.

The responsibility of the corpus luteum is to release estrogen and progesterone. Progesterone maintains the thickening of the endometrium initiated earlier in the cycle by estrogen. Now in the luteal phase, the last days of the menstrual cycle are approaching. It is necessary for a decrease in the number of hormones being released in order to allow the cycle to end. The estrogen and progesterone released by the corpus luteum send a negative feedback signal back to the hypothalamus. This negative feedback decreases the amount of GnRH released by the hypothalamus, which in turn decreases LH and FSH secretion by the anterior pituitary gland. The corpus luteum cannot be maintained when LH concentration decreases, so eventually the secretion of estrogen and progesterone decreases as well. The endometrium thickness will decrease, and menstruation will begin, restarting another cycle.

Menstrual Dysfunction (MD)

Within the Triad spectrum of menstrual function, there is a range from eumenorrhea (normal menstrual function) to functional hypothalamic amenorrhea (Nattiv et al., 2007). Due to environmental or physiological factors, a woman may not always be in a eumenorrheic state (Nattiv et al., 2007). This could be due to fluctuations in the amount and/or timing of reproductive hormone secretion, decreased amount of energy to sustain the physiological process, or other related diseases or medical conditions. Types of menstrual disorders include primary amenorrhea, secondary amenorrhea, oligomenorrhea, anovulation and luteal phase deficiency. Amenorrhea is classified as an absence of menstruation. Primary amenorrhea is diagnosed if an adolescent does not begin menstruating before the age of 16 (Nattiv et al., 2007). Secondary amenorrhea can be diagnosed if a woman's period ceases for 3 months or greater. Oligomenorrhea refers to light or infrequent menstruation. This menstrual disorder is diagnosed if the woman's menstrual cycle is greater than 35 days apart (Nattive et al., 2007). Within the defined menstrual function range, there are subclinical menstrual disorders which can occur, including anovulation and luteal phase deficiency (LPD). While amenorrhea can occur in female athletes, LPD and anovulation are menstrual disorders linked to female athletes (Redman & Loucks, 2005). Anovulation can be defined as the occurrence of a menstrual cycle, but ovulation does not occur (Redman & Loucks, 2005). LPD or luteal suppression occurs if the corpus luteum secretes an inadequate amount of progesterone and has a luteal phase shorter than 11 days (Redman & Loucks, 2005). Anovulation and LPD can occur without a display of observable symptoms, which may make it more difficult to diagnose (Redman & Loucks, 2005).

The theory of the root cause of the female athlete's menstrual function has changed over the years (Slater et al., 2017). It was hypothesized that too low of a body weight or body fat percentage would cause menstrual dysfunction (Slater et al., 2017). Other possible considerations for causes included an excessive or heavy training load, energy availability, and psychological stress which could disrupt normal endocrine processes resulting in menstrual dysfunction (Warren & Perlroth, 2001; Loucks, 2003). In 1962, Dr. Gyula Erdelyi completed one of the first cross-sectional studies observing the affect sport and exercise had on the female athlete's menstrual function. Questionnaires were used to collect data from 729 Hungarian competitive female athletes. Eleven percent of the athletes reported an unfavorable change in menstrual function during exercise. It was concluded that the training load of the athletes could influence menstrual function in athletes (Erdelyi, 1962). Bullen et al., (1985) examined how progressively increasing the female college students' training load would affect menstrual function. They discovered that after the implementation of a 5-week training program, women participating in a vigorous exercise program and lose weight have a greater chance of luteal and ovulation disturbances (Bullen et al., 1985). While the results of these studies and others showed a correlation between MD and exercise training load, the mechanism causing these disturbances in the menstrual cycle was not determined. As research progressed, it became clear that the main factor influencing the health of a female athlete's menstrual function was EA (Nattiv et al., 2007).

MD Prevalence

Evaluating the prevalence of secondary amenorrhea in all types of female athletes (recreational, collegiate, elite), prevalence ranged from 1.0% to 60.0% in 34 studies (n= 5607) and out of 13 studies, primary amenorrhea prevalence ranged from 0.0% to 56.0% (n=

2216) (Gibbs, Williams, & De Souza, 2012). The type of sport, age of athlete, training volume, and body weight affects the prevalence of amenorrhea (Nattiv et al., 2007).

In a study conducted by Torstveit and Sundgot-Borgen (2005) they examined the prevalence of menstrual dysfunction in elite Norwegian athletes (N= 669) and non-athletic females (N= 607) age 13-39 and represented 66 different sports or events. Out of the athlete participants, 31.4% had a type of MD. In a study conducted by Thompson (2007), she surveyed 300 collegiate cross country female athletes to determine characteristics of the Triad in the athletes. Thompson discovered that 77% of the athletes had normal menstruation, 5.3% reported amenorrhea, and 17.7% reported oligomenorrhea. In another study reviewing the prevalence of Triad components in collegiate athletes, 26% of athletes (n=29 out of total N=112) had some type of MD.

In conclusion, menstrual function is an important physiological process and female athletes need to be aware the signs of MD. Though over the years, the theory of the root cause of MD in female athletes changed, there is now research-based evidence that MD in female athletes is caused by low EA. If an athlete does not fuel properly while participating in her sports, menstrual function can be affected. An important part of Triad prevention is educating female athletes on the Triad and why MD is not good for their overall health.

Bone Health

The human skeleton system is the structure that allows athletes to run, jump, and perform all the required physical movements to be successful in their sport. Bones require certain nutrients from food and a certain level of mechanical stress in order to develop into dense bones with a low risk for fracture (Beals & Meyer, 2007, Kohrt, Bloomfield, Little, Nelson, Yingling, 2004). The strength of the bone depends upon the BMD, bone mineral structure, and bone protein quality (Nattiv et al., 2007). While BMD is not the only determining factor of bone health, it is an important factor in measuring bone quality (Brunet, 2005). Stiffness of a bone, or the amount of force needed to bend it, is determined by the bone mineral structure. Bone toughness, or the amount of energy needed to break a bone, is determined by the quality of bone protein (Manore et al., 2007). As a result of the complex nature of bone strength and structure, the risk for fracture in athletes can vary widely (Nattiv et al., 2007).

If an athlete does not properly fuel for their sport they are at a higher risk for low BMD (Beals & Meyer, 2007). The etiology of low BMD in athletes has direct correlation to MD (Nattiv et al., 2007). If the normal menstrual cycle is disturbed due to inadequate energy intake, anovulation will occur, leading to oligomenorrhea, and eventually amenorrhea. When an athlete is in a state of amenorrhea, estrogen production is significantly lower than a eumenorrheic athlete (Warren & Perlroth, 2001). Estrogen is important in the physiological process of bone maintenance because it protects the bones from bone resorption (Beals & Meyer, 2007). Bone resorption is the process of tissue breakdown in bones (Creighton, Morgan, Boardley, & Gunnar Brolinson, 2000).

In a healthy individual there is a balance between bone formation and bone resorption (Walsh, 2017). This process is known as bone remodeling. The purpose of bone remodeling is to maintain bone health and replace old or damaged bone (Sims & Martin, 2014). The two types of osteocytes that regulate bone remodeling, specifically bone formation and bone resorption are osteoblasts and osteoclasts, respectively. Osteoblasts' enable bone remodeling and maintain the bone matrix and BMD. Osteoclasts are cells that aid in bone resorption or the breakdown of bone. Osteoclasts release hydrogen ions and other enzymes that dissolve bone mineralization and breakdown bone matrix (Walsh, 2017). While many factors play a role in bone health and remodeling, estrogen is the focus of Triad research and maintaining bone health in female athletes. Estrogen's role in preventing low BMD is that it inhibits osteoclasts ability to initiate bone resorption. Estrogen also increases apoptosis of osteoclasts, or the death of the osteoclast cells. If estrogen levels are diminished due to MD, the balance of bone formation and bone resorption cannot be maintained resulting in a decrease of BMD (Walsh, 2017).

Prevalence of Low BMD

Studies have shown that athletes, on average, have a higher BMD than their non-athlete counterparts (Torstveit & Sundgot-Borgen, 2005). Torstveit and Sundgot-Borgen found that athletes had 3-20% higher BMD levels than the non-athlete controls. They also discovered that athletes' BMD varied based on the type of sport they participated in. For example, athletes who participated in high impact sports (gymnastics, track and field, basketball, volleyball, etc.) had a higher BMD than athletes who participated in medium impact (long distance running, cross-country skiing, triathlon, etc.) or low impact (cycling, swimming, climbing, etc.) sports.

In a study conducted by Torstveit & Sundgot-Borgen (2005) the prevalence of low BMD in at least one of the five measurement was 11%. Mudd and colleagues (2007) also studied the difference of BMD in collegiate female athletes, using a DXA scan, they measured total BMD as well as lumbar spine, pelvis, and lower leg BMD in their participants. Controlling for menstrual status and bone mass, the runners (n=25) had the lowest overall BMD (total body = 1.079 ± 0.055) and gymnasts (n=8) had the highest overall BMD (total body = 1.173 ± 0.036). Out of all the participants (n=99) runners also had the highest number of athletes with oligomenorrhea (n=7) and amenorrhea (n=4). Mudd et al. concluded that the runners had a lower overall BMD and site-specific BMD due to MD and high training volume coupled with insufficient energy intake.

Diagnostic Criteria for Bone Health

An important component of Triad research is accurately diagnosing athletes with low BMD using standardized methods and criteria. The majority of Triad research is conducted on adolescent and premenopausal female athletes (Nattiv et al., 2007). In this population, there are many factors that influence BMD including bone size, pubertal stage, skeletal maturity, and body composition (Nattiv et al., 2007). The component of osteoporosis is defined as “a disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to enhanced skeletal fragility and increased risk of facture” (Nattiv et al., 2007). The first ACSM Triad position stand used a diagnostic criteria for osteoporosis that was established by the World Health Organization (WHO). An article, summarizing the issues discussed by the WHO expert panel led them to develop osteoporosis diagnostic criteria using z-scores as a way to determine BMD health (Kanis et al., 1994). Table 2.1 displays the criteria set forth by the WHO panel.

Table 2.1 WHO Diagnostic Criteria for BMD Health

	Bone Mineral Density Value
Normal	≤ 1 SD
Osteopenia	1-2.5 SD
Osteoporosis	≥ 2.5 SD
Severe Osteoporosis	≥ 2.5 SD plus the presence of one or more fragility fractures

While these cut points for BMD health shown in Table 2.1 are beneficial in older adults, they may not be applicable to adolescent and premenopausal female athletes due to the age difference and lifestyle habits between the individuals used in the WHO article and the athletes studied in Triad research. The International Society for Clinical Densitometry (ISCD) published a position stand on bone density testing and stated that the WHO classification of osteoporosis should not be applied to healthy premenopausal women and that Z-scores should be used for determining healthy bone density (Leib, Lewiecki, Binkley, & Hamdy, 2004). They recommend that Z-scores be used to account for the age of the female athlete and if they've reached peak bone mass (Leib et al., 2004). Table 2.2 displays the new recommendations for diagnosis bone health in premenopausal athletes. When the new Triad position stand was published in 2007, the ACSM referenced the ISCD's recommendation and addressed the issue that the previous 1997 Position Stand's diagnostic criteria used postmenopausal women standards and applied it to premenopausal women and adolescent athletes. Adjustments needed to be made in order to develop new diagnostic criteria for bone health that would be better suited for young female athletes (Beals & Meyer, 2007).

Table 2.2 Current Recommendations for Diagnosis of Low BMD in Premenopausal Athletes.

	ISCD	**International Olympic Committee (IOC)**
Osteopenia	>20 years of age: ≤ -2	>20 years of age: ≤ -1
Osteoporosis	<20 years of age: ≤ -2	>20 years of age: ≤ -2.5

In the ACSM's update on the Triad, a Z-score < -2 should be referred to as "low bone density below the expected range for age (Nattiv et al., 2007). They recommended that the terms *osteopenia* should not be used for this population and that the term *osteoporosis* should only be used if the athlete has a BMD < -2 plus one or more secondary risk factors including chronic malnutrition, eating disorders, hypogonadism, glucocorticoid exposure, and/or previous fractures. Athletes who participate in weight-bearing sports tend to have a BMD that is 3-20% higher than nonathletes. Athletes on average have a higher BMD, so a Z-score < -1 should warrant further attention and steps should be taken to examine the athlete's risk for fracture (Nattiv et al., 2007).

Dietary Energy Intake

Chronic low EA can lead to MD and low BMD. All of these issues can affect an athlete's performance in their sport and their overall health (Nattiv et al., 2007). The standardized equation for calculating EA is energy intake (EI) – EEE/kg LBM (Nattiv, 2007). The two main variables that determine an athlete's EA are energy intake and energy expenditure (Nattiv et al., 2007). The most common method researchers use to collect data on energy intake of female athletes is self-reporting dietary intake. To collect accurate self-reported data by participants, researchers educate participants on how to keep a food record. Researchers train participants on how to accurately recording the weight or serving sizes of the food (Guebels, Kam, Maddalozzo, & Manore, 2014; Reed, De Souza, & Williams, 2013). Despite researchers' efforts of educating athletes on how to accurately report food intake, a limitation of self-reported food records is athletes' tendency to underreport their intake (Reed et al., 2013).

Exercise Energy Expenditure (EEE)

The other variable affecting EA is EEE. There is not a standardized method for quantifying EEE among Triad research. A review of the current Triad literature, specifically on studies that calculated EEE, indicate that there are a variety of methods that have been used to quantify EEE in an athlete, as well as tools used for data collection (Table 2.3). Tools that have been used to collect EEE data in athletes include indirect calorimetry, the

Ainsworth compendium of physical activity, exercise logs, heart rate monitors, and accelerometers.

Table 2.3 Methods for Calculating EEE

Study	Sport	Sample Size (N)	Methods for calculating energy expenditure	Accelerometer used?
Reed, De Souza, Williams (2013)	Division 1 soccer	19	The data used to collect EA was exercise only energy expenditure; they did not include any usual waking activities; only purposeful exercise Subtracted habitual waking activities from purposeful exercise energy expenditure to get exercise only energy expenditure Three methods were used to capture energy expenditure: 1. Polar Team Software - heart rate monitors were worn during team train sessions such as soccer practice or games, and weight lifting 2. Polar FT4 HR monitors – heart rate monitors were worn during non-team training sessions 3. Purposeful exercise logs – when heart rate monitors were not able to be worn (<10%) Ainsworth compendium of physical activity log was used to determine METs	No
Reed, De Souza, Mallinson, Scheid, Williams (2015)	Recreational exercising college aged women	91	Purposeful exercise sessions longer that 10 minutes in duration with a heart rate higher than 90 beats per minutes for 7 days. They completed the diet logs during the week of the 7 day exercise log. Purposeful exercise included elliptical, running, or strength	No

			training. Daily living activities (e. g. cleaning the house or walking the dog) were not included. Wore a Polar S620 or a RS400 heart rate monitor during the 7 day exercise session; the Own Cal feature was use. Own Cal is a validated tool to calculate EEE from heart rate.	
Guebels, Kam, Maddalozzo, Manore (2014)	Endurance trained women	17	Assessed EEE with 4 methods 1. All planned exercise (all purposeful PA regardless of intensity; not included PA from social games, hobbies, leisure pastimes, or transport related activity. (< 30 min) 2. All planned exercise, bike commute, and all walking 3. All exercise at 4 MET's and greater 4. All exercise greater than 4 METs	Yes
Hoch et al. (2009)	Varsity athletes and sedentary control group	160	EE was calculated from duration of sports participation, intensity of exercise, weight, age, and sex from the Ainsworth compendium of physical activity. Subjects were asked to record the duration and frequency of sport participation over the previous twelve months	No
Ihle and Loucks (2004)	Healthy, young, regularly menstruating women	29	Wore an accelerometer to monitor physical activity to estimate 24-h energy expenditure. TEE for each 24HH was calculated for each treatment day Controlled EE was defined as the TEE during exercise as measured by indirect calorimetry, the sum of the intended EEE; plus the portion of each subject's 24EE	Yes

Melin et al. (2014)	Elite endurance athletes	40	Heart rate monitors and training logs were used to assess EEE; subjects maintained and followed their normal training routine; described each training session in as much detail as possible (type, duration, intensity); wore heart monitor during all training sessions except when swimming or cycling. To calculate EEE, they used the training log and the HR values from each training session	Yes
Loucks and Thuma (2003)	Healthy, young, regularly menstruating women	29	3 treatment days; subject wore accelerometer during all waking hours to estimate 24EE; Controlled EE was the TEE during exercise as measured by indirect calorimetry; Included subjects' habitual walking EE, CEE due to exercise	Yes
Loucks, Verbun, Heath (1998)	Healthy, young, regularly menstruating women	9	EEE was calculated as the controlled EE during exercise minus the subjects' habitual waking EE that would've occurred doing normal activities	No
Day, Wengreen, Heath, and Brown (2015)	Division 1 female runners	27	3-days recording EEE using the Actigraph GTX3 accelerometer Used daily total energy expenditure	Yes

Indirect calorimetry is the gold standard for calculating an individual's EEE. It quantifies energy expenditure by measuring the amount of O_2 consumed and the amount of CO_2 produced. Indirect calorimetry provides accurate readings of EEE, but this method of EEE data collection can be expensive and is best used in a laboratory setting (Loucks &

Thuma, 2003). This method was used many times by Loucks and colleagues as they tested and identified the cutoff values for low EA the affects they have on menstrual function and bone health (Loucks et al., 1998; Louck & Thuma, 2003; Ihle & Loucks, 2004). Loucks and Thuma discovered that as EA decreased below 30 kcal/kg LBM/day, negative effects were seen on LH pulsatility. They found that when EA was restricted between 10-20 kcal/kg LBM/day the LH pulse frequency was suppressed (Louck & Thuma, 2003).

The Ainsworth compendium of physical activity is used to calculate EEE. The compendium was first developed and published in 1993 as a tool that researchers could use to assign metabolic equivalent (MET) values to types of physical activity (Ainsworth et al., 1993). Since then two updates have been made to the Ainsworth compendium which have improved the coding processes and MET value comparisons to physical activity (Ainsworth et al., 2011). Exercise logs are another common way for researchers to collect self-reported exercise data from participants and used them in addition to the Ainsworth compendium (Hoch et al., 2009; Reed et al., 2015; Guebels et al., 2014; Melin et al., 2014). Heart monitors are used to collect EEE data by measuring the intensity of the athlete's exercise through their heart rate. The exercise intensity information can then be converted into EEE (Melin et al., 2014).

The most common methodology used to collect EEE data in Triad studies is either the pairing of heart rate monitors and exercise logs or accelerometers and exercise logs. Though studies use a variety of criteria for quantifying EEE in participants, the criteria used among most studies is all energy expended during purposeful exercise. Purposeful exercise is defined as energy expended during planned exercise sessions minus habitual daily living activities (See Table 2.3).

Accelerometers

A final tool used by researchers to collect EEE data is an accelerometer which is a non-invasive and low burden method for collecting physical activity data from participants in a study (Keadle, Shiroma, Freedson, & Lee, 2014). Accelerometers are worn by participants to measure movements and the data can be used to estimate physical activity information, including activity counts, energy expenditure (kcal), steps, and activity intensity (McMinn et al., 2013). Accelerometers do not measure EEE specifically. Instead the accelerometer measures the acceleration of the individual which then can be used to estimate the EEE (McMinn et al., 2013). Accelerometers can measure acceleration using one, two, or three axes. The possible axes used by accelerometers include vertical (VT) plane, the antero-posterior (AP) plane, and medio-lateral (ML) plane. Accelerometers also use an inclinometer in order to recognize if the wearer is sitting, lying, or standing (McMinn et al., 2013).

EEE Equations

Algorithms are used to process the acceleration data collected by the accelerometer. The Freedson VM3 combination (2011) equation, incorporates the three perpendicular planes of the GT3X+ accelerometer. In a study completed by Lynden, Kozey, Staudenmeyer, and Freedson (2011) they evaluated multiple accelerometers, energy expenditure and MET prediction equations. Their purpose was to measure the accuracy of the prediction equations compared to indirect calorimetry measurements. For the ActiGraph GT3X+ accelerometer, they found that the Freedson Adult (1998) equation tends to underestimate EEE in all activity whether it is an activity of daily living at a light intensity or a higher intensity. The percent of misclassification for the Freedson Adult (1998) equation was 10% for light intensity, 20% for moderate intensity, and 55% for vigorous intensity. Across all three intensity levels the

Freedson Adult (1998) equation underestimates energy expenditure by 30%. Researchers should be aware of the limitation of misclassification by accelerometers when using them in their studies to quantify participants' EEE.

One equation used to calculate EEE from METs data was the Swartz Adult Overground & Lifestyle (2000) equation. Lynden et al. (2011) found that the Freedson Adult (1998) equation consistently underestimated energy expenditure for moderate to vigorous intensity levels. Based on this underestimation of the Freedson Adult (1998) equation, a new prediction equation was developed by Swartz et al. (2000). Based on Lyden et al. (2011) they found that compared to the Freedson MET model, the Swartz equation had an increased accuracy of moderate intensity prediction, but did not improve in the underestimation of vigorous intensity (Swartz et al., 2000).

Wear Time Validation

The activity data collected by accelerometers can be divided into wear and non-wear time. Wear time data includes all waking periods during the days the accelerometer is worn and non-wear time includes periods during the day individuals wear the accelerometer. The purpose of analyzing accelerometer data with a wear time validation algorithm is to ensure the accelerometer was worn for the required time frame during the days they wore the accelerometers.

One equation used to calculate wear time validation is the Choi (2011) equation. In 2011, a study was conducted to test and validate the Choi equation, a new algorithm developed to improve the old wear time validation algorithm. The proposed equation allowed for a 90-min window of time of consecutive zero counts, compared to the old equation which only allowed for a 60-min window of consecutive zeros. This new algorithm also allows for short bursts of nonzero counts lasting less than two minutes. They concluded that the new algorithm better classified wear and non-wear times which will help improve predictions of time spent being sedentary and time spent participating in physical activity. (Choi, Liu, Matthews, & Buchowski, 2011). A valid day of accelerometer wear is defined as wearing the accelerometer for 10 hours or more (Tudor-Locke, Camhi, & Troiano, 2012).

EEE Criteria

Another limitation in current Triad research is there is no standardized EEE criteria that has been identified to use when calculating EA. As seen in table 2.3, some researchers only count energy expended if it is above a certain MET, others only count it if the exercise was purposeful (related to their sport), and some studies use a combination of methods. All of this variation in studies makes it difficult for research to be compared across multiple studies. Guebels et al. (2014) identified this gap in research and conducted a study to identify the best method for calculating EEE to help improve the reproducibility and comparability of Triad research results. The four EEE methods that were tested were (1) all planned exercise regardless of intensity (not including physical activity from social games, hobbies, leisure, or transport-related activity), (2) all planed exercise plus all walking (3.3 METs) and biking (4.0 METs), (3) all exercise ≥ 4 METs, and (4) all exercise > 4 METs. They concluded that the EEE criteria that should be used to measure EA is the fourth method, > 4 METs. They believed that the benefits of the method include that it is less subjective and time consuming, it captures exercise that is moderate to high intensity, and they found in their study that this method did not have significant variation from the other three methods. Despite their conclusions, the EA values produced by the four methods had no significant difference. Also, comparing the four methods for calculating EEE was a secondary purpose. Because of this, limited statistical analysis tests were run to examine the comparison of the four EEE methods.

Low Energy Availability in Females Questionnaire (LEAF-Q)

An important factor of prevention is the ability to screen athletes and identify if they are at risk for developing one or more components of the Triad. With a standardized questionnaire to assess female athlete Triad risk, the ability to compare results between studies would increase as well as the reliability of study results. Slater and colleagues (2017) recognized the need for developing a standardized tool to diagnose low EA and other Triad components. In their review of the historical progression of Triad research and the future directions of prevention, detection, and treatment of low EA in female athletes, they concluded that developing a standardized method of risk identification needs to occur before moving forward with Triad research.

In 2014, Melin and colleagues identified this gap in the research, recognizing there were no screening tools that could be used based on self-reported physiological symptoms to determine an athletes risk for the Triad. They conducted a study with the purpose of developing a screening tool, "designed to identify female athletes at risk for the Triad". The study was conducted in Sweden and Denmark, with a sample size of 84 females, between the ages of 18-39 years of age with a training schedule of ≥ 5 times a week. This study had two parts: first to test the reliability and internal consistency of the questionnaire they developed prior to the study and second to test the validity.

From the results of the study, Melin et al. concluded that the questionnaire had an acceptable sensitivity, specificity, and internal consistency. With these positive results of the study, Melin and colleagues concluded the questionnaire could be considered for use as a screening tool for identifying a female athlete's risk for the Triad. This questionnaire was titled Low Energy Availability in Females Questionnaire (LEAF-Q). The basic premise of the LEAF-Q is that if a female athlete receives a total score ≥ 8, this suggests that they have the potential of having low EA and/or menstrual irregularity and/or low BMD.

Melin et al. (2014) concluded that although the validity and reliability of the LEAF-Q was tested, it was done so only in the group of female endurance athletes used in their study. They recommended that the LEAF-Q should be tested in studies using other sports to increase the validity and reliability of the tool. Overall, the LEAF-Q could be used as a tool to assess risk of female athletes for the Triad and to promote early detection/treatment (Melin et al., 2014). Since the development of the LEAF-Q it has been used in a variety of Triad related studies and Melin et al. (2014) hoped that the LEAF-Q would be used in other studies, so it could be tested in a variety of sports. Further use of the LEAF-Q would also allow for better comparison between studies of the prevalence of Triad risk in female athletes.

Chapter Three

Methods

Subjects

This study was an observational descriptive study of 19 female collegiate athletes at the University of Idaho. Female athletes, ages 18 years and older, participating in a sport that allowed them to wear an accelerometer during exercise were invited to participate in the study. Approval of this study was received from the University of Idaho's Institutional Review Board. Participants met with the primary investigator and received a comprehensive description of all parts of the study. A signed informed consent form was obtained from each participant before data collection began.

Procedures

Questionnaire

Athletes completed a questionnaire that included demographic questions, as well as the Eating Attitudes-26 questionnaire (EAT-26) and Low Energy Availability in Females Questionnaire (LEAF-Q) (Melin et al., 2014). These two questionnaires assessed the athlete's risk for disordered eating and the female athlete triad, respectively. EAT-26 scores higher than 20, or report of pathological disordered eating behaviors are indicative of increased risk for disordered eating. The LEAF-Q included menstrual history, injury history, and gastro-intestinal function questions. LEAF-Q scores ≥ 8 indicated high risk for the Triad.

Using the validated LEAF-Q tool, 13 questions from the questionnaire ask specifically about menstrual history allowing for classification of amenorrhea (absence of a menstrual cycle for 3 months or more), oligomenorrhea (fewer than 11 menstrual cycles per year), or eumenorrhea (normal menstruation). Participants were also asked, "How accurately do you think you can answer questions about your menstrual periods?"

Bone Density

The bone density of athlete participants was determined using Gritman Medical Center's dual-energy x-ray absorptiometry (DXA) (Hologic Discovery, Model C, Marlborough, MA). Bone density at the spine and left hip was measured, and Z-scores were reported. The recommendation by the ACSM is to categorize low BMD as < -2 in premenopausal and adolescent women (Nattiv et al., 2007). They also explained that because athletes have a higher average Z-score than non-athletes, a Z-score of < -1 warrants further investigation (Nattiv et al., 2007). Because of these recommendations, the Z-score of < -1 was used to as the diagnostic criteria for low BMD in this study.

Energy Availability

Energy availability (EA) can be defined as dietary energy intake minus exercise energy expenditure/kg lean body mass. Calculated EA is the surplus energy available after the energy expended during exercise that can be used to maintain other important physiological processes in the body (Nattiv et al., 2007). Optimal energy availability in a female athlete is > 45 kcal/kg LBM/day. (Manore et al., 2007). If an athlete's energy availability is ≤ 30 kcal/kg LBM/day, menstrual function and bone health can be negatively affected (Loucks, Kiens, Wright, 2011; Loucks, 2017; Melin et al., 2014). EA was calculated using three criteria: total activity EEE, structured planned EEE, and METs ≥ 3 EEE.

Energy Intake

To calculate EA, energy intake data and EEE data, and body composition data is needed. Participants were instructed to report dietary intake and exercise for four days (three weekdays and one weekend day). It has been found that a 1-day food record is not enough to make accurate estimations on an individual's diet. Instead it is recommended to collect a multiple-day food record in order to receive a better representation of an individual's diet (Lee & Nieman, 2013). To record dietary intake, athletes used the multi-pass Automated Self-Administered 24 hour Recall System (ASA24), which provides valid estimates of usual dietary intake (https://asa24.nci.nih.gov/). Athletes entered their dietary intake information the day after wearing the accelerometer and completing their activity log (e.g. if the participant started the study on Monday, she would wear the accelerometer and track her activities on Monday but would record her dietary intake for that day [Monday] on Tuesday).

Body Composition

Because EA is reported relative to lean body mass (LBM), body composition was also measured using the Bod Pod (Cosmed, Rome, Italy). One measurement collected from the Bod Pod testing was kg of LBM. This value (kg of LBM) will be used later to calculate EA.

Exercise Energy Expenditure (EEE)

EEE was measured using the ActiGraph GT3X+ accelerometers (Actigraph, Pensacola, FL). These small (4.6 cm × 3.3 cm × 1.5 cm) lightweight (19 g) tri-axial accelerometers monitor human physical activity in three perpendicular planes including the vertical (VT) plane, the antero-posterior (AP) plane, and medio-lateral (ML) plane. The accelerometer uses an inclinometer in order to recognize if the wearer is sitting, lying, or standing. Before data collection, the GT3X+ was initialized to set device parameters specific to the user (e. g. age, weight, height, gender, start/stop time, etc.). This device collects data on steps, calories expended, activity counts, and activity intensity (METs). Acceleration data was collected and recorded in a raw data sample frequency using 50 Hz increments. Epoch length was set at 60 seconds.

Participants were instructed to put on the accelerometer before they went to bed on the day prior to the data collection day. Participants wore the accelerometer on their right hip for the entire day (including when they were sleeping), unless they were showering. Participants were also asked to record the type, duration, and intensity of any planned exercise, unplanned exercise, or physical activity they did during the day (e.g. walking to class, sport specific practice, playing basketball with friends, etc.) lasting 15 minutes or longer (Beals, 2002). The exercise logs were used to cross-reference accelerometer results and to determine which activities were classified as structured, planned exercise.

The participants wore the accelerometer for a total of four non-consecutive days, three weekdays and one weekend day. Matthew et al. (2002) determined that in order to have data that has at least 80% reliability of physical activity measurement, at least three days are necessary. They also found that during the week physical activity differed significantly from weekend physical activity, which led them to recommend future studies using accelerometer data to include weekdays and weekend days in their measurements (Matthews, Ainsworth, Thompson, & Bassett, 2002).

Data Analysis

Data from the accelerometers was downloaded to the software ActiLife, which is compatible with the GT3X+ accelerometers. For this study, the Freedson VM3 Combination (2011), Swartz Adult Overground & Lifestyle (2000), Freedson Adult VM3 (2011) algorithms were used for energy expenditure, METs, and cut points and MVPA, respectively. The Freedson VM3 Combination (2011) equation was used because it incorporates the three perpendicular planes of the GT3X+ accelerometer. The Swartz Adult Overground &

Lifestyle (2000) equation was chosen because it has an increased accuracy of moderate intensity for predicting EEE, compared to the other METs equations. The Freedson Adult VM3 (2011) equation was used because it uses the cut points of the tri-axial vector magnitude (VM3) and to classify physical activity intensity (Sasaki, Dinesh, & Freedson, 2011).

Exercise Energy Expenditure Criteria

Once the accelerometer data was downloaded, processed, and exported to a minute by minute spreadsheet, it was then ready for analysis based on the three EEE criteria used in this study. The three criteria used in this study were total activity EEE, structured planned EEE, and METs ≥ 3 EEE.

Total Activity Exercise Energy Expenditure

Total activity EEE includes all energy expenditure reported from the accelerometer for a given day. Total activity EEE per day is calculated by the ActiLife software and includes all counts above zero.

Structured Planned Exercise Energy Expenditure

Energy expended from structured planned exercise is the sum of all energy expended during structured, planned exercise such as team practices, training sessions, etc. as reported on the participants' activity log (Guebels, Kam, Maddalooz, & Manore, 2014). This criteria does not include any free daily living activities, for example cleaning the house, walking to or from a location, hiking with friends, participating in leisure sports, etc. EEE for each day will be determined by totaling the minute-by-minute energy expenditure of each structured, planned exercise recorded on the athlete's activity log.

METs ≥ 3 Exercise Energy Expenditure

The third criteria evaluated was energy expended during exercise when MET values were greater than or equal to 3. This cut off point was chosen because 3 METs is the minimum number that counts as moderate physical activity. Data spreadsheets from the ActiLife software provide minute-by-minute MET values. Using METs ≥ 3 EEE, EEE for each day was calculated by totaling the minute-by-minute energy expenditure of each physical activity session that had an average MET value of ≥ 3 (Ainsworth et al., 2011). The physical activity session had to last at least ten minutes and METs could not drop below 3 METs for more than two minutes (lyden, Kozey, Staudenmeyer, & Freedson, 2011).

Wear Time Validation

To calculate wear time validation, the Choi (2011) equation used. This new algorithm better classified wear and non-wear times which will help improve predictions of time spent being sedentary and time spent participating in physical activity. (Choi, Liu, Matthews, & Buchowski, 2011). A valid day will be defined as wearing the accelerometer for 10 hours or more (Tudor-Locke, Camhi, & Troiano, 2012).

Statistical Analysis

Based on the research objectives, the data collected in this study was analyzed to determine prevalence of Triad components in the participants, agreement between the three EEE criteria, associations between EA (calculated using the three EEE criteria) and Triad components/risk factors, and associations between the participants' actual Triad component results to the LEAF-Q's prediction. All analyses were preformed using IBM SPSS Statistic 23 software. The statistical test used to test agreement was kappa statistic. Statistical tests used to test association included ANOVA, odds ratio, Spearman correlation, and logistic regression. To test the LEAF-Q prediction ability, sensitivity and specificity were calculated.

Results

Participants Characteristics

Forty participants began the study. However, due to busy schedules, injury, and other factors, not all participants completed all parts of the study. The final sample size used for analysis contained 19 female athletes from the University of Idaho. Participants represented seven sports. On average, participants were very lean; however, over 25% wanted to lose weight. See Table 3.1 for a summary of athlete characteristics.

Table 3.1 Athlete Baseline Characteristics

Participants (*N*= 19)	n (%) / Mean ± SD
Age, yrs	**19.05 ± 1.4**
Height, in	**66.53 ± 3.24**
Weight, kg	**60.8 ± 8.46**
BMI, kg/m^2	**21.17 ± 1.93**
Underweight (<18.5)	**2 (10.5%)**
Normal weight (18.5-24.9)	**16 (84.2%)**
Overweight (25-29.9)	**1 (5.3%)**
Obese (≥ 30)	**0 (0%)**
Body Fat, %	**18.81 ± 4.18**
Lean tissue mass, kg	**49.52 ± 6.59**
BMD, g/cm^2 (z-score)	
Lumbar Spine	**0.21 ± 1.05**
< -1	**3 (16%)**
< -2	**0 (0%)**
Femoral neck	**1.12 ± 0.72**
< -1	**0 (0%)**
< -2	**0 (0%)**
Femur	**1.14 ± 0.79**
< -1	**0 (0%)**
< -2	**0 (0%)**
Main Sport	
Track and Field	**5 (26.3%)**
Cross Country	**5 (26.3%)**
Dance	**3 (15.8%)**
Soccer	**2 (10.5%)**
Volleyball	**2 (10.5%)**
Cheerleading	**1 (5.3%)**
Tennis	**1 (5.3%)**

Wanted to maintain weight	**13 (68.4%)**
Wanted to gain weight	**1 (5.3%)**
Wanted to lose weight	**5 (26.3%)**
Average desired weight loss (lbs)	**-5 ± 4.08**
EAT-26 score	**5.11 ± 4.09**
LEAF-Q score	**7.11 ± 2.96**
Score ≥ 8	**8 (42.1%)**
Score < 8	**11 (57.9%)**

Prevalence of Triad Components

Out of the 19 total participants, 11 participants scored < 8 on the LEAF-Q and 8 participants scored ≥ 8 on the LEAF-Q (Figure 3.1).

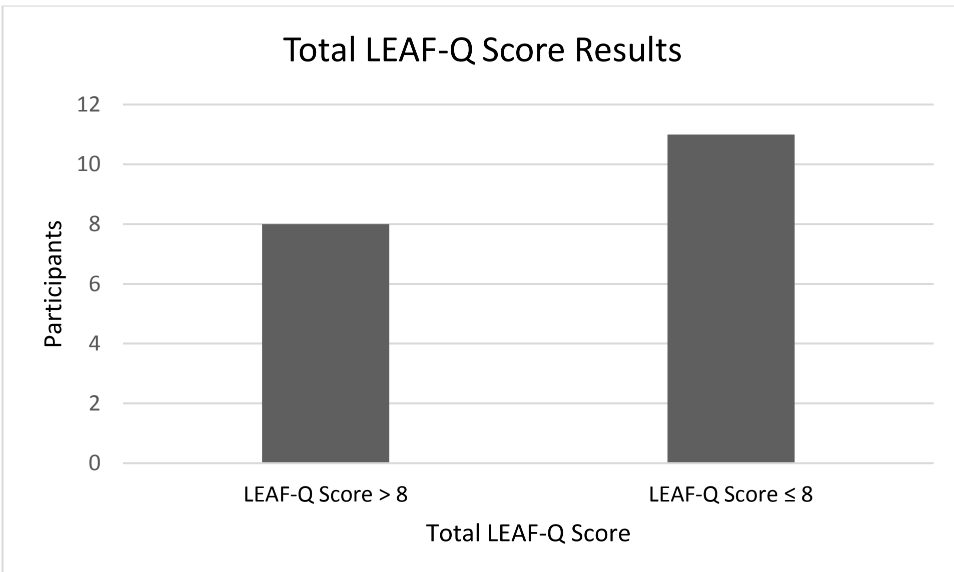

Figure 3.1 Participants (N= 19) total LEAF-Q score divided into groups of a score < 8 or of a score ≥ 8.

Participants received an average score of 1.16 ± 1.54 out of 7 points in the injury section, 1.79 ± 1.51 out of 12 points in the gastrointestinal function section, and 4.21 ± 2.78 points out of 23 in the menstrual function section. The most points received from the gastrointestinal function section was athletes feeling bloated or stomach cramps unrelated to their menstrual cycle. Thirty-seven percent of athletes felt bloated once or twice a week while 26% of athletes felt a stomachache or cramps once or twice a week. On average, only

26% athletes had 12 or more periods in a year, while 47% had less than 12 periods in the past year. Five (26%) participants reported that they could not accurately answer questions about their menstrual periods (See Table 3.2).

Table 3.2 List of LEAF-Q Questions.

Questions	Answer Choices	Possible points for each answer	Answers Frequency
	Injury Section		
A. Have you had absences from your training or participation in competitions during the last year due to injuries?	No, not at all	0	10
	Yes, once or twice	1	8
	Yes, three or four times	2	1
	Yes, five times or more	3	0
A1 If yes, how many days were you absent from training or competition due to injuries in the last year?	1-7 days	1	8
	8-14 days	2	0
	15-21 days	3	0
	22 days or more	4	1
	Answered no to previous question	0	10
If yes, what kind of injuries did you have in the past year?	Participant wrote in their injury	N/A	
Comment or further information regarding injuries.		N/A	
		Average Section Score	1.16 ± 1.54
	Gastrointestinal Function Section		
A. Do you feel gaseous or bloated in the abdomen at times other than during your menstrual period?	Yes, several times a day	3	0
	Yes, several times a week	2	2
	Yes, once or twice a week	1	7
	Rarely or never	0	10
B. Do you get cramps or a stomach ache which cannot be related to your menstruation?	Yes, several times a day	3	0
	Yes, several times a week	2	2
	Yes, once or twice a week	1	5
	Rarely or never	0	12
C. How often do you have bowel movements on average?	Several times a day	1	8
	Once a day	0	9
	Every other day	2	1

	Twice a week	3	0
	Once a week or less	4	1
D. How would you describe your normal stool?	Normal (soft)	0	19
	Diarrhea-like (watery)	1	0
	Hard and dry	2	0
Other comments regarding gastrointestinal function.			
		Average Section Score	1.79 ± 1.51
Menstruation Section			
3.1 A. Do you use oral contraceptives?	Yes	0	5
	No	0	14
A1. If yes, does menstruation stop if you do not use oral contraceptives?	Yes	1	1
	No	0	4
	Answered no to previous question	0	14
3.2 A. How old were you when you had your first period? (If you have never menstruated, skip to question F1.)	12	0	1
	13	0	5
	14	0	7
	15	1	6
	16	1	0
B. Did your first menstruation come by itself?	Yes	0	18
	No	1	1
	I don't remember	1	0
B.1 If no, what kind of treatment was used to start your menstrual cycle?	Hormonal treatment	1	0
	Weight gain	1	0
	Reduced amount of exercise	1	0
	Other	1	0
	Blank	0	19
C. Do you have normal menstruation?	Yes	0	13
	No	2	6
	I don't know	1	0
C1. If yes, when was your last period?	0-4 weeks ago	0	13
	1-2 months ago	1	0
	3-4 months ago	2	0
	5 months ago or more	3	0
	Answered no to previous question	0	6
C2. If yes, are your periods regular? (Every 28th to 34th day?)	Yes, most of the time	0	12
	No, mostly not	1	1
	Answered no to question 3C	0	6
C3. If yes, for how many days do you normally bleed?	1-2 days	1	0
	3-4 days	0	6
	5-6 days	0	5
	7-8 days	0	2

	9 days or more	0	0
C4. If yes, have you ever had problems with heavy menstrual bleeding?	Yes	0	5
	No	0	9
	Answered no to question 3C	0	5
C5. How many periods have you had during the last year?	12 or more	0	5
	9 to 11	1	7
	6 to 8	2	1
	3 to 5	3	0
	0 to 2	4	1
	Blank	0	5
C6. If no, or "I don't remember", when did you have your last period?	2-3 months ago	1	2
	4-5 months ago	2	2
	6 months ago or more	3	1
	I'm pregnant and therefore do not menstruate	0	0
	Answered yes to 3C	0	13
D. Have your periods ever stopped for 3 consecutive months or longer?	No, never	0	10
	Yes, it has happened before	1	5
	Yes, that is the situation right now	2	4
E. Does your menstruation change when you increase your exercise intensity?	Yes	1	11
	No	0	8
E1. If yes, how?	I bleed less	1	3
	I bleed more	0	0
	I bleed fewer days	1	3
	I bleed more days	0	0
	My menstruation stops	2	5
	Answered no to previous question	0	8
		Average Section Score	4.21 ± 2.78

Exercise Energy Expenditure

Of the three EEE criteria, the total activity EEE produced the largest average EEE (see Figure 3.2). When comparing the total average EEE produced using the three EEE criteria, total activity EEE and METs ≥ 3 EEE had a 186.5 kcal difference. Total activity EEE and structured planned EEE had a 387.85 kcal difference. There was almost a 200 kcal difference between total activity EEE and METs ≥ 3 EEE, which is statistically significant

(Table 3.3). The calorie difference between METs ≥ 3 EEE and structured planned EEE, is also about a 200 kcal, which is statistically significant (Table 3.3).

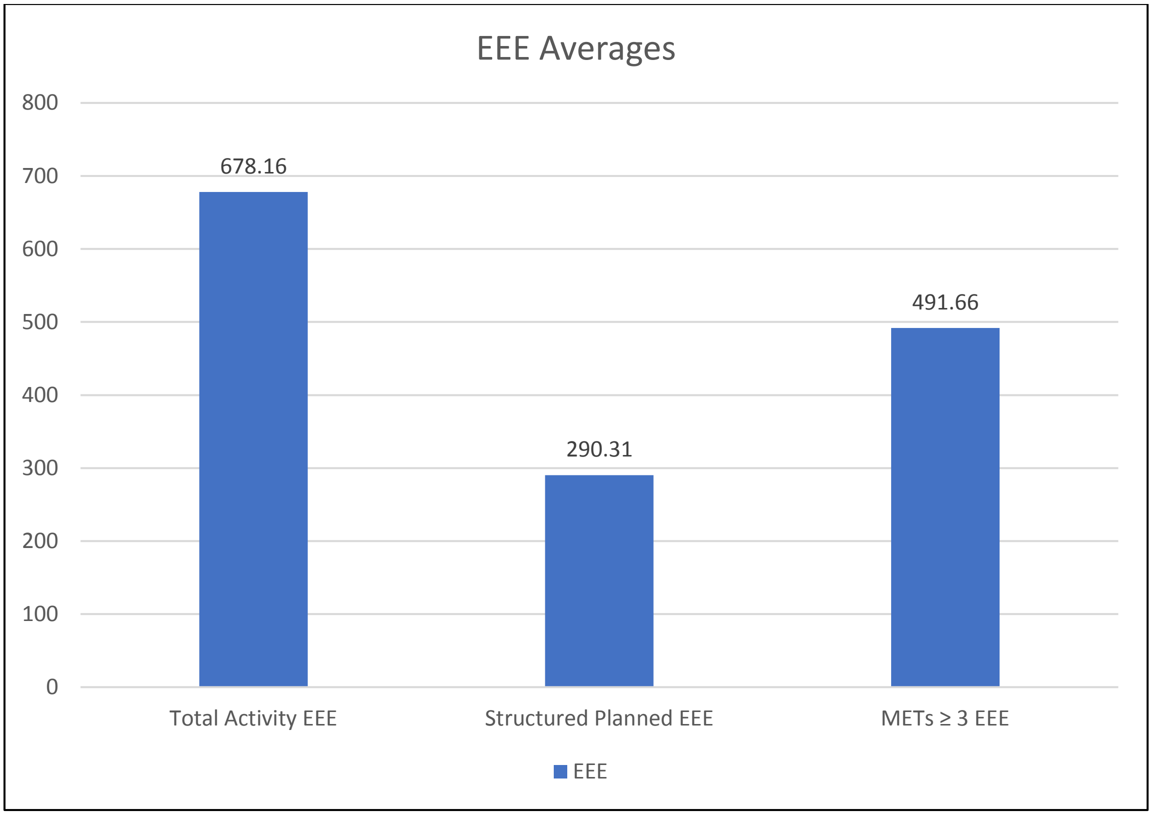

Figure 3.2 Average EEE for Total Activity EEE, METs ≥ 3 EEE, and Structured Planned EEE

Table 3.3 Significance of Differences in Average EEE when Comparing Total Activity EEE, METs ≥ 3 EEE, and Structured Planned EEE

Exercise Energy Expenditure Criteria	p – value
Total Activity EEE vs METs ≥ 3 EEE	< 0.001
Total Activity EEE vs Structured Planned EEE	< 0.001
METs ≥ 3 EEE vs Structured Planned EEE	< 0.001

Energy Availability

Out of the three EEE criteria, the average EA were all above the low EA standard (≤ 30 kcal/kg LBM/day) (see Figure 3.3). While this is true, none of the average EAs are above the standard that is considered optimal EA (> 45 kcal/kg LBM/day). When analyzing the average EA, the data shows about a four point difference between the two criteria, total activity EEE and METs ≥ 3 EEE. This pattern is also seen when comparing METs ≥ 3 EEE to structured planned EEE. These differences between the average EA for the three EEE criteria are statistically significantly different (Table 3.4).

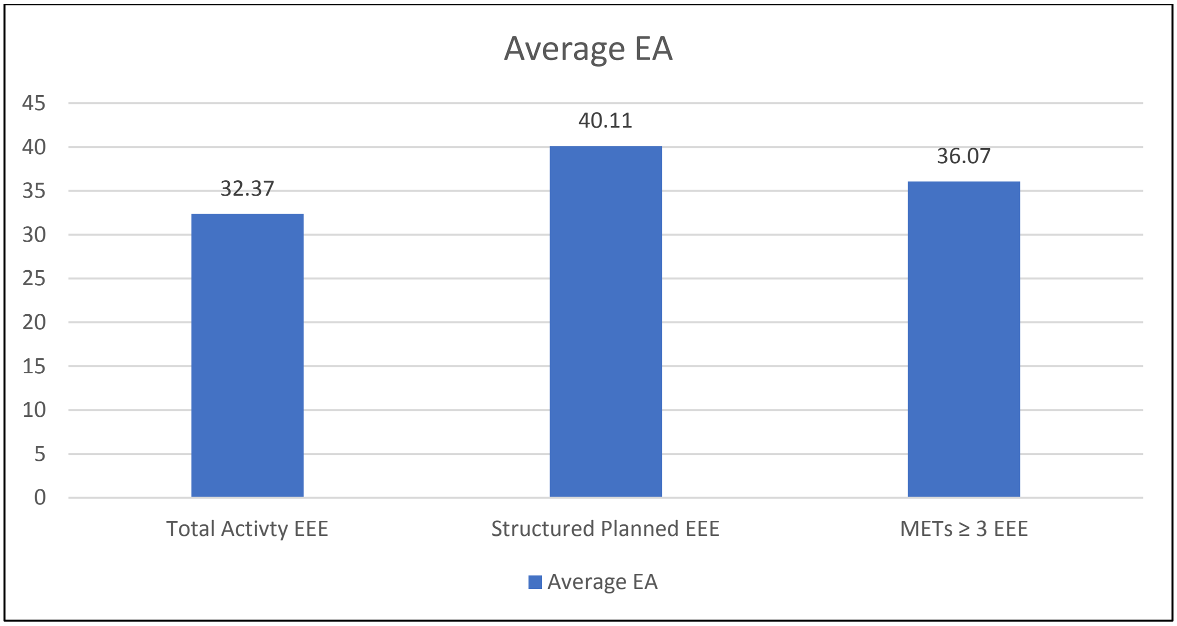

Figure 3.3 Average EA When Using Total Activity EEE, METs ≥ 3 EEE, and Structured Planned EEE Criteria

Table 3.4 Significance of Differences in Average EA when Comparing Total Activity EEE, METs ≥ 3 EEE, and Structured Planned EEE Using a T-test.

Exercise Energy Expenditure Criteria used for EA Comparisons	p - value
EA using Total Activity EEE vs EA using METs ≥ 3 EEE	< 0.001
EA using Total Activity EEE vs EA using Structured Planned EEE	< 0.001
EA using METs ≥ 3 EEE vs EA using Structured Planned EEE	< 0.001

Prevalence

Presence of Triad Components

The prevalence of low EA ranged from 26% to 53% and varied based on which EEE criteria was used. The prevalence of MD was 26%. The prevalence of low BMD was 16% (see Table 3.5). The most common component of the Triad that the participants had was low EA. This varied depending on the EEE criteria that was used to calculate EA (Table 3.5). The total activity EEE criteria produced the largest number of participants with a low EA (n= 10). Out of the 19 participants, five participants presented with a low EA in all three EEE criteria. Two participants had low EA in both the total activity EEE and METs $\geq$ 3 EEE criteria. Three athletes had low EA in only the total activity EEE criteria.

The variation in the results of participants who had the presence of one or more Triad components was due to the change in EA values when using the three EEE criteria. In this study, 58-68% of all participants had at least one component of the Triad. The percentage of participants with at least two components of the Triad ranged from 11-21%. Out of the 19 athletes who participated in this study, zero participants had all three components of the Triad (Table 3.6). In Table 3.7, prevalence of EA below the optimal range is shown, ranging from 63-84%.

Table 3.5 Presence of Triad Components (N=19)

Energy Availability	
Exercise Energy Expenditure Criteria	**Participants with Low EA (out of N= 19)**
Total Activity EEE	10
Structured Planned EEE	5
METs ≥ 3 EEE	7
Bone Mineral Density	
Participants with Low BMD	3
Menstrual Function	
Type of Menstrual Dysfunction (MD)	**Participants with MD**
Primary Amenorrhea	0
Secondary Amenorrhea	4
Oligomenorrhea	1

Table 3.6: Presence* of One, Two, or Three Triad Components When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA (N=19)

	Total Activity EEE	**Structured Planned EEE**	**METs ≥ 3 EEE**
Presence of 1 Triad component	13	10	11
Presence of 2 Triad component	4	2	3
Presence of 3 Triad component	0	0	0

*** The participants could have the components of low energy availability, menstrual irregularity, low bone mass density, or a combination of the three components.**

Table 3.7 Prevalence of EA < 45 kcal/kg LBM/day When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA (N=19)

	Total Activity EEE %(n)	**Structured Planned EEE %(n)**	**METs ≥ 3 EEE %(n)**
EA < 45 kcal/kg LBM/day	84% (n=16)	63% (n=12)	79% (n=15)

Agreement

Kappa Statistic

The highest level of agreement was observed when comparing the structured planned EEE and METs ≥ 3 EEE criteria (Table 3.8). Total activity EEE and METs ≥ 3 EEE had moderate agreement. However, total activity EEE and structured planned EEE had low agreement (Table 3.8)

Table 3.8 Agreement between EA When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA

	Kappa Value	p-value
Total Activity EEE vs Structured Planned EEE	0.486	0.013
Total Activity EEE vs METs ≥ 3 EEE	0.689	0.002
Structured Planned EEE vs. METs ≥ 3 EEE	0.759	0.001

There was no statistical significant agreement between high LEAF-Q scores and low EA when using any of the three EEE criteria ($p < 0.05$) (Table 3.9).

Table 3.9 Agreement between LEAF-Q score ≥ 8 and Presence of Low EA When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA

	Kappa Value	p-value
LEAF-Q Score ≥ 8 vs low EA via Total Activity EEE	-0.044	0.845
LEAF-Q Score ≥ 8 vs low EA via Structured Planned EEE	-0.251	0.243
LEAF-Q Score ≥ 8 vs low EA via METs ≥ 3 EEE	-0.208	0.361

Association

There were no significant differences in average EA when comparing those with high LEAF-Q scores, and those with low LEAF-Q scores (See Table 3.10).

Table 3.10 Average EA Among Participants With LEAF-Q Scores < 8 and ≥ 8.

	Average EA for participants with a LEAF-Q Score < 8	Average EA for participants with a LEAF-Q Score ≥ 8	p – value
Total Activity EEE	31.22 ± 15.28 kcal/kg LBM/day	33.93 ± 8.75 kcal/kg LBM/day	0.660
Structured Planned EEE	39.17 ± 15.74 kcal/kg LBM/day	41.40 ± 9.88 kcal/kg LBM/day	0.729
METs ≥ 3 EEE	34.68 ± 14.49 kcal/kg LBM/day	37.98 ± 8.90 kcal/kg LBM/day	0.577

Odds Ratio

The comparisons made using the odds ratio statistical test included determining if there were associations between the participants' risk for having one or more Triad components and their total LEAF-Q scores or scores from each LEAF-Q section (Table 3.11, 3.12, 3.13, 3.14, 3.15). Due to wide confidence intervals that include the number one, results of the tests showed that there were no significant odds of having one or more component of the Triad when participants had a LEAF-Q score that categorized them at being at risk for having a Triad component.

Table 3.11 Odds Ratio Values LEAF-Q score and calculated EA When Total Activity EEE, Structured Planned EEE, or METs ≥ 3 EEE Was Used to Calculate EA

	Total Activity EEE	Structured Planned EEE	METs ≥ 3 EEE
Odds Ratio Value for LEAF-Q score ≥ 8	0.571	0.250	0.400

Table 3.12 Odds Ratio Values for Gastrointestinal Function Section Score ≥ 2 and Low EA When Total Activity EEE, Structured Planned EEE, or METs ≥ 3 EEE Was Used to Calculate EA

Section	Variable	Odds Ratio (95% CI)
Gastrointestinal Function Section ≥ 2	Low EA	
➢ **Total Activity EEE**		1.200 (0.194 to 7.144)
➢ **Structured Planned EEE**		1.125 (0.141 to 8.995)
➢ **METs ≥ 3 EEE**		2.500 (0.341 to 18.332)

Table 3.13 Odds Ratio Values for Injury Section Score ≥ 2 and Low BMD

Section	Variable	Odds Ratio (95% CI)
Injury Section	Low BMD	0.500 (0.037 to 6.683)

Table 3.14 Odds Ratio Values for Participants with Low EA and a Type of Menstrual Dysfunction When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA

	Total Activity EEE	Structured Planned EEE	METs ≥ 3 EEE
Odds Ratio Value (95% CI)	1.500 (0.189 to 11.927)	0.625 (0.052 to 7.457)	1.200 (0.147 to 9.768)

Table 3.15 Odds Ratio Values for LEAF-Q score and Prevalence of 1 and 2 Triad Components

	Odds ratio (95% CI)
Presence of 1 Triad component	
➢ **Total Activity EEE**	1.714 (0.23 to 12.89)
➢ **Structured Planned EEE**	2.000 (0.31 to 12.84)
➢ **METs ≥ 3 EEE**	1.389 (0.216 to 8.92)
Presence of 2 Triad components	
➢ **Total Activity EEE**	6.000 (0.49 to 73.45)
➢ **Structured Planned EEE**	1.429 (0.08 to 26.89)
➢ **METs ≥ 3 EEE**	3.333 (0.25 to 45.11)

Correlation and Logistic Regression

There were no significant correlations (Table 3.16) between LEAF-Q scores and presence of one or more Triad component ($p < 0.05$) (Table 3.16). Results from the logistic regression test did not prove effective in predicting the presence of any Triad components based on participants' EA using any of the three criteria.

Table 3.16 Correlations between Total LEAF-Q score and Presence of 1 or 2 Triad Components Using Spearman Correlation Test

Total Activity EEE		
EEE Criteria	**Correlation**	**p-value**
Presence of 1 component of the Triad	0.121	0.623
Presence of 2 components of the Triad	0.344	0.149
Structured Planned EEE		
Presence of 1 component of the Triad	0.169	0.490
Presence of 2 components of the Triad	0.055	0.824
METs ≥ 3 EEE		
Presence of 1 component of the Triad	0.080	0.746
Presence of 2 components of the Triad	0.215	0.376

Sensitivity and Specificity

After comparing the athletes' EA to their LEAF-Q scores, using the ANOVA test, results did not show a significant association. To further examine associations between athletes' EA and LEAF-Q scores, sensitivity and specificity was calculated. The sensitivity and specificity tests were calculated to determine how often the LEAF-Q scores were predicting true positives and true negatives, respectively.

In this study, 11 participants had a LEAF-Q score < 8 and 8 participants with a LEAF-Q score ≥ 8. This indicates that, according to the LEAF-Q, 8 participants should have a low EA and 11 participants should not. Table 3.17 shows the number of participants who had a normal EA out of the number of participants that the LEAF-Q predicted would have normal EA and does the same for low EA.

Table 3.17: Predicted prevalence of normal and low EA based on the total LEAF-Q score compared to the actual EA of the participants using the three EEE criteria.

EEE Criteria	Normal EA (>30 kcal/kg LBM/day)	Low EA (≤ 30 kcal/kg LBM/day)
Total Activity EEE	5	4
Structured Planned EEE	7	1
METs ≥ 3 EEE	6	2
Predicted by the LEAF-Q Score	11	8

Table 3.18 Sensitivity and Specificity of the LEAF-Q's Prediction of Low EA When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA

	Total Activity EEE	Structured Planned EEE	METs ≥ 3 EEE
Sensitivity	50%	12.5%	25%
Specificity	45.5%	63.6%	54.5%

When comparing the sensitivity and specificity percentage for the three EEE criteria shown in table 3.18, total activity EEE criteria produced the highest sensitivity rate (50%) and the structured planned EEE criteria produced the highest specificity rate (63.6%). This sensitivity percentage means that when using total activity EEE, the LEAF-Q predicts a low EA correctly 50% of the time in this study. And when using structured planned EEE, the LEAF-Q predicts a normal EA correctly about 64% of the time, in this study. These results indicate that the LEAF-Q's true positive rate for predicting low EA was highest when using total activity EEE. And the LEAF-Q's true negative rate for predicting normal EA was highest when using structured planned EEE.

Sensitivity and specificity were also used to determine the accuracy of the LEAF-Q score in predicting if a participant had one, two, or all three components of the Triad. According to the results listed in Table 3.19, sensitivity was highest (75%) using total activity EEE when predicting if the participant has at least one component of the Triad. This total activity EEE sensitivity percentage was cut in half (37.5%), when sensitivity was calculated on the LEAF-Q's ability to predict if a female athlete had at least two components of the Triad. For all three EEE criteria, the sensitivity percentage was higher when predicting if a participant had just one component of the Triad, compared to predicting if a participant had two components of the Triad.

With regards to specificity, structured planned EEE had the highest specificity percentage of 54.5%, when predicting if the participants had at least one component of the Triad. This percentage indicates that the LEAF-Q can predict a normal EA 54.5% of the time when using structured planned EEE. Total activity EEE had the lowest specificity percentage (36.4%), when predicting if the participants had at least one component of the Triad. This means that 36.4% of the time the LEAF-Q correctly predicted that the participant did not have at least one component of the Triad. The specificity percentage for predicting two Triad components, all three criteria produced a 90.9 percentage.

Table 3.19 Sensitivity and Specificity of the LEAF-Q's Prediction of 1 or 2 Triad Components When Total Activity EEE, METs ≥ 3 EEE, or Structured Planned EEE Was Used to Calculate EA

	Sensitivity	Specificity
Total Activity EEE Criteria		
➢ One Triad Component	75%	36.4%
➢ Two Triad Components	37.5%	90.9%
Structured Planned EEE Criteria		
➢ One Triad Component	62.5%	54.5%
➢ Two Triad Components	12.5%	90.9%
METs ≥ 3 EEE Criteria		
➢ One Triad Component	62.5%	45.5%
➢ Two Triad Components	25%	90.9%

Discussion

Low EA may lead to MD and low BMD, which can have negative impacts on a female athlete's sport performance, as well her immediate and lifetime health. Though studies evaluating EA in a lab setting have standard protocols for measuring and quantifying EEE (Loucks, Verdun, & Heath, 1998; Loucks & Thuma, 2003; Ihle & Loucks, 2004), there is not a standardized method for measuring and quantifying EEE in free-living people. Though Guebels, et. al (2014) compared EA using various methods for classifying EEE (e.g. METS ≥4, all planned exercise), this is the first study to compare total activity EEE to other methods. Results of this current study suggest that METs ≥ 3 EEE may be a better choice as a standardized method for calculating EA. This current study provided additional data to support the findings of Melin et al. (2014), indicating that a LEAF-Q score ≥ 8 cannot predict low EA, but it can be useful in identifying female athletes who have one or more Triad components.

Prevalence

Breaking down the prevalence results into the three EEE criteria categories, total activity EEE produced the highest prevalence of at least one Triad component in participants (n=13, 68%) which was higher than when using METs ≥ 3 EEE (n=11, 58%) or structured planned EEE (n=10, 53%). These numbers dropped when assessing the prevalence of at least two Triad components present in participants. Prevalence rates were 21% (n=4), 11% (n=2), and 16% (n=3), for total activity EEE, structured planned EEE, and METs ≥ 3 EEE criteria, respectively. More athletes, in this study, had just one Triad component. Because of this, it was important to know which Triad component was the most prevalent among the participants in this study. These results relate to the conclusions made by the 2007 ACSM Triad Position Stand, which explain that an athlete does not need to display signs of all three Triad components to be considered as having the Triad. Rather, the Triad is considered to be a spectrum of health and the athlete can fall within the spectrum of health and disease depending on their dietary and exercise habits (Nattiv et al., 2007). Though an athlete may show signs for low EA, depending on how long they've been in a state of low EA, the athlete may not have the symptoms of MD or low BMD.

The most prevalent Triad component among participants in this study was low EA, reaching as high as 10 participants with low EA. Only 15% (n=3) had low BMD and 26% (n=5) of participants had a type of MD. What these results do not show is how long these participants have had low EA. Those athletes who qualified for low EA but do not have MD or low BMD are at risk for developing these Triad components if necessary steps are not taken by the athlete to increase their energy intake and thus normalizing their EA.

This finding is important because of the influential power EA has on menstrual function and BMD in athletes. While many factors influence EA in an athlete, energy expended through exercise is a key factor in determining how many calories an athlete needs to consume in a day to sustain exercise and physiological function. Low EA can be easily manipulated by an athlete if they change their daily energy intake or EEE. Applying this finding to practice, because EA is so easy to manipulate, it would be beneficial to incorporate checking an athlete's EA levels once or twice a year, as young female athletes approach the age of menarche. It also may be beneficial to test athletes during their season and off season. Reed, De Souza, and Williams (2013) discovered that in division 1 soccer players, prevalence of low EA was lower in the off season than mid-season.

Prevalence rates of low EA in this study for the three EEE criteria include 53% (n=10) for total activity EEE, 26% (n=5) for structured planned EEE, and 37% (n=7) for METs ≥ 3 EEE. The low EA prevalence rates in this study were comparable to rates reported in previous studies, which ranged from as low as 26% to as high as 63% (Melin et al., 2014; Slater et al., 2016; Reed et al., 2013; Day, Wengreen, Heath, & Brown, 2015). In the study with the lowest prevalence, they only measured EEE during purposeful exercise, which is similar to the structured planned EEE criteria (Reed et al., 2013). The study with the highest prevalence of low EA, the method used to calculate EEE was using the daily mean EEE (Melin et al., 2014).

Results from the menstrual function section in the LEAF-Q indicate that all five participants who qualified for MD had a LEAF-Q score ≥ 8. This was similar to the findings of Melin et al. (2014); MD was the most prevalent Triad component. Of the 45 total participants, 29 participants had a type of MD. Out of those 29 participants, 23 had a total LEAF-Q score ≥ 8. This could be due to the fact that, on average, participants earned the most points on the LEAF-Q from the menstrual function section. In addition, female athletes may be more aware of their menstrual cycle history and therefore may be better able to report menstruation in comparison to other components of the LEAF-Q.

Agreement

Identifying agreement between the three EEE criteria was an important component of this study. It was hypothesized that there would not be significant agreement between the three EEE criteria. If the three EEE criteria had a significant agreement, then it would suggest that any of the three criteria used for quantifying EEE may be acceptable to use in Triad research. But if there was not significant agreement between the three EEE criteria, this would indicate a need for the development of a standardized method for calculating EEE data in Triad studies. Findings from the current study highlighted that total activity EEE and METS $\geq$ 3 EEE had moderate agreement, and total activity EEE and structured planned EEE criteria had the lowest agreement. These results are significant because there was not unanimous agreement for all three methods of calculating EEE. A reason why the EEE criteria did not have perfect agreement is the inclusion criteria for each EEE criteria. Structured planned EEE only captured the participants' planned exercise for sport. The athletes in this study participated in other physical activity outside of their planned exercise, which increased the athletes' daily EEE amount collected by the total activity EEE criteria and, depending on the intensity level of the other exercise, it could have added to the METs $\geq$ 3 EEE collection as well. In a study comparing four EEE methods for calculating EA, showed EA values varying up to ~30% depending on which EEE method was used (Guebels, Kam, Maddalozzo, & Manore, 2014). This variation in EA when using different EEE criteria, both in this study and other studies, indicate the need for a standardized method which would allow for the opportunity to better compare Triad study results between studies.

Association

A surprising finding from the ANOVA test was that there were no significant differences between the average EA for the three EEE criteria when comparing participants who scored ≥ 8 on the LEAF-Q and participants who scored < 8. It was hypothesized that total activity EEE would have a significantly lower EA than structured planned EEE and that participants with a LEAF-Q score ≥ 8 would have a significantly lower EA. The insignificant results may be due to the small number of participants in the study. It is possible that if the sample size was bigger, there would be a significant difference between average EA in the three criteria. However, Melin and colleagues (2014), when testing the reliability and validity of the LEAF-Q, found that when comparing the average EA of participants with a LEAF-Q score ≥ 8 (n=28) to participants who had a LEAF-Q score < 8 (n=17), there was no significant difference in average EA.

Sensitivity and Specificity

When comparing participants' actual EA using the three EEE criteria to LEAF-Q score ≥ 8, the sensitivity ranged from 12.5-50% and specificity ranged from 45.5-63.6%. Total activity EEE produced the highest sensitivity (50%) for correctly classifying if a participant had low EA. Structured planned EEE produced the highest specificity (63.6%) for correctly classifying if a participant had normal EA. It is not surprising the EEE method that produced the highest frequency of low EA would have the highest sensitivity percentage. This idea also applies when evaluating the specificity of the three EEE criteria. The structured planned EEE criteria was the most restrictive when identifying which physical activities could be used when quantifying participants' daily EEE, producing higher EAs on average than the other two EEE criteria.

Another comparison tested in this study was if participants had one or more components of the Triad compared to their LEAF-Q score. For example, if they had a LEAF-Q score ≥ 8, would this correspond with the participant having either low EA and/or MD and/or low BMD? The sensitivity for participants having at least one Triad component for the three EEE criteria ranged from 62.5-75% and specificity ranged from 36.4-54.5%. Second, when looking at participants who had at least two Triad components, sensitivity ranged from 12.5-37.5% and specificity was 90.9% for all three EEE criteria. This difference in sensitivity between the two categories could be due to the fact that the number of participants who had at least one Triad component in this study was much higher than the number of participants who had two Triad components. The specificity for participants having two components of the Triad was 90.9% could be due to that most of the participants did not have two components of the Triad, which raised the probability that if a participant had a LEAF-Q score < 8 they were more likely to not have two components of the Triad.

In the Melin article published in 2014, they also tested the sensitivity and specificity of the total LEAF-Q score and its prediction ability. In their study, they had a sensitivity rate of 78% and a specificity rate of 90% when predicting if an athlete had either low EA and/or MD and/or low BMD. When they excluded participants with PCOS and other MD besides oligomenorrhea or amenorrhea, the sensitivity and specificity percentages were 83% and 90%, respectively. They were testing the ability of the LEAF-Q to predict if an athlete had either low EA and/or MD and/or low BMD, which is similar to the second comparison made in this study. Unlike the Melin article, this study produced multiple sensitivity and specificity percentages because three EEE criteria were used. The EEE criteria that produced a sensitivity closest to the sensitivity in the Melin article was total activity EEE when screening if an athlete has at least one Triad component.

One key purpose of this study was to compare agreement between the three EEE criteria to gain further insight into which method for quantifying EEE could be used for a standardized method for all Triad research. Each criteria had its pros and cons. The process of calculating EEE using total activity EEE was very straightforward which could lead to less error and increased efficiency. It also provided a full picture of how many calories participants were expending each day. Guebels et al. (2014) discussed the idea that athletes are more likely to participate in physical activity outside of their planned sport practices. If inclusion criteria for EEE is limited to EEE during training session related to their sport, significant EEE data could be missed if participants are free to engage in other physical activities and exercise throughout the day (Guebels et al., 2014). Though, total activity EEE produced the highest sensitivity rates, it produced the lowest specificity, which suggest that using this EEE criteria could lead to false positive predictions of an athlete being at risk for the Triad and may overestimate an athlete's daily EEE.

This is why the criteria METs ≥ 3 EEE, should be considered as a possible choice for the standardized method for quantifying an athlete's daily EEE. It would incorporate exercise the athlete participates in throughout the day, besides their structured planned sport practices, but it would leave out any activity that was only light intensity. Using METs ≥ 3 EEE could also help researchers distinguish between if an athlete is participating in physical activity or if they are participating in exercise. While physical activity and exercise are often used interchangeably, they are not the same. Physical activity is "any bodily movement produced by skeletal muscles that results in energy expenditure" (Casperson, Powell, & Christenson, 1985). Exercise is defined as “physical activity that is planned, structured, repetitive, and purposive in the sense that improvement or maintenance of one or more components of physical fitness is an objective” (Casperson, Powell, & Christenson, 1985). Based on these definitions of physical activity and exercise, total activity EEE would capture energy expended from both exercise and physical activity, thus leading to the overestimation of an athlete’s daily EEE. Using METs $\geq$ 3 EEE, which only counts energy expended from bouts lasting longer than 10 minutes and do not drop below three METs for more than two minutes, may capture energy expended more from exercise rather than physical activity which may lead to a more accurate estimate of daily EEE in an athlete. Further research should be conducted to identify if both exercise and physical activity of an athlete has a significant effect on an athlete’s EA and subsequent Triad risk or if it is sufficient to just measure an athlete’s energy expenditure during exercise.

The final objective of this study was to evaluate the LEAF-Q as a tool to predict Triad risk in female athletes. Overall, the LEAF-Q has the ability to predict an athlete’s risk for one or more Triad components. Results of this study showed that the LEAF-Q is better suited for generally assessing risk of the Triad in female athletes than specifically identifying which component of the Triad a female athlete could be at risk for. The LEAF-Q had the best results when total activity EEE was used, compared to the other two EEE criteria.

It is necessary that the results of this study are evaluated and retested by future researchers to further understand the best method for quantifying EEE in female athletes and to further refine the LEAF-Q so that it may become a standardized tool used for assessing Triad risk in female athletes.

Limitations

A limitation of this study was that the duration of the study was short term, recording only four days of the participants' energy intake and expenditure. A long-term study could provide a greater insight into an athlete's long term average EA.

The sample size of this study was also a limiting factor. Despite this limitation, there have been many published Triad research studies that had small sample sizes. Guebels et al. (2014) published a study on methods for quantifying EEE with a sample size of 22 participants. Another study by Reed and colleagues (2013) evaluated changes in EA for division 1 soccer players with a sample size of 19 participants. Loucks, Verdun, and Heath (1998) conducted a study on the influence of low EA and its effects on LH pulsatility with a sample size of 9 participants. Later, Loucks and Thuma (2003) conducted a study with a sample size of 29. Having a larger sample size for this study would have been beneficial and helped increase the reliability and generalizability of the results of the study.

Another limitation was using a self-reported method to determine if the participants had a type of MD. Also, no verification was made that the MD was exercise related and not due to other factors (e. g. PCOS or other MD disorders). The self-reporting method for dietary intake was also a limitation. Though the tool used for data collection (ASA-24) was a validated tool, it is difficult to control for unintentional or intentional underreporting or not reporting all the food consumed.

There are also limitations to utilizing accelerometers to estimate EEE. Accelerometers worn on the hip to not provide accurate EEE estimates of upper-body exercises. In relation to the accelerometers, there were two participants who rode a stationary bike for exercise. Accelerometers in general underestimate the energy expended for biking

(McMinn et al., 2013). While the participants may have a lower EEE if they biked, there is no bias between the EEE criteria.

Conclusions

In conclusion, there was a high prevalence of participants having at least one Triad component among the athletes in this sample. Out of the three Triad components, the most prevalent Triad component was low EA.

Results of the agreement between the three EEE criteria, indicate that total activity EEE and structured planned EEE had the lowest agreement. When analyzing the associations between the average EA using the three EEE criteria, there was no significant association. Overall, it was concluded that METs ≥ 3 EEE did the best job at capturing an accurate estimate of a participant's daily EEE. METs ≥ 3 EEE may be the better choice as a standardized method for quantifying EA in athletes because it could distinguish between an athletes' physical activity and their exercise. Further research studies should be completed using these EEE criteria to identify which criteria to use as the standardized method for Triad researchers when quantifying their participants' EEE.

Results from this study indicate that though the LEAF-Q does have the ability to predict if an athlete is at risk for having one or more Triad components, the questionnaire is not able to predict the specific Triad component an athlete may have.

Implications for Future Research

Recommendations for future research based on the results of the study are first, when conducting a study examining the relationship between the LEAF-Q and EEE criteria, include a menstrual assessment of the participants to collect objective data on the participant's menstrual status. Second, would be to recreate the study conducted by Loucks et al. (1998) that examined the effects of regulating EA in a controlled laboratory setting, but utilizing accelerometers to measure energy expended in exercise and other physical activities throughout the day. Recreating this study using accelerometers in free-living setting using the total activity EEE criteria would give researchers the opportunity to compare their results to the results of the Loucks et al. (1998) study.

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Appendix A

University *of* Idaho

Office of Research Assurances

Institutional Review Board

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To: Katie Brown

From: Jennifer Walker
Chair, University of Idaho Institutional Review Board
University Research Office
Moscow, ID 83844-3010

Date: 6/4/2015 10:12:17 AM

Title: Refueling Station Food and Beverage Consumption and Risk for the Female Athlete Triad among Female Collegiate Athletes

Project: 15-796
Approved: June 04, 2015
Renewal: June 03, 2016

On behalf of the Institutional Review Board at the University of Idaho, I am pleased to inform you that the protocol for the above-named research project is approved as offering no significant risk to human subjects.

This study may be conducted according to the protocol described in the application without further review by the IRB. Every effort should be made to ensure that the project is conducted in a manner consistent with the three fundamental principles identified in the Belmont Report: respect for persons; beneficence; and justice.

This IRB approval is not to be construed as authorization to recruit participants or conduct research in schools or other institutions, including on Native Reserved lands or within Native Institutions, which have their own policies that require approvals before Human Participants Research Projects can begin. This authorization must be obtained from the appropriate Tribal Government (or equivalent) and/or Institutional Administration. This may include independent review by a tribal or institutional IRB or equivalent. It is the investigator's responsibility to obtain all such necessary approvals and provide copies of these approvals to ORA, in order to allow the IRB to maintain current records.

As Principal Investigator, you are responsible for ensuring compliance with all applicable FERPA regulations, University of Idaho policies, state and federal regulations.

This approval is valid until June 03, 2016.

Should there be significant changes in the protocol for this project, it will be necessary for you to submit an amendment to this protocol for review by the Committee using the Portal. If you have any additional questions about this process, please contact me through the portal's messaging system by clicking the ‘Reply’ button at the top of this message.

ATT00001.bin

Jennifer Walker

Appendix B

Female Athlete Health Questionnaire

Please be sure to mark your answers **DARK ENOUGH TO READ** and correctly as shown.

Answer Selection: Correct = ● Incorrect = ☒ ✓ ⊖

<table>
<tr><td>Sport

O Soccer
O Cross-county
O Volleyball
O Swimming and Diving
O Golf
O Tennis
O Basketball
O Track and Field

What is your position?____________________</td><td>Age

O 18
O 19
O 20
O 21
O 22
O Other_______________</td></tr>
<tr><td>Height ____________</td><td>Weight______________</td></tr>
<tr><td>Rate your usual level of hunger when you <u>begin</u> eating

O 1 - Empty
O 2 - Ravenous
O 3 - Stomach Pangs
O 4 - Slightly Hungry
O 5 - Neutral
O 6 - Satisfied
O 7 - Full
O 8 - Overly Full
O 9 - Stuffed
O 10 - Sick</td><td>Rate your usual level of hunger when you <u>finish</u> eating

O 1 - Empty
O 2 - Ravenous
O 3 - Stomach Pangs
O 4 - Slightly Hungry
O 5 - Neutral
O 6 - Satisfied
O 7 - Full
O 8 - Overly Full
O 9 - Stuffed
O 10 - Sick</td></tr>
<tr><td>How often do you eat during your training and competition season?

Every ___________ hours</td><td>Are you trying to gain or lose weight?

O Yes, gain weight
O Yes, lose weight
O No

If yes, how much? __________lbs.</td></tr>
<tr><td colspan="2">1. Injuries</td></tr>
<tr><td>A. Have you had absences from your training or participation in competitions during the last year due to injuries?

O No, not at all
O Yes, once or twice
O Yes, three or four times
O Yes, five times or more</td><td>A1. If yes, how many days were you absent from training or competition due to injuries in the last year?

O 1-7 days
O 8-14 days
O 15-21 days
O 22 days or more</td></tr>
<tr><td>If yes, what kind of injuries did you have in the past year?</td><td>Comments or further information regarding injuries.</td></tr>
</table>

2. Gastrointestinal Function	
A. Do you feel gaseous or bloated in the abdomen at times other than during your menstrual period? O Yes, several times a day O Yes, several times a week O Yes, once or twice a week O Rarely or never	B. Do you get cramps or a stomach ache which cannot be related to your menstruation? O Yes, several times a day O Yes, several times a week O Yes, once or twice a week O Rarely or never
C. How often do you have bowel movements on average? O Several times a day O Once a day O Every other day O Twice a week O Once a week or less	D. How would you describe your normal stool? O Normal (soft) O Diarrhea-like (watery) O Hard and dry Other comments regarding gastrointestinal function:
3. Menstruation etc.	
3.1 A. Do you use oral contraceptives? O Yes O No	A1. If yes, does menstruation stop if you do not use oral contraceptives? O Yes O No
3.2 A. How old were you when you had your first period?______________ **<u>*If you have never menstruated, skip to question F1.</u>**	B. Did your first menstruation come by itself? O Yes O No O I don't remember
B1. If no, what kind of treatment was used to start your menstrual cycle? O Hormonal treatment O Weight gain O Reduced amount of exercise O Other	C. Do you have normal menstruation O Yes O No (go to question C6) O I don't know (go to question C6)
C1. If yes, when was your last period? O 0-4 weeks ago O 1-2 months ago O 3-4 months ago O 5 months ago or more	C2. If yes, are your periods regular? (Every 28th to 34^{th} day) O Yes, most of the time O No, mostly not
C3. If yes, for how many days do you normally bleed? O 1-2 days O 3-4 days O 5-6 days O 7-8 days O 9 days or more	C4. If yes, have you ever had problems with heavy menstrual bleeding? O Yes O No

C5. How many periods have you had during the last year? O 12 or more O 9-11 O 6-8 O 3-5 O 0-2 **Skip to D**	C6. If no, or “I don’t remember”, when did you have your last period? O 2-3 months ago O 4-5 months ago O 6 months ago or more O I pregnant and therefore do not menstruate
D. Have your periods ever stopped for 3 consecutive months or longer? O No, never O Yes, it has happened before O Yes, that is the situation right now	E. Does your menstruation change when you increase your exercise intensity frequency or duration? O Yes O No
E1. If yes, how? (select all that apply) O I bleed less O I bleed more O I bleed fewer days O I bleed more days O My menstruation stops	How accurately do you think you can answer questions about your menstrual periods? O Very accurately. I usually know when my monthly cycle will start. O Fairly accurately. I don’t know exactly when it will start, but I would notice if I haven’t had a menstrual cycle in a while. O Not very accurately. I don’t usually pay attention to my menstrual cycle.

F. Health Knowledge and Attitudes

Please rate your agreement to the following questions:

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree	Don’t Know
1. Skipping or losing my period while playing sports is normal and healthy.	O	O	O	O	O	O
2. A menstrual cycle typically occurs every 28 +/- 7 days.	O	O	O	O	O	O
3. I’m not old enough to have weak bones that fracture easily.	O	O	O	O	O	O
4. Female athletes who skip their period may have difficulty become pregnant.	O	O	O	O	O	O
5. Not eating enough calories could cause me to lose or skip my period.	O	O	O	O	O	O

6. Stress fracture risk is not influenced by the amount of calories I consume.	O	O	O	O	O	O
7. An athlete should eat every 2-3 hours during times of training and competition.	O	O	O	O	O	O
8. Stress fractures (very small bone cracks or breaks) occur more often in girls that skip their period.	O	O	O	O	O	O
9. There is no set body fat percentage that is required for optimal athletic performance.	O	O	O	O	O	O
10. Bone loss that occurs when I am in my teens and early twenties is completely reversible.	O	O	O	O	O	O
11. I have a busy schedule.	O	O	O	O	O	O
12. I can prepare healthy meals.	O	O	O	O	O	O
13. I consume enough calories from my diet to support my health and physical activity.	O	O	O	O	O	O
14. I restrict my dietary intake.	O	O	O	O	O	O

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

Ever used laxatives diet pills or diuretics (water pills) to control your weight or shape?	O	O	O	O	O	O
Exercised more than 60 minutes a day to lose or control weight?	O	O	O	O	O	O
Lost 20 pounds or more in the past 6 months	Yes	O	No	O		
*Defined as eating much more than most people would under the same circumstances and feeling that eating is out of control						