Describing the Components of the Female Athlete Triad and Resting Metabolic Rate in a Cohort of Middle-Upper Class Adolescent Female Athletes: A Cross-Sectional Study

A Thesis
Presented in Partial Fulfillment of the Requirements for
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Abstract

**Background:** The female athlete triad is defined as “a spectrum of abnormalities in energy availability, menstrual function, and bone mineral density”.¹ This spectrum of abnormalities refers to a range of severity from normal to varying degrees of pathology for each component of the triad.¹ The female athlete triad has been studied extensively in adult women, but few studies have evaluated the triad and its associated conditions, namely suppressed resting metabolic rate, among adolescent female athletes.

**Objectives:** To describe the components of the female athlete triad and resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes.

**Methods:** A convenience sample of adolescent female participants ages 14-18 was recruited from a middle-to-upper class suburban high school in Ohio. Each participant attended one, individual one-hour laboratory visit. During this visit, bone mineral density measurements were conducted for total body, femoral neck, lumbar spine, and ultra-distal radius using a General Electric Lunar iDXA. Resting metabolic rate was estimated using a Korr Medical Technologies ReeVue Indirect Calorimeter. Prior to the laboratory visit, each participant received a VioScreen Food Frequency Questionnaire® to estimate caloric energy intake and a compendium of questionnaires generated using the Research Electronic Data Capture (REDCap) application to self-report and estimate physical activity and menstrual function.² Energy availability was estimated using estimates of energy intake and exercise energy expenditure derived from the questionnaires, as well as measured kilograms of lean body mass. Participants were categorized into one of two menstrual function categories: dysmenorrheic and eumenorrheic. Bone mineral density z-scores were reported for total body less head (TBLH) and lumbar spine. The Cunningham equation and the
Harris-Benedict equation were used to produce two estimated RMR values for each participant. These estimated rates were compared to the measured RMR to determine if there were significant differences between predicted and measured RMR among this sample of adolescent female athletes. Descriptive statistical analysis was performed to describe the components of the female athlete triad and resting metabolic rate. Spearman’s correlation was used to describe the potential correlations between variables; correlations were significant if $p \leq 0.05$. The Mann-Whitney $U$ test was used to assess differences between menstrual function categories. The Related-Samples Wilcoxon Signed Rank Test was used to compare median differences between RMR measured by indirect calorimetry and RMR predicted from the Cunningham equation and the Harris-Benedict equation individually.

**Results:** Nineteen participants were recruited; 17 participants provided complete data sets. Nine participants (56.3%) exhibited low energy availability (less than 30 kcal/kg LBM/day), 2 participants (14.3%) exhibited menstrual dysfunction, and 4 participants (21.1%) exhibited low lumbar spine BMD z-scores. One participant exhibited all three components of the female athlete triad. Based on the 2007 diagnostic criteria, 11 participants exhibited one or more pathologic triad components and would therefore be diagnosed with the female athlete triad. Data analysis revealed a significant correlation between TBLH BMD z-score and lumbar spine BMD z-score. Both total mass and lean body mass were significantly correlated with TBLH z-score and lumbar spine z-score. A significant correlation was observed between lumbar spine BMD z-score and osteogenic potential (Ost score). Dysmenorrheic participants trended toward lower energy availability than eumenorrheic participants, however this trend was not significant. A significant correlation was observed between energy availability and daily energy intake. Though no significant correlations were observed between RMR and other variables, the median of differences between measured RMR and HB-predicted RMR was significantly different. This difference was not observed between measured RMR and Cunningham-predicted RMR.
Conclusion: Each component of the female athlete triad was present among this cohort of adolescent female athletes. Additional research is needed to more thoroughly describe the prevalence and severity of the components of the female athlete triad and resting metabolic rate in the adolescent population. This additional research will aid in the development of educational curricula and protocols to address screening, prevention, and treatment of the triad. These resources and tools are necessary to protect the present and future health of the adolescent female athlete.
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List of Abbreviations

AN  Anorexia nervosa
BN  Bulimia nervosa
BMD Bone mineral density
DE  Disordered eating
DSM-IV Diagnostic and Statistical Manual – IV
DSM-V Diagnostic and Statistical Manual – V
EA  Energy availability
ED  Eating disorder
ED-NOS Eating disorder not otherwise specified
EEE Exercise energy expenditure
EI  Energy intake
E1G  Estrone-1-glucuronide
E2  Estradiol
FSH  Follicle-stimulating hormone
GnRH Gonadotropin-releasing hormone
HAZ Height-for-age z-scores
HB  Harris-Benedict equation
hCG  Human chorionic gonadotropin
HWHSFAS Healthy Wisconsin High School Female Athletes Survey
HPA Hypothalamic-pituitary-adrenal axis
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Insulin-like growth factor binding protein 3</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>Insulin-like growth factor binding protein 1</td>
</tr>
<tr>
<td>IOC</td>
<td>International Olympic Committee</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>lbs</td>
<td>Pounds</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>NTX</td>
<td>N-telopeptide</td>
</tr>
<tr>
<td>OC</td>
<td>Osteocalcin</td>
</tr>
<tr>
<td>OSFED</td>
<td>Other specified feeding or eating disorder</td>
</tr>
<tr>
<td>PdG</td>
<td>Pregnanediol glucuronides</td>
</tr>
<tr>
<td>PICP</td>
<td>Procollagen carboxy-terminal propeptide</td>
</tr>
<tr>
<td>P4</td>
<td>Progesterone</td>
</tr>
<tr>
<td>REE</td>
<td>Resting energy expenditure</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
</tr>
<tr>
<td>TBLH</td>
<td>Total body less head</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>UFED</td>
<td>Unspecified feeding or eating disorder</td>
</tr>
<tr>
<td>U-CTX-I</td>
<td>C-terminal telopeptide</td>
</tr>
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List of Definitions

Amenorrhea – absence of menstrual cycles for more than 90 days.¹

Anovulation – a menstrual cycle without ovulation.¹

Disordered eating – various abnormal eating behaviors, including restrictive eating, fasting, frequently skipped meals, diet pills, laxatives, diuretics, enemas, overeating, binge-eating and then purging (vomiting).¹

Dysmenorrhea – abnormal menstrual function; encompasses both oligomenorrhea and secondary amenorrhea, 0-8 menstrual periods in the previous 12 months.

Eating disorder (ED) – a clinical mental disorder defined by DSM-V³ and characterized by a persistent disturbance of eating or eating-related behavior that results in the altered consumption or absorption of food; significantly impairs physical health or psychosocial functioning.

Energy availability (EA) – dietary energy intake (EI) minus exercise energy expenditure (EEE) normalized to lean body mass (LBM), i.e., EA = (EI - EEE)/LBM, in units of kilocalories or kilojoules per kilogram of lean body mass.¹

Eumenorrhea – menstrual cycles at intervals near the median interval for young adult women. In young adult women, menstrual cycles recur at a median interval of 28 days that varies with a standard deviation of 7 days.¹

Exercise energy expenditure (EEE) – strictly, the energy expended during exercise training in excess of the energy that would have been expended in non-exercise activity during the same time interval.¹

Female athlete triad – relationships among energy availability (EA), menstrual function, and bone mineral density (BMD) that may have clinical manifestations including eating disorders, functional hypothalamic amenorrhea, and osteoporosis.¹

Food security – having physical and economic access to food that meets dietary needs as well as food preferences.⁴

Lean body mass (LBM) – total fat-free mass; the sum of muscle mass and bone mass

Low bone mineral density (BMD) – bone mineral density z-score between −1.0 and −2.0 for physically active and athletic premenopausal women and children.¹

Oligomenorrhea – menstrual cycles at intervals longer than 35 days, i.e., greater than the median plus one standard deviation.¹
Osteoporosis – a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture; bone mineral density z-score ≤ -2.0 together with secondary risk factors for fracture (e.g., undernutrition, hypoestrogenism, prior fractures).¹

Primary amenorrhea – failure to reach menarche by the age of 15.¹

Resting metabolic rate – the metabolic rate of the body at relative rest defined as fasted for at least 4 hours and awake, preferably but not necessarily in the morning and without the requirement for no movements in the hours prior to measurement; a rest recovery period is required immediately prior to the measurement.⁶

Secondary amenorrhea – amenorrhea beginning after menarche.¹

Z-score – densitometry score to compare the bone mineral density of an individual to those of age, race, and sex-matched controls.¹
Chapter 1: Introduction

Since the 1970s, the opportunity for adolescent females to participate in sports has grown exponentially. With an increase in opportunity came an increase in participation, with almost 3 million girls participating in high school athletics by the year 2006. As participation rates have continued to increase, a grouping of interrelated conditions has presented itself among this population of athletes. The female athlete triad, first recognized in 1992, is defined as a spectrum of abnormalities in energy availability, menstrual function, and bone mineral density. Several other conditions have since been associated with the triad, including decreased resting metabolic rate, all of which have been observed primarily among females participating in sports which emphasize a lean body type. As the severity of each triad component increases, the risk of sport-related injury, reproductive and skeletal consequences, and a number of other health concerns have been shown to increase correspondingly.

Sport participation among adolescent females reaps incredible benefits including the optimization of muscular strength and flexibility; maintenance of a healthy body weight; attainment of peak bone mass; development of neuromuscular awareness; optimization of cognition, mental health, mood, sleep, and academic performance; and the improvement of overall wellness and social behavior. In light of these benefits, the answer to preventing the development of the female athlete triad and its associated conditions is not to discourage sport participation among this population. Rather, it is necessary to investigate the disorder itself and determine how to best screen for, prevent, and treat each of the triad components and associated conditions. With this, it is important to educate athletes, coaches, and sports medicine practitioners about the condition itself and the long-term consequences of undiagnosed and
untreated triad components. Few studies address the complete female athlete triad and associated conditions among adolescent female athletes. In order to develop an educational curriculum and protocol to address screening, prevention, and treatment, more research is needed to describe the prevalence and severity of the female athlete triad and associated conditions among this population.

**Objectives**

The primary objective of this cross-sectional descriptive study was to:

- Describe the components of the female athlete triad, namely energy availability, menstrual function, and bone mineral density, as well as resting metabolic rate, among a cohort of middle-to-upper class adolescent female athletes.
Chapter 2: Review of the Literature

While athletic participation can be incredibly beneficial for adolescent females, there exists a risk of developing skeletal, reproductive, metabolic, and other serious health conditions as participation rates in this population increase and the level of competition continues to rise. The female athlete triad, a spectrum of abnormalities in energy availability, menstrual function, and bone mineral density, is a prevalent and serious concern for adolescent female athletes. A review of the literature regarding female athletic participation, the history of the female athlete triad, each of the three interrelated conditions, and other health concerns associated with the female athlete triad will provide a foundation for analyzing the data and interpreting the results of this study.

Adolescent Female Athletic Participation

“No person in the United States shall, on the basis of sex, be excluded from participation in, be denied the benefits of, or be subjected to discrimination under an education program or activity receiving Federal financial assistance”. This excerpt from Title IX of the Educational Amendments of 1972 has provided access for adolescent females to participate in organized sports to the same degree as their male counterparts. This act of equality increased the opportunities available for female adolescents to participate in sports, and participation rates grew exponentially. In 1972, approximately 294,000 female adolescents participated in high school sports. By 2006, this number had grown to over 2,953,000 participants. This 904% increase in participation demonstrates the historical significance of Title XI, and the remarkable shift toward equality of access to participate in organized sports among high school athletes. As more adolescent females have engaged in sport, a high incidence of menstrual dysfunction has
stimulated research on these female athletes, revealing several problems associated with participation in sport among this population.

Problems Associated with Increased Sport Participation Among Adolescent Females

Menstrual Dysfunction

An increase in participation has been accompanied by an increase in sport-related health complications. Menstrual irregularity has been found to be prevalent in the athletic population, especially compared to the general population. In a study conducted by Beals and Manore in 2002, 31% of a sample of 425 female collegiate athletes ages 17 to 21 reported “irregular [menstrual] cycles” and more than 41% reported having less than 12 menstrual cycles per year. In 2010, Rauh, Nichols, and Barrack found that, among a group of 163 female high school athletes, 25% experienced menstrual dysfunction. Nichols et al. reported a similar incidence of menstrual dysfunction among 170 female high school athletes ages 13 to 18, with 23.5% of the athletes reporting menstrual irregularities. In 2013, Gibbs and her colleagues reported a higher menstrual dysfunction incidence of 60% among a group of 75 exercising women ages 19 to 27. Compared to sedentary high school students, Hoch et al. found that high school athletes suffered more menstrual abnormalities, with 21% of sedentary students and 54% of athletes experiencing menstrual disturbances. These results suggest increased physical activity and sport participation play a role in the disruption of menstrual function.

Sport-related Injuries

An increase in the prevalence of sport-related injuries has also been observed, especially in the athletes presenting with menstrual dysfunction. In the 2002 study, Beals and Manore also documented the prevalence of musculoskeletal injuries in a large sample of female collegiate athletes. Of their anonymous sample of collegiate athletes participating in aesthetic, endurance, and anaerobic sports, 65.9% of the total sample reported a muscle injury and 34.3% reported a bone injury during their collegiate career. When comparing athletes with and without menstrual
dysfunction, more muscle and bone injuries were reported by the athletes with menstrual dysfunction than by those without.  

<table>
<thead>
<tr>
<th>% Reporting bone injury</th>
<th>Regular (n = 211)</th>
<th>Irregular (n = 95)</th>
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<tr>
<td></td>
<td>31.4</td>
<td>40.0</td>
</tr>
<tr>
<td>% Reporting muscle injury</td>
<td>60.8</td>
<td>67.4</td>
</tr>
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Table 1 - Relationship Between Those Athletes Self-Reporting Regular and Irregular Menstrual Cycles and Musculoskeletal Injuries

Rauh et al. reported similar results among a sample of 163 high school female athletes competing in 8 interscholastic sports. During the season of interest, 37.4% of the athletes suffered at least one sport-related musculoskeletal injury severe enough to prevent them from participating in practice or competition. In relation to menstrual function, 56.1% of athletes experiencing menstrual dysfunction reported an injury while only 31.1% of athletes with normal menstrual function reported an injury. These findings among high school athletes were supported by a cross-sectional study conducted by Thein-Nissenbaum and her colleagues in 2012. Among a sample of 249 high school female athletes competing in 33 interscholastic sports, 63.1% experienced a musculoskeletal injury during the study period. Athletes who reported menstrual dysfunction experienced a higher percentage of severe injuries than those with normal menstrual function. It is seemingly intuitive that as athletic participation among adolescent females increases, the number of sport-related injuries also increases. The consistently higher prevalence of injury among athletes experiencing menstrual dysfunction however proves more complex and
demands investigation into the causes of menstrual dysfunction and musculoskeletal injuries among adolescent female athletes.

*Eating Disorders and Disordered Eating*

The prevalence of eating disorders among athletes versus non-athletes has been studied extensively and the results of these studies have been consistent. Sundgot-Borgen and Torstveit in 2004 sought to examine the prevalence of anorexia nervosa (AN), bulimia nervosa (BN), anorexia athletica (AA), and eating disorders not otherwise specified (ED-NOS) in Norwegian elite athletes and a representative sample from the general Norwegian population. Among the female athletes in the study, 21% were classified as at-risk for an eating disorder and 20% of those classified as at-risk met the diagnostic criteria for EDs.16 Among the control subjects, 14% were classified as at-risk and of these subjects, 9% met the diagnostic criteria for EDs. The authors concluded that the prevalence of eating disorders is higher among elite athletes than controls. Sundgot-Borgen collaborated with Martinsen in 2013 to conduct a similar study among adolescent elite athletes. Although a greater percentage of control subjects than athletes were classified as “at risk” for an eating disorder, a higher percentage of athletes than control subjects were estimated to meet the diagnostic criteria for EDs. Eating disorders are consistently found to be more prevalent among athletes than the general population.16,17

It is necessary to make a distinction between clinical and subclinical eating disorders. Diagnosis of a clinical eating disorder must meet all of the criteria described in the *Diagnostic and Statistical Manual of Mental Disorders (DSM-5).* Some individuals meet several but not all of the diagnostic criteria and are deemed subclinical eating disorders. Current literature indicates that the prevalence of subclinical eating disorders among female athletes is greater than that of clinical eating disorders.22–24 It is important to take the whole spectrum of disordered eating into consideration when observing changes in disordered eating patterns among female athletes.
As defined by the American College of Sports Medicine in The Female Athlete Triad Position Stand, disordered eating refers to “various abnormal eating behaviors, including restrictive eating, fasting, frequently skipped meals, diet pills, laxatives, diuretics, enemas, overeating, binge-eating and then purging”. The prevalence of disordered eating among female athletes has been assessed in various populations using an assortment of tools. In 2002, Beals and Manore used the Eating Attitudes Test (EAT-26) and the Eating Disorder Inventory Body Dissatisfaction Subscale (EDI-BD) to assess the risk of disordered eating among a group of 425 female collegiate athletes from 7 National Collegiate Athletic Association (NCAA) universities. The percentage of athletes scoring above the cut-off scores on the EAT-26 and EDI-BD was 15.2% and 32.4% respectively, indicative of being “at-risk” for disordered eating. Rosendahl et al. also utilized the EAT-26 to screen for disordered eating among a group of high school athletes and non-athletes. The results of this study revealed a greater percentage of non-athletes scoring above the designated cut-off scores on the EAT-26 versus athletes. However, 26% of the athletes were considered “at-risk” for disordered eating based on their EAT-26 scores. The outcomes of these studies are highly dependent on the cohort studied and the setting in which the research was conducted. Having said this, the percentages of “at-risk” athletes are relatively similar among samples.

The Eating Disorder Examination Questionnaire (EDE-Q) is a tool that has been used more extensively to identify disordered eating attitudes and assess eating behaviors among athletes and non-athletes. Nichols et al. reported an 18.2% and 20.0% prevalence of disordered eating among two different cohorts of female high school athletes based on EDE-Q scores. Pernick et al. and Rauh et al. published similar results among female high school athletes, reporting a prevalence of 19.6% and 16% respectively. In the published literature to date, the reported prevalence of disordered eating among high school female athletes ranges from 16% to 35%. 
Many of these studies assessing disordered eating among female athletes attempted to identify which athletes may be at a greater risk for disordered eating based on sport type. In 1993, Sundgot-Borgen assessed 522 elite female athletes, finding that a significantly higher number of athletes than controls suffered from disordered eating, particularly athletes competing in sports where a specific weight or a lean body type was favored. Beals and Manore assessed the eating behaviors and attitudes of 425 female collegiate athletes divided into three categories based on the physiological nature and competitive requirements of their individual sports: aesthetic, endurance, and team/anaerobic. Athletes participating in aesthetic sports (i.e. cheerleading, diving, and gymnastics) scored significantly higher on the EAT-26 than athletes participating in endurance or team/anaerobic sports. Furthermore, significantly more athletes participating in aesthetic sports scored above the cut-off score on the EAT-26. Thein-Nissenbaum et al. also classified subjects into either aesthetic, endurance, or team/anaerobic sports and reported that a greater percentage of athletes participating in aesthetic sports were “at-risk” for disordered eating compared to athletes participating in endurance or team/anaerobic sports. In these cases, athletes participating in aesthetic sports were more likely to experience menstrual irregularities than athletes participating in endurance or team/anaerobic sports. Nichols et al. categorized a group of 423 high school female athletes into lean-build and non-lean build sports. Though there was no difference found between the groups for disordered eating, the prevalence of menstrual irregularities was greater among athletes in lean-build sports versus non-lean build sports. Though prevalence estimates vary, it is clear that disordered eating and its associated conditions are of great concern, especially among athletes participating in aesthetic and lean-build sports where a specific weight or lean body type is preferred.

Initially recognized as a relationship between disordered eating, amenorrhea, and musculoskeletal injuries, the female athlete triad has been studied extensively among various populations. New literature has led to a more accurate definition of the triad, defining it as “a
spectrum of abnormalities in energy availability, menstrual function, and bone mineral density”.¹

Over the years, the female athlete triad has emerged as a serious problem among female athletes, particularly during the adolescent years due to the significant growth and development that occurs during this time. Understanding the history, risk factors, and individual components of the triad will help sports medicine professionals, as well as coaches, parents and athletes, to identify, prevent, and treat these interrelated conditions.

**The Female Athlete Triad**

*History of the Female Athlete Triad*

In 1992, the Task Force on Women’s Issues of the American College of Sports Medicine (ACSM) assembled and presented the term *female athlete triad* to describe the condition characterized by disordered eating, amenorrhea, and osteoporosis.²⁹ At this time, diagnosis of the female athlete triad required the presence of all three triad components: an eating disorder, amenorrhea, and osteoporosis.²⁹ Because of this, many female athletes struggling with one or two of the components remained undiagnosed and thus went untreated.³⁰

In 2007, a group of experts from ACSM made modifications to the definition of the female athlete triad and published an updated position statement. The new definition presented the triad as “a spectrum of abnormalities in energy availability, menstrual function, and bone mineral density”.¹ This spectrum of abnormalities refers to a range of severity from normal to varying degrees of pathology for each component of the triad.¹ The spectrum of each component is presented in Figure 1.
The 2007 diagnostic criteria requires the presence of at least one triad component of pathologic degree (i.e. low energy availability with or without an eating disorder, functional hypothalamic amenorrhea, or osteoporosis) for diagnosis. This definition and accompanying diagnostic criteria is detailed in “The American College of Sports Medicine Position Stand for the Female Athlete Triad”. It stands as the current working definition and diagnostic criteria for the triad.

Components of the Female Athlete Triad

Energy Availability

Energy availability is operationally defined as dietary energy intake (EI) minus exercise energy expenditure (EEE) normalized to lean body mass (LBM), i.e., $EA = (EI - EEE)/LBM$ LBM/day. This represents the amount of dietary energy that remains for other bodily functions after exercise. The spectrum of energy availability ranges from optimal energy availability to low
energy availability with or without an eating disorder. Energy availability may be reduced to any degree with or without an eating disorder along the spectrum. This component of the triad was originally defined as “disordered eating” but has since evolved into “energy availability” to include athletes who may experience reduced or low energy availability apart from disordered eating.\(^1\)

Reduced or low energy availability may be the result of one or several energy-restricting behaviors. An increase in exercise energy expenditure greater than dietary energy intake will result in decreased energy availability. Alternatively, reducing dietary energy intake to such a degree that it does not meet exercise energy expenditure results in low or reduced energy availability. Abnormal eating behaviors that have a detrimental effect on energy availability include fasting, binge-eating and purging, and more severely clinical eating disorders including anorexia nervosa, bulimia nervosa, other specified feeding or eating disorder (OSFED), and unspecified feeding or eating disorder (UFED).\(^3\) Other behaviors that limit energy availability are the inappropriate use of diet pills, laxatives, diuretics, and enemas to achieve or maintain a low weight.\(^1\) Participation in one or more of these behaviors places an individual along the spectrum from optimal energy availability to low energy availability.

Low or reduced energy availability poses a threat to the female athlete because when energy is not available to support normal function for all body systems, physiological mechanisms direct available metabolic fuels toward life-sustaining processes; these fuels are therefore not available for other processes such as cellular maintenance and repair, thermoregulation, growth, and reproduction.\(^31\) Though this shift promotes survival, it has negative effects on the systems being deprived of energy and can greatly impair growth and development. This is especially concerning in the adolescent population, as this is an important time of growth and development; for the athlete, the energy needed to fuel sport participation in addition to that needed for growth and development can threaten the athlete’s ability to achieve and maintain
optimal energy availability and can place this athlete at risk for low or reduced energy availability.\textsuperscript{31}

The negative health effects associated with a prolonged state of low or reduced energy availability are troublesome. Low EA is associated with decreased bone mineral density and failure to reach optimal bone mineral density during childhood and adolescence.\textsuperscript{5,32} It is also associated with subclinical and clinical menstrual dysfunction.\textsuperscript{33–36} A decline of bone mineralization and disruption of menstrual function have been observed below an energy availability threshold of 30 kcal/kg LBM per day in adult female athletes.\textsuperscript{32–34,37} Loucks and colleagues conducted a randomized, repeated-measures, prospective cohort experiment among 29 regularly menstruating, habitually sedentary, young women to compare and contrast the incremental effects of balanced and restricted energy availability on luteinizing hormone (LH) pulsatility and selected markers of bone turnover.\textsuperscript{34} Participants were randomized into three groups and within each group women were further divided into two groups: balanced EA at 45 kcal/kg LBM per day and restricted EA at 10, 20, or 30 kcal/kg LBM per day. The experiment was conducted twice so each woman would participate at a restricted EA and a balanced EA. Researchers measured LH pulse frequency as a measure of menstrual function, plasma osteocalcin (OC) and serum type 1 procollagen carboxy-terminal propeptide (PICP) as markers of bone formation, and urinary N-telopeptide (NTX) as a marker of bone resorption. The results identify a suppression of LH pulse frequency, and thus a disruption of reproductive function, at an EA of 10 and 20 kcal/kg LBM per day, but not at 30 kcal/kg LBM or a balanced EA of 45 kcal/kg LBM.\textsuperscript{34} Furthermore, a dose-response relationship was identified between EA and bone marker concentrations. Restricted EA at 10, 20, and 30 kcal/kg LBM per day reduced both OC and PICP, with greater reductions observed with greater EA restriction. An EA of 10 kcal/kg LBM per day increased both NXT and two indices of resorption/formation uncoupling. These results support the theory that decreased bone mineralization and menstrual dysfunction occur
below a threshold of 30 kcal/kg LBM per day.\textsuperscript{34} Several other adult women studies support these findings,\textsuperscript{1,38–40} however others have failed to identify such a threshold and dose-response relationship.\textsuperscript{20,41} No studies have investigated such a threshold among adolescent females.

Low or reduced energy availability can have negative effects on the body beyond the triad itself. From their early work, Beals et al. suggest low or reduced energy availability can lead to decreased glycogen stores, decreased lean body mass, chronic fatigue, micronutrient deficiencies, dehydration, anemia, and electrolyte and acid-base imbalances.\textsuperscript{42} The normal function of several body systems can be disrupted by low or reduced energy availability, including the cardiovascular, endocrine, reproductive, skeletal, gastrointestinal, renal, and central nervous systems.\textsuperscript{1} If low energy availability is associated with disordered eating, an underlying psychological problem such as decreased self-esteem, anxiety, depression, and suicidal tendencies may exist.\textsuperscript{43} All of this said, overall health and well-being is dependent upon adequate energy availability, and the risk of experiencing serious health consequences is increased when energy availability is reduced below the amount of energy required to support normal bodily function.

\textit{Menstrual Function}

The American College of Sports Medicine presents menstrual function as a spectrum ranging from eumenorrhea to functional hypothalamic amenorrhea.\textsuperscript{1} Eumenorrhea is defined as “menstrual cycles at intervals near the median interval for young adult women”, that is, 28 days with a standard deviation of 7 days.\textsuperscript{1} Amenorrhea is defined as the “absence of menstrual cycles for more than 90 days” or three months.\textsuperscript{1} Primary amenorrhea refers to the failure to reach menarche by the age of 15 while secondary amenorrhea refers to the occurrence of amenorrhea after menarche.\textsuperscript{1} Subclinical menstrual disorders fall between these two extremes, ranging from less severe luteal phase defects and anovulation, which exhibit no perceptible symptoms, to more severe oligomenorrhea, which is recognized by menstrual cycles occurring at longer intervals
than 35 days. The continuum of menstrual disturbances is presented in the context of the Female Athlete Triad in Figure 1 and as its own entity displaying each subclinical menstrual disorder in Figure 2.

Figure 2 – Continuum of Menstrual Disturbances in Athletes

From a mechanistic perspective, hypothalamic menstrual disturbances occur when the pulsatile secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus is disrupted. This disruption in GnRH causes a disruption in luteinizing hormone (LH) pulsatility; the pituitary gland fails to secrete pulses of LH at the correct frequency. In 1998, Loucks, Verdun, and Heath conducted an experiment to test two hypotheses about the disruption of LH pulsatility in exercising women. The first hypothesis, the “exercise stress hypothesis”, suggested that exercise training is a chronic stressor that activates the hypothalamic-pituitary-adrenal (HPA) axis and that one or more of the central and peripheral mediators of the HPA axis disrupts the GnRH pulse generator. The second hypothesis, the “energy availability hypothesis”, suggested that the GnRH pulse generator is disrupted by “a signal that dietary energy intake is inadequate for the energy costs of both reproduction and locomotion”. To test the exercise stress hypothesis, researchers assayed LH in blood samples drawn from habitually sedentary women on
days 8, 9, and 10 of two menstrual cycles after 4 days of intense exercise. They then compared LH pulsatility of these women to those previously reported in women with similar energy availability who had not exercised.\textsuperscript{39} To test the energy availability hypothesis, researchers controlled the subjects’ dietary energy intakes to produce energy availabilities at 45 and 10 kcal/kg LBM/day during the two trials and recorded LH pulsatility in each of the energy availability scenarios.\textsuperscript{39} Loucks and her colleagues found that the exercising women with low energy availability had a 10% reduction in LH pulse frequency and a 36% increase in LH pulse amplitude.\textsuperscript{39} Interestingly, the previously reported non-exercising women had a 60% reduction in LH pulse frequency when the stress of exercise was not present and low energy availability was caused by dietary restriction alone.\textsuperscript{39} Among the exercising women controlled to 45 kcal/kg LBM/day, the stress of exercise neither reduced LH pulse frequency nor increased pulse amplitude.\textsuperscript{39} These results led the researchers to conclude that low energy availability, not stress of exercise, alters LH pulsatility in exercising women.\textsuperscript{39}

Several studies were conducted in the years following Loucks’ investigation providing support for the energy availability hypothesis. In 2001, Williams et al. performed a longitudinal study using cynomolgus monkeys to examine the role of low energy availability on the development and reversal of exercise-induced amenorrhea.\textsuperscript{48} Eight adult female monkeys were subjected to a 7- to 24-month running program. Daily exercise was increased to 12.3 ± 0.9 km/day, at which the monkeys developed amenorrhea, defined as an absence of menses for at least 100 days, with low and unchanging concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone (P4).\textsuperscript{48} During the development of amenorrhea, food intake remained constant. To test whether the amenorrhea was caused by low energy availability, four of the monkeys were given additional calories equal to 138-181% of their initial calorie amount as they continued daily exercise training.\textsuperscript{48} All of the monkeys given supplemental calories resumed ovulation and experienced an increase in reproductive hormone
levels within 12-57 days.\textsuperscript{48} From these results, Williams and her colleagues concluded that exercise-induced amenorrhea is the result of low energy availability, and that the resumption of normal menstrual function can be accomplished by increasing energy availability with supplemental calories.\textsuperscript{48}

Lagowska replicated the results of Williams et al. in a study conducted with human subjects. In this 2014 study, an increase in energy intake and energy availability among 45 professional athletes with menstrual irregularity resulted in a rise in LH concentrations and LH to FSH ratios during a three-month dietary intervention.\textsuperscript{49} Though the menstrual cycle was not restored during this short intervention, these improvements in hormone concentrations indicate improved reproductive function and authors suggest that menstrual function may have been restored had the intervention continued.\textsuperscript{49} Several other studies support this idea that menstrual function can be restored through an increase in energy availability.\textsuperscript{41}

In 2012, Arends et al. conducted a retrospective chart review of Division I collegiate female athletes to determine if menses could be restored through an increase in energy availability in human subjects.\textsuperscript{50} Of the 373 charts reviewed, 51 athletes were found to have menstrual dysfunction.\textsuperscript{50} All of the athletes who were identified with menstrual disturbances were advised to increase energy availability with either an increase in dietary intake or a decrease in exercise energy expenditure with the guidance of a physician and a sports dietitian.\textsuperscript{50} At a 5-year follow-up, 26 athletes had been lost to follow-up, had failed to partake in the intervention, or could not be included in the final results because they had initiated the use of oral contraceptives. However, of those 25 remaining, 36% experienced a restoration of menses.\textsuperscript{50} Looking at the anthropometric and dietary data of those who experienced a restoration of menses and those who did not, those who resumed menstruation experienced a significant increase in body weight (5.3 ± 1.1 kg) and BMI (1.9 ± 0.4 kg/m\textsuperscript{2}) during the study period compared to those who did not resume menstruation (1.3 ± 1.1 kg; 0.5 ± 0.4 kg/m\textsuperscript{2}).\textsuperscript{50} They also had a higher absolute and percentage
weight gain and a greater change in BMI than those who did not experience a restoration of menses. Furthermore, energy, protein, fat, and calcium intakes were greater among those who experienced a restoration of menses versus those who did not. Had all of the athletes received the same intervention and increased energy availability to the degree as those who experienced a resumption of menses, it could be hypothesized that more athletes may have been successful in restoring menstrual function. From these results, researchers concluded that non-pharmacologic intervention, that is, an increase in energy availability through an increase in dietary intake or a reduction in exercise energy expenditure, in college athletes with menstrual disturbances could restore regular menstrual cycles.

In 2014, Williams collaborated with De Souza and several others to assess the impact of energy deficiency on menstrual function in adult women. Controlled feeding and supervised exercise regimens were conducted over four menstrual cycles in untrained, eumenorrheic women. Participants were randomly assigned to an “exercising control group” or one of three “exercising energy deficit groups”. Throughout the course of the training, menstrual cycle length and changes in urinary concentrations of estrone-1-glucuronide (E1G), pregnanediol glucuronide (PdG), and mid-cycle LH were assessed. Results indicate that the overall sum of disturbances was greater in the exercising energy deficit groups than in the exercising control group. They also observed a dose-response relationship between the magnitude of energy deficiency and the frequency of exercise-related menstrual disturbances. However, the degree of energy deficiency did not determine the severity of the menstrual disturbances in this group of women. Reed et al. reported similar findings in a 2015 study in which 91 exercising women were categorized as amenorrheic, oligomenorrheic, or eumenorrheic based on menstrual status. Energy availability was calculated using diet logs, heart-rate monitors and/or exercise logs, and body composition data from DXA. Researchers found that EA did distinguish the eumenorrheic subjects from the amenorrheic subjects; the eumenorrheic subjects displayed a mean of 37.6
kcal/kg LBM/day while the amenorrheic subjects displayed a mean of 30.9 kcal/kg LBM/day. EA however did not distinguish between subclinical menstrual disturbances; as observed in Williams’ study, the degree of energy deficit did not determine the severity of subclinical menstrual disturbances.

Though much literature supports the energy availability hypothesis, more research needs to be conducted to determine whether an energy availability threshold exists at which menstrual disturbances begin to occur or menstrual function is restored. Furthermore, it is unclear how or if the restriction of energy availability to various degrees correlates with the severity of subclinical menstrual dysfunction. Finally, there is a lack of published research that addresses the relationship between energy availability and menstrual function in the adolescent population.

**Bone Mineral Density**

The spectrum of bone mineral density identified by the American College of Sports Medicine ranges from optimal bone health to osteoporosis. Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Though bone strength and the associated risk of fracture depend on several factors, namely density and internal structure of bone mineral and the quality of bone protein, the screening and diagnosis of osteoporosis is based on bone mineral density alone. Dual-energy x-ray absorptiometry (DXA) is considered the gold standard for conducting these BMD measurements. In premenopausal women and children, BMD measurements are expressed as z-scores, which compare individuals to age and sex-matched controls. For diagnostic purposes, a z-score below -2.0 standard deviations is deemed “low bone mineral density below the expected range for age” in premenopausal women and “low bone density for chronological age” in children. The International Society for Clinical Densitometry (ISCD) recommends that osteoporosis should be diagnosed in these populations only when low BMD is present with secondary clinical risk factors associated with bone loss and fracture, including chronic
malnutrition, eating disorders, hypogonadism, glucocorticoid exposure, and previous fractures. In the context of the female athlete triad, the American College of Sports Medicine defines “low BMD” as “a history of nutritional deficiencies, hypoestrogenism, stress fractures, and/or secondary risk factors for fracture together with a BMD z-score between -1.0 and -2.0” and “osteoporosis” as “secondary clinical risk factors for fracture with BMD z-scores less than or equal to -2.0”.

When studying an adolescent population, several researchers suggest height status should be considered in order to accurately measure BMD and calculate z-scores. A DXA BMD measurement produces a two-dimensional image, which does not incorporate the depth or shape of the bone being measured. Because of this, a small bone may appear to have a lower areal BMD compared to a larger bone with a comparable volumetric BMD. This inaccurate measurement would theoretically produce a low z-score. In 2009, Zemel et al. conducted a study in five clinical centers in the United States to compare various methods to adjust BMD for height in healthy children. Of all the methods used to adjust BMD z-scores, adjustments using height-for-age z-scores (HAZ) were the least biased for estimating the effect of stature on BMD. However, the authors note further validation of this approach is needed to fully evaluate the validity of this approach. At this time, no validated method of height-adjusting BMD z-scores in the adolescent population exists.

Historically, chronic hypoestrogenism has been accepted as the major cause of bone loss in women. Estradiol (E2), a form of estrogen, plays an important role in bone formation and maintenance among adolescent and premenopausal women. Estrogen serves to suppress the activity of osteoclasts, the cells responsible for bone resorption, allowing the work of osteoblasts to lay down bone tissue and increase bone mass. The bone must be exposed to adequate estrogen to optimize this process.
In the presence of menstrual dysfunction, reduced estrogen levels can inhibit bone formation and contribute to bone loss, a failure to achieve peak bone mass, and poor bone mineral density as a risk factor for osteoporotic fractures among amenorrheic women. Many studies have observed a correlation between poor bone mass and menstrual dysfunction, pointing to hypoestrogenism as an underlying mechanism by which bone mass is compromised. In 1990, Drinkwater and her colleagues conducted an important benchmark study to examine the relationship between present bone mineral density, menstrual history, and current menstrual status of active women. Ninety-seven active women completed questionnaires to report menstrual history and current menstrual status, the latter of which was confirmed by drawing blood samples and assaying for estradiol and progesterone levels. Single- and dual-photon absorptiometry were used to measure bone density at 7 sites. The women were categorized into 3 groups based on past and present menstrual status: 1) women who had always had regular menses, 2) women who had experienced periods of oligomenorrhea or amenorrhea interspersed with periods of regularity including current oligomenorrheics, and 3) women who were currently amenorrheic or had been amenorrheic or oligomenorrheic in the past. Results indicated a significant difference in vertebral BMD among groups, with women in Group 1 having the greatest BMD and women in group 3 having the poorest. Women in Group 1 and 2 also had a higher femoral shaft BMD than women in Group 3. These group differences were also seen in estradiol and progesterone levels. Women in Group 1 had the highest estradiol and progesterone levels while women in Group 3 had the lowest. From these results it was concluded that a relationship exists between bone mineral density and past and present menstrual status due to the hypoestrogenism that accompanies oligomenorrhea, amenorrhea, and late menarche. Other studies have drawn similar conclusions among girls who experienced late menarche, suggesting that decreased estrogen exposure during adolescence led to decreased bone mineral density later
in life.\textsuperscript{55-57} All of these studies suggest poor bone mass results from decreased estrogen exposure occurring during menstrual dysfunction.

Hypoestrogenism, however, has not been supported as a stand-alone mechanism by which bone loss occurs. Several observations point to factors other than hypoestrogenism that prevent adequate bone formation and lead to poor bone mass. For example, several studies found that women with hypothalamic amenorrhea did not experience a complete recovery of bone mass when administered oral contraceptives and estrogen, suggesting that a factor besides estrogen may play a role in bone formation.\textsuperscript{58-60} Others have reported a failure to restore bone mass when menses were restored among previously amenorrheic athletes.\textsuperscript{61,62} Additionally, poor bone mass has been observed among regularly menstruating women, suggesting that apparently normal levels of estrogen do not preclude poor bone mass.\textsuperscript{63}

In light of these findings, researchers began to suspect that a chronic energy deficiency may also impair bone formation through an estrogen-independent mechanism.\textsuperscript{32} In 1995, Grinspoon et al. were the first to demonstrate that markers of bone formation, namely serum osteocalcin (OC) and procollagen carboxyl-terminal propeptide (PICP), decline significantly with short-term fasting among a group of healthy women.\textsuperscript{64} This same group of researchers were also the first to demonstrate that bone formation falls rapidly with acute caloric deprivation in normal women.\textsuperscript{65} Looking at these results, Ihle and Loucks conducted a randomized, repeated-measures, prospective cohort experiment among regularly menstruating, habitually sedentary, young women to describe the incremental effects of balanced (45 kcal/kg LBM/day) and three restricted (10, 20, and 30 kcal/kg LBM/day) energy availability treatments on OC, PICP, and NTX.\textsuperscript{32} As previously discussed, Ihle and Loucks found a dose-response relationship between energy availability and bone turnover among this population.\textsuperscript{32} At all levels of energy restriction, OC and PICP were significantly reduced, indicating the suppression of bone formation.\textsuperscript{32} It was only at the lowest energy availability that NTX, the marker of bone resorption, was affected, rising significantly at
an energy availability of 10 kcal/kg LBM/day.\textsuperscript{32} Interestingly, estradiol was also unaffected until energy restriction reached 10 kcal/kg LBM/day.\textsuperscript{32} These results indicate that severe energy restriction limits bone formation and increases bone resorption, leading to the uncoupling of these two factors and the reduction of bone mineral density.\textsuperscript{32} They also indicate that bone formation can be impaired at an energy availability as high as 30 kcal/kg LBM/day despite no change in estradiol concentrations at this energy restriction.\textsuperscript{32} It was therefore concluded that, through an estrogen-independent mechanism, bone formation is dependent on energy availability in regularly menstruating young women, and that an energy availability as high as 30 kcal/kg LBM/day has the potential to prevent young women from achieving their genetic potential for peak bone mass despite adequate levels of estradiol.\textsuperscript{32}

In 2008, De Souza et al. conducted a study to evaluate the independent and combined effects of estrogen deficiency and energy deficiency on bone formation and bone resorption in premenopausal exercising women.\textsuperscript{66} Forty-four premenopausal women were observed for a 2- to 3-month time period. Researchers evaluated energy status using resting energy expenditure (REE) and metabolic hormones including triiodothyronine ($T_3$), leptin, and ghrelin.\textsuperscript{66} Estrogen status was determined by daily measurements of estrone-1-glucuronide (E1G), pregnanediol glucuronides (PdG), and luteinizing hormone (LH). Measurements of type I procollagen carboxy-terminal propeptide (PICP) and osteocalcin (OC) were evaluated as markers of bone formation, while measurements of C-terminal telopeptide (U-CTX-I) were evaluated as markers of bone resorption.\textsuperscript{66} Researchers also assessed bone mineral density using dual-energy X-ray absorptiometry (DXA), dietary energy intake using 3-day nutritional logs, eating behaviors using the Eating Disorders Inventory (EDI) and the Three Factor Eating Questionnaire, and peak aerobic capacity using a progressive treadmill test to volitional exhaustion.\textsuperscript{66} Researchers found that osteocalcin levels were higher among the energy replete women than the energy deficient women, suggesting that bone formation was suppressed in the presence of an energy deficit.\textsuperscript{66}
Looking at the estrogen status of the energy deficient women, those who had adequate levels of estrogen had higher levels of osteocalcin than those who were estrogen deficient, suggesting that energy and estrogen have a combined effect on osteocalcin levels.\textsuperscript{66} Similarly, the women with both an energy deficiency and an estrogen deficiency exhibited the greatest U-CTX levels, indicating that bone resorption was greatest among this group of women.\textsuperscript{66} From these results, the authors concluded that when energy status is adequate, disruption in bone formation and bone resorption is not a concern, regardless of estrogen status. When an energy deficiency is present, however, bone formation can be suppressed, even when estrogen levels are adequate.\textsuperscript{66} When an estrogen deficiency exists in the presence of an energy deficiency, bone loss can occur as the result of both a decrease in bone formation and an increase in bone resorption.\textsuperscript{66} The results of this study support the idea that estrogen status and energy status together affect bone turnover and skeletal health in exercising women.\textsuperscript{66}

As De Souza and Williams so eloquently stated, “the effects of caloric restriction on BMD may be mediated by endocrine factors that may include an estrogen-dependent pathway, but may also include an estrogen-independent pathway that involves some of the metabolic-related hormones that are altered in exercising women with severe menstrual disturbances and that impact bone turnover”.\textsuperscript{54} This “estrogen-dependent pathway” refers to the relationship between menstrual dysfunction and decreased levels of estradiol. As previously discussed, decreased levels of estradiol are associated with greater bone resorption and loss of bone mass.\textsuperscript{32} The “estrogen-independent pathway” on the other hand refers to the relationship between an energy deficiency and changes in bone trophic factors. In the presence of an energy deficit, the body becomes hypometabolic in an attempt to conserve available energy.\textsuperscript{54} In this hypometabolic state, alterations in metabolic hormones have been observed.\textsuperscript{46,47} Changes in many of these metabolic hormones, particularly a decrease in IGF-1 and leptin, have been found to play a role in altered bone turnover and contribute to bone loss.\textsuperscript{46,47,54} Therefore, bone loss can occur
independently from estrogen as a result of changes in metabolic hormones including total triiodothyronine (T₃), leptin, insulin, glucose, IGF-1, IGFBP-3, IGFBP-1, ghrelin, growth hormone, and cortisol.⁴⁶,⁴⁷ De Souza and Williams suggest that these mechanisms of bone loss work in tandem, however more research must be conducted to tease out the differential effects of hypoestrogenism and energy deficiency.⁵⁴

Aside from the effects of estrogen and energy on bone, the osteogenic response to the mechanical stresses of exercise must be considered. In 1974, Dalen and Olsson conducted a study “to examine whether increased physical activity can raise the amount of bone mineral in the skeleton, or, at least, retard the reduction with age.”⁶⁷ Two groups of participants were examined in this study: a long-term physical activity group consisting of 15 male cross-country runners who had been practicing the sport for at least 25 years, and a control group consisting of 31 men subjected to a three-month increase in physical activity.⁶⁷ Using x-ray spectrophotometry, bone mineral content was measured at seven sites: radius and distal ulna; radius and mid-shaft ulna; humeral head; third lumbar vertebra; femoral neck; mid-shaft femur; and calcaneus.⁶⁷ The mean bone mineral content of the cross-country runners was greater than the controls, with BMC of appendicular sites containing trabecular bone showing a significant 20% advantage.⁶⁷ These results set a foundation for future research investigating the effect of physical activity on bone and raised questions about the generalizability of these results to other populations.

In 1996, Etherington et al. conducted a retrospective cohort study to estimate the changes in bone mineral density as a consequence of exercise in female ex-athletes and age-matched controls.⁶⁸ Eighty-three ex-elite female athletes (middle and long distance runners and tennis players) and 585 age-matched females were recruited.⁶⁸ Investigators measured BMD of the lumbar spine, femoral neck, and forearm using DXA. Levels of physical activity were assessed using a modified Allied Dunbar Fitness Survey. Analysis of covariance was used to adjust for differences in age, weight, height, and smoking between groups.⁶⁸ Results indicated that athletes
had significantly greater femoral neck and lumbar spine BMD than controls when adjusted for age, height, weight, and smoking status. Interestingly, tennis players had significantly higher BMDs at the lumbar spine than runners. Furthermore, among the tennis players, total BMD of the dominant arm was significantly greater than that of the non-dominant arm when adjusted for age, weight, and height. The authors concluded that, in this middle-aged female population sample, long-term weight bearing exercise was associated with an increase in BMD. They discussed the differences observed between runners and tennis players, noting that tennis “involves intermittent sudden torsional strains on the limbs and spine which may be a greater stimulus for bone mineral development than a sport that produces a repetitive high frequency axial stress such as running.”

Investigators suggested in the general population, long-term exercise of 1 hour/week has the potential to increase BMD. They also recognized weight-bearing exercise as an important modifiable risk factor in this population for the regulation of bone mass and fracture prevention.

Similar results have been found among adolescent females. In 2003, Nurmi-Lawton and her colleagues conducted a study to investigate the long-term influences of impact-loading exercise on bone quantity and quality in young females. Forty-five gymnasts and 52 normally active controls 8-17 years of age were recruited for this study. Anthropometry, diet, physical activity, and quantitative ultrasound were measured for three consecutive years. DXA was used to assess total body and lumbar spine BMC and BMD three times during the study at one-year intervals. Analysis of covariance was used to assess adjusted mean differences between groups. No differences were found between groups in energy, calcium, or protein intake. The gymnasts, however, had significantly greater QUS, BMC, and BMD than controls after adjusting for maturity, height, weight, protein intake, and energy intake. The authors suggest these results support impact-loading exercise as a means to attaining optimal bone density and content.

In 2004, The American College of Sports Medicine published a position stand on Physical Activity and Bone Health. This publication describes the adaptive response of bone
stimulated by overloading forces, this stimulus to bone being a “[literal] physical deformation of bone cells.” From the literature, the ACSM summarized:

“Weight bearing physical activity has beneficial effects on bone health across the age spectrum. Physical activities that generate relatively high-intensity loading forces, such as plyometrics, gymnastics, and high-intensity resistance training, augment bone mineral accrual in children and adolescents. Further, there is some evidence that exercise-induced gains in bone mass in children are maintained into adulthood, suggesting that physical activity habits during childhood may have long-lasting benefits on bone health.”

Though the authors suggest it is not yet possible to prescribe in detail an exercise program to optimize peak bone mass, they do suggest participation in impact activities and sports that involve running and jumping likely are of benefit. Recommendations include participation in high intensity activity involving bone-loading forces 3 days per week for 10-20 minutes per day. These statements and recommendations are supported by a list of foundational research studies conducted in the 1990s and 2000s.

In 1999, Buell conducted a physical activity analysis on data from a four-year study to adopt or create a five-year retrospective questionnaire and methodology to adequately evaluate the relationship between physical activity and bone mineral status in adolescent females. The study measured sport participation and the annual time spent in particular sports or activities and used this data to evaluate the role of impact and weight-bearing activity in bone growth and mineral accretion. At the time of the study, a compendium of values to represent the degree of impact or weight-bearing capacity of each activity studied was not available. The study included the creation of a questionnaire completed by ten certified athletic trainers (raters) to categorize sports and activities into similar groups by weight-bearing, linear, lateral cutting, and jumping (loading) characteristics. Raters were asked to categorize sports and activities into six categories: non-weight bearing (NWB), WB-two legs, WB-single limb, WB-cutting, WB jumping, and
weight-loading. The responses for each sport and activity were tallied and the tally for each category was multiplied by a weight-bearing factor specific to the category. These values were summed and divided by the number of tallies for each sport or activity to produce an average and weighted value of osteogenic potential (Ost). The Ost score for each sport or activity was multiplied by the annual hours of participation of each sport or activity. These annual Ost scores for each sport or activity were summed as the total annual Ost score for each participant. The study found that average Ost scores consistently appeared as significant predictors of bone mineral density among adolescent female athletes. These findings support the positive influence of weight-bearing and impact activities on bone mineral density, and the validity of an Ost score or similar representation of osteogenic potential of exercise as a predictor of bone mineral density among adolescent females.  

In 2000, Witzke and Snow published results that compliment Buell’s findings in adolescent girls. These researchers sought to investigate the effects of plyometric jump training on bone mineral content (BMC), lower extremity performance, and static balance in adolescent girls. Participants trained for 30-45 minutes three times per week for 9 months. The program, designed to load the lower extremities, included squats, lunges, and calf raises using weighted vests, as well as plyometric exercises including hopping, jumping, bounding, and box depth jumps. These exercisers were matched to controls for age and months past menarche. At baseline and at 9 months, participants were assessed for BMC, strength by isokinetic dynamometry, power, and static balance. At the end of the program, t-tests for independent samples revealed both groups experienced significant increases in BMC compared to zero for the whole body, femoral neck, lumbar spine, and femoral shaft, but only the exercisers improved BMC of the greater trochanter. Additionally, knee extensor strength and medial/lateral balance were significantly improved in the exercise group, while no changes were observed among the controls. From these results, the authors concluded that plyometric jump training continued over a
longer period of time during adolescent growth may increase peak bone mass. These results lend support to the implementation of plyometric and weight-loading exercise in the adolescent population to improve bone strength and aid in the achievement of peak bone mass. 

**Damage Beyond the Female Athlete Triad?**

*Relative Energy Deficiency in Sport (RED-S)*

In 2014, the International Olympic Committee (IOC) introduced the term “relative energy deficiency in sport (RED-S)” as “a broader and more comprehensive term for the condition previously known as female athlete triad.” The IOC defined RED-S as “impaired physiological function including, but not limited to, metabolic rate, menstrual function, bone health, immunity, protein synthesis, [and] cardiovascular health caused by relative energy deficiency.” They suggest, “the clinical phenomenon is not a ‘triad’ of three entities of energy availability, menstrual function, and bone health, but rather a syndrome that affects many aspects of physiological function, health, and athletic performance.” The authors of this consensus statement acknowledge the triad, but propose the effects of a relative energy deficiency span far beyond menstrual dysfunction and poor bone health. They suggest the triad falsely limits these health consequences to females, and therefore the new concept of RED-S acknowledges the health consequences of a relative energy deficiency among male athletes as well. The authors go on to discuss the need for additional research among racially diverse athletic populations and disabled athletic populations. In light of this updated and expanded definition, the IOC presents new screening, diagnostic, and treatment strategies to encompass all of the health consequences associated with RED-S, as well as a “Risk Assessment” model, a “Return-to-Play” model, and recommendations for future research in this area. Figure 3 and Figure 4 respectively present the health consequences and potential performance effects associated with RED-S as defined by the IOC.
Figure 3 – Health consequences of Relative Energy Deficiency in Sport (RED-S). As an expanded version of the Female Athlete Triad, this model addressed a wider range of health consequences associated with a relative energy deficiency to include both female and male athletes. 

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Figure 4 – Potential performance effects of Relative Energy Deficiency in Sport (RED-S)\textsuperscript{12}
Immediately following the publication of the IOC’s consensus statement, a group of established and respected researchers in the field including Mary Jane De Souza, Nancy Williams, Anne Loucks, Michelle Barrack, Jeanne Nichols, and several others responded with an article titled “Misunderstanding the Female Athlete Triad: Refuting the IOC Consensus Statement on Relative Energy Deficiency in Sport (RED-S).” The authors state, “this new terminology (RED-S) is insufficiently supported by scientific research to warrant adoption at this time.” They go on to highlight “several major concerns and errors with the IOC consensus statement”, including the “poorly developed ‘hub-and-spoke’ diagrams”, the lack of supporting evidence for the development and implementation of the risk assessment and return to play models, and the inaccurate referencing and misinterpretation of scientific literature. “While we appreciate the need for improved thinking in medical science,” state the authors, “we strongly believe it is unwise and misleading to propose a new approach based on faulty science.” Despite the criticism, the proposition of the RED-S paradigm presents countless opportunities for more research, which may in fact lead to a more comprehensive, well supported definition of the female athlete triad and relative energy deficiency in sport (RED-S) in the coming years.

Resting Metabolic Rate

In the presence of menstrual dysfunction, a decrease in resting metabolic rate (RMR) has been observed. In 1991, Myerson et al. investigated metabolic and nutritional factors associated with exercise induced menstrual dysfunction. Three groups of women were studied: amenorrheic runners, eumenorrheic runners, and eumenorrheic sedentary controls. The results indicated that RMR was significantly lower in the amenorrheic runners compared to the eumenorrheic runners and controls. They also found that amenorrheic women scored higher than eumenorrheic runners and controls on a scale of abnormal eating patterns. Though the amenorrheic runners seemed to have less adequate diets than the eumenorrheic runners and controls, there was no significant weight difference between the groups.
In 1999, Myburgh reported similar findings among a cohort of ballet dancers. The authors investigated the relationship between nutritional status and menstrual function by calculating energy intake, determining the incidence of abnormal eating attitudes, assessing body composition, and measuring RMR and serum thyroid hormone concentrations. Of the 21 ballet dancers studied, 13 had a history of menstrual irregularity and 8 had always been regular. Those with a history of menstrual irregularity had significantly lower resting metabolic rates than those who had always been regular, however there were no significant differences in body weight or composition. This same relationship between menstrual dysfunction and low RMR was also reported by Lebenstedt et al in 1998.

More recent studies have evaluated changes in metabolic hormones in the presence of menstrual dysfunction. In the study conducted by Williams et al. in 2001, changes in the metabolic hormone triiodothyronine (T₃) were measured at various levels of energy availability. It was found that at low levels of energy availability, animal subjects developed amenorrhea and exhibited significant decreases in plasma T₃ levels. When adequate energy availability was established and menstrual function was restored, animal subjects exhibited significant increases in plasma T₃ levels. The authors concluded that a tight correlation exists between changes in reproductive function and the metabolic hormone T₃, as dictated by the status of energy availability. Though RMR was not directly measured, it is clear that metabolic function was suppressed in the presence of an energy deficiency.

De Souza and her colleagues drew similar conclusions from their study conducted in 2008. The purpose of this study was to evaluate the effects of energy deficiency and estrogen deficiency on bone turnover markers. Resting energy expenditure was measured along with the metabolic hormones T₃, leptin, and ghrelin. Women were retrospectively categorized into “Energy Replete + Estrogen Replete”, “Energy Replete + Estrogen Deficient”, “Energy Deficient + Estrogen Replete”, and “Energy Deficient + Estrogen Deficient” based on energy and estrogen
Researchers found that resting energy expenditure was lower among the “Energy Deficient” and “Estrogen Deficient” groups compared to the “Energy Replete” and “Estrogen Replete” groups. They also found that the “Energy Deficient + Estrogen Deficient” women had lower levels of T₃ and higher levels of ghrelin than the others. Furthermore, these women exhibited the lowest levels of bone formation markers and the highest levels of bone resorption markers, indicative of increased bone loss. The changes observed in REE and metabolic hormone levels have been deemed “energy deficiency-related [metabolic] adaptations” and point to an energy conservation mechanism at work among these energy deficient women.

The correlation between changes in metabolic hormones and changes in bone turnover markers in the presence of an energy and estrogen deficiency has been eloquently summarized by De Souza and her colleagues:

The additional observation in [Williams’ 2001 study] that changes in circulating total T₃ was correlated with both the induction and reversal of amenorrhea lends support to the idea that the suppression of reproductive function is linked with adaptive mechanisms to reduce energy expenditure in the face of inadequate caloric intake. Additional evidence for the relationship between energy availability and exercise-associated menstrual disturbances is found in cross-sectional studies of metabolic hormones and substrates that illustrate adaptive changes similar to that observed during episodes of chronic undernutrition. These studies in amenorrheic athletes, combined with studies exposing metabolic endocrine signs of an energy deficit in exercising women with subtle menstrual disturbances such as luteal phase defects and anovulation provide strong evidence that a hypometabolic state includes reductions in resting metabolic rate, total T₃, leptin, insulin, glucose, IGF-1, and IGFBP-3 and elevations in IGFBP-1, ghrelin, growth hormone, and cortisol. Changes in some of these metabolic hormones are
documented to play a role in altered bone turnover that can contribute to bone loss, particularly a decrease in IGF-1 and leptin.⁸⁵

Though metabolic status is not specifically addressed in the context of the triad, there is clearly a correlation between metabolic status and each of the triad components. In the RED-S consensus statement published by the IOC, a change in metabolic status is listed as one of several health consequences associated with RED-S beyond the triad itself.¹² Despite the controversy surrounding RED-S, literature supporting the effects of low energy availability on metabolic status does exist. In conducting more research and more thoroughly studying the relationship between energy availability and metabolic status, a more rigorous conceptual framework can be developed to capture metabolic status in the context of the female athlete triad.

Review of the Literature Conclusions

In conclusion, the literature clearly demonstrates the interrelationships between low energy availability, menstrual dysfunction, and poor bone mineral density among female athletes. However, literature demonstrating the simultaneous occurrence of all three triad components among adolescent female athletes is currently lacking and the reported prevalence of each triad component varies between studies.⁸⁶ Additionally, the far-reaching effects of an energy deficiency beyond the triad are difficult to study, thus not well supported or constructed.¹³ In light of these gaps in the literature, there is a need for additional descriptive studies to gain a better understanding of the triad components individually and synergistically, as well as the effects of an energy deficiency beyond the triad itself. In doing so, the concept of the female athlete triad can continue to be shaped and expanded upon. Evidence-based implications and recommendations can then be made for screening, preventing, and treating the female athlete triad and its associated conditions. Because of the incredible benefits of athletic participation among adolescent females, it is necessary to gain a greater understanding of the triad and its associated conditions, not to discourage adolescent girls from competing in sports, but rather to
protect the health and well being of these girls in the midst of greater participation and competition.
Chapter 3: Methods

The purpose of this descriptive, cross-sectional study was to describe the components of the female athlete triad, namely energy availability, menstrual function, and bone mineral density, as well as resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes. The protocol presented in this report was approved by the Nationwide Children’s Hospital Institutional Review Board (IRB14-00564).

Participants

A convenience sample of adolescent female participants was recruited from a middle-to-upper class suburban high school in Ohio during the autumn semester of the 2014-2015 academic school year.

Inclusion Criteria

To be included in this study, participants must have been female student athletes at the designated high school between 14-18 years of age at the time of recruitment.

Exclusion Criteria

Participants were excluded from the study if they competed in a winter sport at the designated high school.

De-identification of Participants

Identification numbers were assigned to participants in the order in which they scheduled laboratory visits. All data was collected, archived, and analyzed by identification number.

Recruitment

Several methods were used in the recruitment of participants for this study. Recruitment fliers were placed throughout the halls of the designated high school during the autumn semester.
of the 2014-2015 academic school year. Recruitment continued via word of mouth from athletic trainers, coaches, parents, and student athletes. Two recruitment meetings were held by the study physician to inform student athletes and parents or legal guardians about the purpose and methodology of the study and obtain assent and consent from student and guardian respectively. Once assent and consent were obtained, participants scheduled individual laboratory visits. No monetary incentives were used in the recruitment of participants, however participants received a free biometric screen including height, weight, bone mineral density, and resting metabolic rate, as well as the opportunity to participate in a personal training program under the supervision of a strength coach and athletic trainer.

**Methodology**

*Laboratory Visits*

In December 2014 and January 2015, each participant attended one individual, 1-hour laboratory visit at The Ohio State University Sports Nutrition Laboratory at Martha Morehouse Medical Plaza.

*Anthropometric Measurements*

Height and weight were measured to the nearest tenth centimeter and kilogram on a standardized and calibrated Health-O-Meter stadiometer scale (Model 500KL). Body composition and bone mineral density were estimated using a General Electric densitometer (Lunar iDXA, Encore 14.0 GE) following manufacturer protocol for total body, non-dominant hip, lumbar spine, and non-dominant radius BMD. Prior to testing, participants were required to provide a urine sample to ensure urine was negative for human chorionic gonadotropin (hCG). Negative results were required for testing to proceed.

*Questionnaires*

Two questionnaires were sent to participants prior to the laboratory visit. A Vioscreen Food Frequency Questionnaire® was used to assess foods frequently consumed, consumption...
frequency of each food, and the typical serving size of each food consumed in the preceding 3 months. From this data, an estimate of daily energy intake for each participant was calculated.

A compendium of validated questionnaires was generated using the Research Electronic Data Capture (REDCap) application. This application was used to deliver, collect, and store data in a confidential manner. This questionnaire will be referred to as the “REDCap questionnaire” throughout this report. The REDCap questionnaire was used to collect information regarding physical activity and menstrual function. This questionnaire assessed frequency of physical activity and sport participation. Metabolic equivalents (METS) of activities reported were retrieved from The 2011 Compendium of Physical Activities, reported in Appendix A. Frequency data was multiplied by respective MET values to estimate total annual MET minutes of exercise and average daily exercise energy expenditure. The REDCap questionnaire was also used to categorize participants by past and present self-reported menstrual status. The questionnaire asked participants “Have you started your period?”, “How old were you when you started your period?”, “Have you missed any periods in the last three to four months?”, and “Have you been taking the pill (oral contraceptives)?”. It also asked participants to estimate how many periods they had in the past 12 months.

Describing Components of the Female Athlete Triad

Energy Availability

Energy availability, operationally defined as:

(caloric energy intake – caloric exercise energy expenditure) / kilograms lean body mass

was estimated using data collected in the laboratory and via questionnaire. The Vioscreen Food Frequency Questionnaire® was used to estimate energy intake. The REDCap questionnaire was used to estimate exercise energy expenditure. Lean body mass was estimated by the Lunar iDXA. Using the equation above, a value of energy availability was estimated for each participant.
**Menstrual Function**

Data from the REDCap Questionnaire were used to categorize participants by menstrual function into one of two categories: eumenorrheic or dysmenorrheic (oligomenorrheic and secondary amenorrheic); no participants self-reported primary amenorrhea, thus this category was not considered. Participants were classified as eumenorrheic if they reported having 9 or more menstrual periods in the previous 12 months. Participants were classified as dysmenorrheic if they reported 0-8 menstrual periods in the previous 12 months.

**Bone Mineral Density**

Bone mineral density was estimated using a General Electric densitometer (Lunar iDXA, Encore 14.0 GE) following manufacturer protocol for total body, non-dominant hip, lumbar spine, and non-dominant radius BMD. One investigator conducted and analyzed all bone mineral density measurements. Data were exported from the machine for statistical analysis.

To capture the osteogenic response to the weight bearing capacity and impact of each activity reported by the participants, responses to the REDCap questionnaire were quantified in terms of the probable osteogenic potential (Ost). Participants were asked to list which sports and activities they had participated in in the previous 12 months and the amount of time spend participating in each of these activities individually. Total annual hours of each activity were multiplied by the Ost value specific to that activity. Annual Ost scores for each activity were calculated and summed to produce an annual Ost score. This annual Ost score was then normalized to body weight. The values of osteogenic potential are reported in Appendix B.

**Resting Metabolic Rate**

The KORR Medical Technologies ReeVue indirect calorimeter was used to estimate resting metabolic rate. Participants were instructed to arrive to the laboratory fasted, having not
eaten since midnight the previous night. A 10-minute rest period in a reclined state was required prior to testing. The machine was calibrated following manufacturer protocol. Two investigators and several trained laboratory assistants conducted RMR measurements. Data were hand-recorded into the computer and double-checked for accuracy by two separate laboratory staff.

The Cunningham equation and the Harris-Benedict equation were used to produce two estimated RMR values for each participant. These estimated rates were compared to the measured RMR to determine if there were significant differences between predicted and measured RMR among this sample of adolescent female athletes.

Validation of Tools

The General Electric densitometer (Lunar iDXA, Encore 14.0 GE), a total-body dual x-ray absorptiometry system, was used to measure bone mineral density and body composition. In October 2005, the U.S. Food and Drug Administration approved this system for use as an aid in the detection and diagnosis of osteoporosis, and for monitoring subsequent treatment.90 DXA is the current gold standard in the measurement of BMD and the GE Lunar iDXA has been used widely to assess both BMD and body composition.91

In a 2012 review, Toombs et al. evaluated the trueness and precision of several densitometers, including the GE Lunar iDXA, in evaluating body composition.91 Results reported by Hind et al. and Rezzi et al. demonstrated precision of body composition measurements on the Lunar iDXA to be 0.4-0.5% for lean mass, 0.7-0.8% for fat mass, and 0.6-0.9% for percent body fat.92,93 A pilot study conducted by the reviewers produced similar results, demonstrating precision of body composition measurements on the Lunar iDXA to be 0.4% for lean mass, 1.0% for fat mass, and 0.9% for percent body fat.91 These coefficients of variation were smaller than those of the GE Lunar Prodigy, Hologic QDR-1000W, Hologic QDR-4500A, Norland XR 26, Mark II HS, GE Lunar DPX, and GE Lunar GE DPX-L. Therefore, the current evidence suggests that the Lunar iDXA shows excellent precision for body composition measurements and slightly
better precision than a densitometer of a previous generation.\textsuperscript{91} It is therefore an appropriate tool to measure both BMD and body composition in the context of this study.

The ReeVue indirect calorimeter was used to estimate resting metabolic rate. After the designated rest period, each participant was instructed to wear a nose clip and breathe exclusively through a simple mouthpiece. All the exhaled air was collected and the concentration of oxygen breathed out was directly measured.\textsuperscript{94} The indirect calorimeter determines the volume of oxygen consumed and estimates calorie consumption using the correlation between oxygen consumed and calories burned (4.813 calories for every mL of oxygen consumed).\textsuperscript{94} Using this information, an estimate of resting metabolic rate was determined for each participant. The ReeVue indirect calorimeter was approved by the FDA and validated by the University of Southern California in 2009.\textsuperscript{95} The calories from the machine output are utilized for this analysis.

The Vioscreen Food Frequency Questionnaire\textsuperscript{®} was used to estimate daily energy intake. In 2014, Kristal et al. conducted an evaluation of web-based, self-administered, graphic food frequency questionnaires including the Vioscreen FFQ\textsuperscript{®}.\textsuperscript{96} In this NIH-funded validation study, this tool received high scores for reliability, accuracy, and ease of use.\textsuperscript{96} The inter-method reliability for the Vioscreen FFQ\textsuperscript{®} was higher than paper FFQs in major epidemiological research studies.\textsuperscript{87,96} Additionally, 100\% of the 74 participants reported that Vioscreen\textsuperscript{®} was easy to use. Vioscreen\textsuperscript{®} was rated as either great or excellent by 93\% of the participants and 95\% of the participants agreed that the food photos helped in selecting portion sizes.\textsuperscript{87} This study validates the use of the Vioscreen FFQ\textsuperscript{®} as a web-based dietary questionnaire.\textsuperscript{96}

**Statistical Analysis**

Data were collated to a master excel spreadsheet and imported into IBM\textsuperscript{®} SPSS Statistics Version 23.0 (International Business Machines Corp., Armonk, New York) for analysis. Descriptive analysis was performed to include mean, standard deviation, and range of variables. Due to the size of the sample (n=19; 17 complete data sets), Spearman’s correlation was used to
describe the potential correlations between variables; correlations were significant if \( p \leq 0.05 \). The Mann-Whitney \( U \) test was used to assess differences between the two independent menstrual function categories. The Related-Samples Wilcoxon Signed Rank Test was used to compare median differences between RMR measured by indirect calorimetry and RMR predicted from the Cunningham equation and the Harris-Benedict equation individually.
Chapter 4: Results

Nineteen participants were recruited for this descriptive study. All 19 participants completed the anthropometric, bone mineral density, and RMR tests. Two of the participants failed to complete the questionnaires, leaving seventeen participants with estimates of energy intake, exercise energy expenditure, energy availability, and menstrual function. Anthropometric characteristics are presented in Table 2. Data analysis revealed a significant correlation between weight and lean body mass (p≤0.01).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>16.3 (1.19)</td>
<td>14.5</td>
<td>18.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.7 (5.7)</td>
<td>151.1</td>
<td>171.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.8 (8.6)</td>
<td>43.8</td>
<td>81.9</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>40.3 (4.2)</td>
<td>35.5</td>
<td>52.1</td>
</tr>
</tbody>
</table>

Table 2 – Anthropometric Characteristics of Adolescent Female Athletes (N=19)

Descriptive analysis revealed a single outlier, displayed in terms of exercise energy expenditure and energy availability in Figure 5 and Figure 6 respectively. This participant was removed from descriptive analysis of annual exercise as well as osteogenic potential, exercise energy expenditure, and energy availability as the reported annual exercise was used in the calculation of these other variables. Self-reported annual exercise and osteogenic potential are presented in Table 3.
Figure 5 – Exercise Energy Expenditure Distribution with Outlier

Figure 6 – Energy Availability Distribution with Outlier
Table 3 – Self-Reported Physical Activity (min/year) and Osteogenic Potential of Adolescent Female Athletes (N=16)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Exercise (min/year)</td>
<td>29137 (17703)</td>
<td>7805</td>
<td>73659</td>
</tr>
<tr>
<td>Osteogenic Potential (Ost/kg/year)</td>
<td>55.6 (31.2)</td>
<td>13.7</td>
<td>109.56</td>
</tr>
</tbody>
</table>

Energy availability data are presented in Table 4. The mean energy availability estimated for this population of adolescent female athletes was 30.7 kcal/kg LBM/day. Excluding the outlier, 56.3% of the sample fell below the energy availability threshold of 30 kcal/kg LBM/day proposed by Loucks et al. among adult female athletes. A significant correlation was observed between EA and daily energy intake (p≤0.01). Because energy availability is dependent on both EI and EEE, this significant correlation suggests daily energy intake and EA are more closely correlated than exercise energy expenditure and EA (p=0.362).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Intake (kcal/day)</td>
<td>1736.2 (546.9)</td>
<td>1084.7</td>
<td>2734.0</td>
</tr>
<tr>
<td>Exercise Energy Expenditure (kcal/day)</td>
<td>516.4 (321.6)</td>
<td>130</td>
<td>1288</td>
</tr>
<tr>
<td>Energy Availability (kcal/kg LBM/day)</td>
<td>30.7 (14.8)</td>
<td>4.8</td>
<td>51.7</td>
</tr>
</tbody>
</table>

Table 4 – Estimated Energy Availability of Adolescent Female Athletes (N=16)

Analysis of menstrual status and oral contraceptive use is presented in Table 5. None of the participants reported a late onset of menarche, thus primary amenorrhea was not included.

Three participants were either currently taking or had previously taken oral contraceptives. These three participants were excluded from analysis due to this confounding variable. Two (14.3%) participants reported experiencing less than 9 menstrual periods in the past 12 months and were categorized as “dysmenorrheic”. The remaining 12 participants reported experiencing 9 or more menstrual periods in the past 12 months and were categorized as “eumenorrheic”. The distribution of these participants is shown in Table 5.

<table>
<thead>
<tr>
<th>Total Sample (N=14)</th>
<th>Dysmenorrheic</th>
<th>Eumenorrheic</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 5 – Self-Reported Menstrual Function of Adolescent Female Athletes (N=14*)

*Excludes three participants reporting oral contraceptive use: 2 eumenorrheic and 1 dysmenorrheic
After excluding the three participants who reported oral contraceptive use and the outlier, a Mann-Whitney U test was conducted to assess difference between menstrual function categories. This test revealed no significant differences between menstrual function groups in terms of weight, LBM, exercise energy expenditure, daily energy intake, energy availability, osteogenic potential, RMR, lumbar spine BMD z-score, and TBLH BMD z-score. Figure 7 displays the results of the Mann-Whitney U test, testing the null hypothesis concerning energy availability. Though the difference in energy availability between menstrual function groups was not statistically significant (p=0.154), it is noteworthy that the only dysmenorrheic participant included in this test exhibited the lowest daily energy intake (1106 kcal/day) and the lowest energy availability (20 kcal/kg LBM/day) of the 13 participants.

![Figure 7 – Mann-Whitney U Test Results: Menstrual Status and Energy Availability](image)

Bone mineral density measurements for this sample are presented in Table 6. All participants reported a dominant right leg; therefore all hip BMD measurements were conducted on the left hip. Two participants and 17 participants reported a dominant left arm and right arm.
respectively; therefore results include 2 right forearm BMD measurements and 17 left forearm BMD measurements. $Z$-scores are reported for total body less head (TBLH) and lumbar spine. Of the 19 participants, 4 participants (21.1%) exhibited lumbar spine $z$-scores indicative of low BMD. A significant correlation was observed between TBLH $z$-score and lumbar spine $z$-score ($p\leq0.01$). Total mass and lean body mass were significantly correlated with TBLH $z$-score ($p=0.001$ and $p\leq0.01$) and lumbar spine $z$-score ($p=0.003$ and $p=0.24$). No significant correlations were observed between EA and BMD $z$-scores. After excluding the outlier, a significant correlation was observed between lumbar spine $z$-score and Ost score ($p=0.047$).

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean BMD (SD)</th>
<th>Minimum $Z$-score</th>
<th>Maximum $Z$-score</th>
<th>“Low BMD” n (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBLH</td>
<td>1.03 (0.09)</td>
<td>-0.29</td>
<td>2.93</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.73 (0.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>1.06 (0.11)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>1.15 (0.12)</td>
<td>-1.22</td>
<td>2.31</td>
<td>4 (21.1)</td>
</tr>
<tr>
<td></td>
<td>-0.03 (0.99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius UD</td>
<td>0.39 (0.05)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6 – Bone Mineral Density and BMD $Z$-scores of Adolescent Female Athletes (N=19)

* represents only $z$-score diagnostic criteria for “low BMD”; to be diagnosed with low BMD, participants would also require a history of nutritional deficiencies, hypoestrogenism, stress fractures, and/or secondary risk factors for fracture.

** $z$-scores cannot be used to express femoral neck and radius UD BMD; normative data for the adolescent population does not exist.
Descriptive analysis of resting metabolic rate is presented in Table 7. All participants reported a 12-hour fast prior to indirect calorimetry measurement. No significant relationships were observed between RMR and other variables in this study.

Using the Cunningham and Harris-Benedict equations, two predicted resting metabolic rates were calculated for each participant. Descriptive analysis of these two predicted rates are also presented in Table 7. A Related-Samples Wilcoxon Signed Rank Test was used to compare median differences between RMR measured by indirect calorimetry and RMR predicted from the Cunningham equation and the Harris-Benedict equation individually. This test revealed that the median of differences between measured and Cunningham-predicted RMR was not significantly different from 0 (p=0.573). However, the median of differences between measured and HB-predicted RMR was significantly different from 0 (p<0.01).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured RMR (kcal/day)</td>
<td>1402.8 (180.0)</td>
<td>1022</td>
<td>1714</td>
</tr>
<tr>
<td>Cunningham Predicted RMR*</td>
<td>1386.0 (92.9)</td>
<td>1279</td>
<td>1645</td>
</tr>
<tr>
<td>(kcal/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB Predicted RMR**</td>
<td>1168.4 (84.9)</td>
<td>1036</td>
<td>1414</td>
</tr>
<tr>
<td>(kcal/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7 – Measured and Predicted Resting Metabolic Rate of Adolescent Female Athletes (N=19)

*Cunningham equation (1980): RMR (kcal/day) = 500 + 22 (LBM)\(^7\)

**Harris-Benedict equation (1919): RMR (kcal/day) = 655.1 + 9.56 (wt) + 1.85 (ht) − 4.68 (age)\(^7\)

Ht: cm; Wt: kg; LBM: kg; Age: years
For visual comparison of participants, the raw data is presented in Table 8.

<table>
<thead>
<tr>
<th>Participant</th>
<th>EA (kcal/ kg LBM/day)</th>
<th>MF</th>
<th>BMD TBLH (z-score)</th>
<th>BMD Lumbar Spine (z-score)</th>
<th>Ost Score (Ost/kg/ year)</th>
<th>RMR (kcal/ day)</th>
<th>HB RMR (kcal/ day)</th>
<th>C RMR (kcal/ day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>17.3</td>
<td>D*</td>
<td>-0.29</td>
<td>-1.13</td>
<td>93.2</td>
<td>1656</td>
<td>1109</td>
<td>1313</td>
</tr>
<tr>
<td>02</td>
<td>44.5</td>
<td>E</td>
<td>0.07</td>
<td>-0.61</td>
<td>109.6</td>
<td>1339</td>
<td>1177</td>
<td>1335</td>
</tr>
<tr>
<td>03</td>
<td>28.5</td>
<td>E</td>
<td>0.02</td>
<td>-0.34</td>
<td>13.7</td>
<td>1238</td>
<td>1126</td>
<td>1316</td>
</tr>
<tr>
<td>04</td>
<td>36.0</td>
<td>E</td>
<td>2.04</td>
<td>0.49</td>
<td>30.7</td>
<td>1714</td>
<td>1277</td>
<td>1541</td>
</tr>
<tr>
<td>05</td>
<td>-27.9</td>
<td>D</td>
<td>1.87</td>
<td>-0.07</td>
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<td>1267</td>
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<td>1426</td>
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<td>-0.14</td>
<td>-</td>
<td>1584</td>
<td>1037</td>
<td>1307</td>
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Table 8 – Female Athlete Triad, Ost Score, and RMR of Adolescent Female Athletes

* = Taking oral contraceptives

MF = Menstrual Function

E = Eumenorrheic; D = Dysmenorrheic

HB = Harris-Benedict Predicted RMR; C = Cunningham Predicted RMR
Chapter 5: Discussion

The purpose of this descriptive, cross-sectional study was to describe the components of the female athlete triad, namely energy availability, menstrual function, and bone mineral density, as well as resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes. The study explored the severity of each triad factor and the prevalence of pathologic triad factors in this cohort of adolescents. Additionally, we explored the relationships between individual triad components, as well as the relationship between resting metabolic rate and each triad component individually. Upon analysis of physical activity data, one participant was excluded as an outlier, reporting statistically greater levels of physical activity than the other participants. Of the 16 participants with usable data sets, 9 (56.3%) exhibited low energy availability (less than 30 kcal/kg LBM/day). After excluding the three participants reporting oral contraceptive use, 2 (14.3%) participants reported dysmenorrhea. Of the 19 participants with BMD data, 4 (21.1%) exhibited low lumbar spine BMD z-scores. One participant exhibited all three components of the female athlete triad, however it should be noted that she reported previously or currently taking an oral contraceptive. Using the 2007 definition of the female athlete triad, which requires only one pathologic triad component for diagnosis, 11 of the participants in this study would be diagnosed, however it can be hypothesized that this number may be greater if complete and reliable data sets were provided for all 19 participants. Data analysis revealed a significant correlation between TBLH BMD z-score and lumbar spine BMD z-score. After excluding the outlier, a significant correlation was observed between lumbar spine z-score and Ost score. Lean body mass was significantly correlated with both TBLH z-score and
lumbar spine z-score. A difference in energy availability was observed between eumenorrheic and dysmenorrheic groups, however this difference was not significant.

Energy Availability

Few studies have evaluated energy availability in adolescents. Of the studies that have been conducted, disordered eating has been assessed as a marker of low energy availability. This study, however, calculated energy availability for each participant using questionnaires to assess physical activity and energy intake. Though a threshold of energy availability has not been proposed for adolescents, these calculated EA values can be compared to the energy availability threshold of 30 kcal/kg LBM/day for adult women proposed by Loucks and Thuma. In this study, 56.3% of participants fell below this threshold. According to Loucks and Thuma, menstrual dysfunction presents below this threshold. Both participants reporting menstrual dysfunction fell below this threshold, however a total of 9 participants fell below the threshold, all of whom should theoretically experience menstrual dysfunction. This suggests several possibilities: the self-reported physical activity or menstrual function from this group of adolescent female athletes is inaccurate, the threshold is different in an adolescent population, or menstrual dysfunction occurs at a different EA for each individual and the threshold is therefore not applicable. If the threshold is different for an adolescent population, one could intuitively suggest the EA threshold is greater than 30 kcal/kg LBM/day due to the increased energy needs for growth and development in this population of athletes. Additional research is needed to evaluate a potential threshold of energy availability in an adolescent population. If menstrual dysfunction occurs at a different EA for each individual, menstrual function should be closely monitored to ensure proper and adequate fueling among adolescent female athletes.

Menstrual Function

The 14.3% prevalence of menstrual dysfunction in this cohort of adolescent female athletes is lower than that reported in the literature. It is more comparable to a prevalence of
15.9% reported by Fischer et al. among a sample of 491 adolescent female athletes presenting to a pediatric primary care sports medicine clinic in the same geographical location as the present study. Table 9 displays the prevalence of menstrual dysfunction previously reported among female high school athletes; a range of 18.8 – 54% is discernable among similar populations.

<table>
<thead>
<tr>
<th>Author</th>
<th>Participants</th>
<th>Prevalence (%)</th>
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<tbody>
<tr>
<td>Nichols et al., 2006</td>
<td>170</td>
<td>23.5</td>
</tr>
<tr>
<td>Nichols et al., 2007</td>
<td>423</td>
<td>20.1</td>
</tr>
<tr>
<td>Thein-Nissenbaum et al., 2011</td>
<td>331</td>
<td>18.8</td>
</tr>
<tr>
<td>Hoch et al., 2009</td>
<td>80</td>
<td>54.0</td>
</tr>
</tbody>
</table>

Table 9 –Reported Prevalence of Menstrual Dysfunction in High School Female Athletes

The accuracy of the self-reported menstrual function must be considered. In a recent manuscript titled “Challenges of Menstrual Dysfunction Screening Using Pre-Participation Physical Exam”, Fischer and her colleagues sought to determine if the pre-participation physical exam (PPE) was sufficient to screen for menstrual dysfunction among female high school and middle school athletes when compared to validated research protocols. In this observational, cross-sectional study, researchers approached female students attending a PPE to participate. Prior to the PPE, all students and/or their parents completed the Ohio High School Athletic Association (OHSAA) PPE Form, which included demographic information such as age and current medications. It also asked the student, “Have you ever had a period?” and “How many periods have you had in the last 12 months?” Participants also completed the Healthy Wisconsin High School Female Athletes Survey (HWHSFAS). This questionnaire included the same
questions from the PPE form, but also asked directly “Are you taking birth control pills” and “Are you taking any medications prescribed by a doctor other than birth control pills which were given to you to regulate your menstrual periods?” This questionnaire went on to ask how competitive training influenced time between menstrual periods, length of menstrual periods, and intensity of flow. Finally, this questionnaire asked participants if they missed periods during competitive training. Answers to this questionnaire were compared to those of the PPE form. Of the 15 girls recruited for this study, 47% had at least one discrepancy between the answers on the PPE form and HWHSFAS. The results of this study led the authors to conclude that menstrual dysfunction reported using the PPE form may be underreported and therefore undertreated. The authors suggest the use of menstrual cycle tracking devices such as calendars or phone applications to better identify menstrual dysfunction and risk for the female athlete triad among adolescent female athletes. The discrepancies reported in Fischer’s study suggest that discrepancies may exist between the true menstrual function and that reported using the REDCap questionnaire in the present study. As in Fischer’s study, it is possible that menstrual function was underreported among this cohort of adolescent female athletes. This becomes more of a possibility when considering the prevalence reported in this study falls below the prevalence range reported in the literature. Tools such as those recommended by Fischer and her colleagues could help to better identify menstrual dysfunction among this population.

Assuming the self-reported data of the present study is accurate, the results support the energy availability hypothesis and are consistent with the adult literature in that the dysmenorrheic participant ranked below eumenorrheic participants in EA. Because the present study did not evaluate differences in clinical and subclinical menstrual disturbances, conclusions cannot be made about the severity of menstrual function relative to EA. In adult studies, energy availability discriminated clinical menstrual status, however the magnitude of energy deficit did not predict the severity of the menstrual disturbances. A more
comprehensive study is needed to evaluate the relationship between EA and the severity of menstrual dysfunction in this population.

Noteworthy is the oral contraceptive use among the participants. Three participants reported previously or currently taking oral contraceptives, however the questionnaire did not ask for the specific oral contraceptive used by each of these participants. Two of these participants reported normal menstrual function while one of these participants reported menstrual dysfunction. No conclusions can be made about menstrual function among these individuals. Additionally, the possibility of discrepancies between actual and reported contraceptive use must be considered.

**Bone Mineral Density**

The prevalence of low BMD among this cohort of adolescent female athletes is comparable to that reported by Rauh et al.\(^\text{19}\) In the 2010 study, 36 of the total 163 female high school athletes (28.3%) exhibited low total body or lumbar spine BMD z-scores (\(z \leq -1\)).\(^\text{19}\) The present study reported a prevalence of 21.1%; all participants included in this percentage exhibited low lumbar spine BMD z-scores. It is possible that this value inaccurately represents the prevalence of low BMD. The GE Lunar iDXA produces a two-dimensional image which does not incorporate the depth or shape of the bone.\(^\text{53}\) A small bone may therefore appear to have a lower areal BMD, and a lower z-score, compared to a larger bone with a similar volumetric BMD.\(^\text{53}\) It has been suggested that z-scores be height-adjusted to correct for these differences in bone length and discrepancies in z-scores, however no validated method of height-adjusting BMD z-scores in the adolescent population exists. Therefore, z-scores in this present study were not height-adjusted. Further validation of height-adjustment techniques is needed before this is possible.

As was predicted, a significant correlation was observed between lumbar spine BMD z-scores and Ost score. BMD z-scores would be expected to increase with greater scores of
osteogenic potential, as observed\textsuperscript{68–70,78,79}. These results lend support to the recommendation of weight-bearing physical activity in the development and maintenance of bone mass. More comprehensive measures of physical activity should be used to gain a greater understanding of the relationship between physical activity and osteogenic potential.

Surprisingly, a significant correlation was not observed between BMD $z$-scores and menstrual function. The questionnaire used to assess menstrual function may not be the most reliable tool to determine menstrual status given the self-report nature of the questionnaire. With additional resources, urinary concentrations of E1G, PdG, LH, and other markers of menstrual status could be measured to assess both clinical and sub-clinical menstrual dysfunction. These biological markers would improve the accuracy of menstrual function data and generate more significant results. More rigorous testing is needed to accurately evaluate the relationship between energy availability, BMD, and menstrual function.

The significant correlations between BMD $z$-scores and body composition measures are consistent with the results reported by Lima et al.\textsuperscript{74} The authors of this 2001 study reported significant correlations between weight and total BMD ($p < 0.001$), femoral neck BMD ($p < 0.001$), and lumbar spine BMD ($p < 0.001$).\textsuperscript{74} The present study also demonstrated a significant correlation between weight and TBLH BMD ($p = 0.001$), TBLH BMD $z$-score ($p = 0.001$), total body BMD ($p = 0.006$), total body BMD $z$-score ($p < 0.001$), lumbar spine BMD ($p = 0.003$), and lumbar spine BMD $z$-score ($p = 0.003$). A significant correlation was also observed between LBM and all these BMD measures and corresponding $z$-scores. This is intuitive in light of the correlation between weight and LBM; as lean body mass increases, total mass will also increase.

This correlation between lean body mass and total mass presents a challenge in the context of the female athlete triad. Often, female athletes engage in behaviors to decrease body weight for appearance or performance. This weight loss occurs through a reduction in EA. Though girls may believe they are losing fat mass and maintaining lean body mass, the
correlation between weight and LBM, supported by the literature, suggests that a reduction in weight will concurrently reduce LBM. This reduction in weight and LBM will then lead to a reduction in BMD. This reduction in EA to decrease weight and the resulting decrease in LBM and BMD present as the female athlete triad; in these circumstances, menstrual dysfunction would also be expected. The idea of gaining weight to prevent such dramatic consequences is certainly a barrier to preventing or recovering from the female athlete triad, especially in an adolescent population. Because of this, education of athletes, parents, coaches, and sports medicine professionals is critical.

Resting Metabolic Rate

Interestingly, no significant correlations were observed between RMR and other variables. Additionally, significant differences in RMR were not observed between menstrual function groups. In 1991, Myerson et al. reported a significantly lower RMR in amenorrheic runners compared to eumenorrheic runners and controls. Similar results were reported by Lebenstedt et al. in 1998 and Myburgh in 1999. Correlations between RMR and EA have also been reported, yet were not observed in this study. All these studies, however, were conducted in adult women and cannot be generalized to include the adolescent female athletes of the present study. Additionally, it must be noted that menstrual function and data used to estimate energy availability in the present study were self-reported. More research and more accurate tools to assess menstrual status and energy availability are needed to properly assess the relationship between RMR and these variables.

Currently, the prediction equations developed by Cunningham and Harris-Benedict are recommended by the American College of Sports Medicine (ACSM) for estimation of RMR in athletes. However, neither of these equations were developed for an athletic population, nor were they developed for an adolescent population. Several studies have concluded that RMR can be accurately predicted among adult athletes using the Cunningham equation, but data on
adolescents is lacking. Several other equations have been recommended in the estimation of RMR among healthy adults, such as the equations developed by Owen in 1986 and 1987, the World Health Organization equations developed in 1985, and the Schofield equations also developed in 1985. Like the Cunningham and Harris-Benedict (HB) equations, however, these equations were not developed for an athletic or adolescent population and it is unclear whether these equations are valid in estimating RMR in an athletic adolescent population. This is important to note when considering the significant differences observed between measured RMR and HB-predicted RMR in the present study. A validated equation to estimate RMR among adolescent athletes is needed to truly assess the differences between predicted and measured RMR in this population.

Relative Energy Deficiency in Sport

The results of this small study were not able to support the RED-S paradigm. Though correlations were observed between EA and menstrual function, no significant correlations were observed between triad components or RMR. This study did not address the additional components of the RED-S model and therefore cannot speak to the relationships beyond the triad and RMR. There remains, however, the opportunity to develop a more comprehensive, well-supported definition of the female athlete triad and RED-S as more rigorous research is conducted.

Limitations

Several limitations exist in this study. With 19 participants recruited, 17 participants providing complete data sets for analysis, and the exclusion of one participant as an outlier, the statistical power of the study is low. Significant correlations, however, were observed and reported. Additionally, much of the data (menstrual status, energy intake, and physical activity) was self-reported. Energy intake was estimated using the VioScreen Food Frequency Questionnaire®. Food logs or diaries may have provided more valid estimates of daily energy
intake. Exercise energy expenditure was estimated using answers to the questions generated using the REDCap application. A series of calculations were performed to estimate MET-hours of weekly activity, which was further converted to daily exercise energy expenditure in kilocalories. The questionnaire inquired about months per year, days per week, and minutes per day of participation in each sport and activity, but failed to ask how many weeks per month each sport or activity was performed. Because of this, it was assumed that the sport or activity was performed all weeks of the indicated months (i.e. 4.5 weeks/month). This could have led to an over-estimation of exercise energy expenditure. This over-estimation could have been prevented by including a question to determine weeks per month of participation in each sport or activity. Regardless, the use of pedometers, heart rate monitors, and physical activity logs may be more robust in estimating daily exercise energy expenditure. Menstrual status was also self-reported via the REDCap questionnaire. From this data, it was possible to distinguish between amenorrhea and eumenorrhea, but it was not possible to differentiate sub-clinical menstrual disturbances. This inability prevented researchers from estimating where on the spectrum of menstrual dysfunction the dysmenorrheic participants fell. Blood samples of LH and FSH would allow for this differentiation and would add validity to the menstrual status data. Unfortunately, limited resources and a lack of funding prevented the use of these more advanced tools and methods. Future research should consider a larger population and the use of these tools and methods to promote significance and validity.

The socioeconomic status of the population studied serves as another limitation in this study. The adolescent female athletes were recruited from a middle-to-upper class high school in Central Ohio. It can be assumed that this population is food secure, having both physical and economic access to food that meets dietary needs as well as food preferences. In theory, these participants have the ability to consume adequate energy to maintain optimal energy availability and adequate dietary calcium to promote bone health. They also have the access and opportunity
to participate in weight-bearing and impact sports and activities to promote bone mineralization. Lesser socioeconomic populations may not be food secure and therefore may not have access to adequate and nutrient-dense foods. These individuals may not be able to maintain optimal energy availability and consume recommended levels of dietary calcium due to a lack of resources. These populations may not have the access or opportunity to participate in weight-bearing and impact sports and activities. Because of this, the prevalence of the female athlete triad may be greater in these lower socioeconomic populations than in this sample of adolescent female athletes.

Furthermore, the lack of normative BMD data in the adolescent population prevented the researchers from describing femoral neck BMD, radius UD BMD, total femur BMD, and total radius BMD in terms of Z-scores. This lack of normative data prevented the comparison of these BMD measurements from this sample to a larger population of adolescent females. Additionally, the z-scores of this study were not height adjusted for bone length. There is currently no validated method to height-adjust BMD z-scores in the adolescent population; this validation is needed before these adjustments can accurately be made.

Similarly, the literature does not support the use of a single equation to estimate RMR among adolescent athletes. Because of this, it is challenging to accurately predict RMR among this population. A validated predictive equation for adolescent athletes must be developed to truly assess the differences between predicted and measured RMR in this population.

Finally, the exclusion criterion of this study, which excluded winter sport athletes from participation, decreased the athletic diversity among the participants. By excluding winter sport athletes, several groups of athletes were not represented in the sample, namely basketball players and swimmers. This limits the generalizability of the results.
Future Research

Though a high prevalence of the female athlete triad was not observed in this cohort of adolescent female athletes, individual components of the female athlete triad were observed across the spectrum of severity, and significant relationships were observed among several variables. In light of the existing literature concerning the female athlete triad, these observations evoke the need for additional research. As a pilot study, funding and resources were limited. With greater funding and resources, future research should consider a greater population of adolescent female athletes, including athletes from all sports and activities to produce a diverse and all-encompassing sample of adolescent female athletes. More comprehensive tools should be used to collect diet and physical activity data; diet analysis and fitness software, heart rate monitors, pedometers, and the like may be considered. Blood samples should be used to measure reproductive and metabolic hormones as well as markers of bone formation and resorption. Other hormones, such as cortisol and leptin, may also be measured to further evaluate the complex relationships of the triad and RED-S. Future research should also investigate differences between adolescent female athletes and adolescent controls in terms of energy availability, disordered eating, menstrual function, bone mineral density, and RMR. Differences in triad components and RMR between lean and non-lean sports in the adolescent population should also be studied. The effect of weight bearing and impact loading exercise on BMD over time should be considered. The effect of “time since menarche” on BMD should also be analyzed. Finally, the eating attitudes and behaviors studied but not evaluated by the present study should be explored.

Conclusion

This study described the components of the female athlete triad and resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes. Results identify the presence of all three female athlete triad components among this small sample of adolescent females and add to the literature more evidence of this ever-present condition. In light of the serious health
consequences associated with the female athlete triad, it is crucial that athletes, parents, coaches, and sports medicine practitioners are informed about the triad and take the necessary steps to screen, prevent, and treat low energy availability, menstrual dysfunction, and low bone mineral density. Sport participation among adolescent females reaps incredible benefits. However, in order to support these females in their athletic endeavors, an educational curriculum and protocol to address screening, prevention, and treatment of the female athlete triad must be developed. This descriptive study lends support to the need for these programs and resources. As additional research is conducted, these programs and resources can be developed. With the support and dedication of the entire team of parents, coaches, and sports medicine professionals, adolescent female athletes will have the tools and resources they need to thrive physically, mentally, and athletically.
References


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67


87. VioScreen - VIOCARE®.


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90. Wakanine, Yael. FDA Approvals: Lunar iDXA, Urgent PC, MimirX.


94. KORR Medical Technologies. ReeVue Indirect Calorimeter. 2015.


# APPENDIX A – METABOLIC EQUIVALENT (MET) REFERENCE DATA

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<th>Activity</th>
<th>Description</th>
<th>2011 Compendium Code</th>
<th>MET equivalent</th>
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<td>8</td>
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<td>Basketball, general</td>
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<td>Bicycling, general</td>
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<td>Wrestling</td>
<td>Wrestling (one match = 5 minutes)</td>
<td>15730</td>
<td>6</td>
</tr>
<tr>
<td>Yoga</td>
<td>Yoga, Hatha</td>
<td>02150</td>
<td>2.5</td>
</tr>
<tr>
<td>Martial Arts</td>
<td>Martial arts, different types, slower pace, novice</td>
<td>15425</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>performers, practice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barn Work</td>
<td>Farming, light effort (e.g., cleaning animal sheds, preparing</td>
<td>11147</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>animal feed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rock Climbing</td>
<td>Rock climbing, ascending or traversing rock, low-to-moderate</td>
<td>15537</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>difficulty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kayaking</td>
<td>Kayaking, moderate effort</td>
<td>18100</td>
<td>5</td>
</tr>
<tr>
<td>Elliptical</td>
<td>Elliptical trainer, moderate effort</td>
<td>02048</td>
<td>5</td>
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</table>
## APPENDIX B – OSTEOGENIC POTENTIAL (Ost) REFERENCE DATA

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Ost value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobics</td>
<td>Aerobics</td>
<td>5.41</td>
</tr>
<tr>
<td>Band/Drill</td>
<td>Marching band</td>
<td>3.71</td>
</tr>
<tr>
<td>Basketball</td>
<td>Basketball</td>
<td>11.04</td>
</tr>
<tr>
<td>Bicycling</td>
<td>Biking</td>
<td>2.42</td>
</tr>
<tr>
<td>Bowling</td>
<td>Bowling</td>
<td>3.42</td>
</tr>
<tr>
<td>Cheerleading</td>
<td>Cheerleading</td>
<td>10.6</td>
</tr>
<tr>
<td>Dance Class</td>
<td>Dance</td>
<td>8.38</td>
</tr>
<tr>
<td>Field Hockey</td>
<td>Field hockey</td>
<td>6.73</td>
</tr>
<tr>
<td>Football</td>
<td>Football</td>
<td>8.93</td>
</tr>
<tr>
<td>Garden/Yard Work</td>
<td>Yard/garden</td>
<td>1</td>
</tr>
<tr>
<td>Gymnastics</td>
<td>Gymnastics</td>
<td>11.57</td>
</tr>
<tr>
<td>Hiking</td>
<td>Hike</td>
<td>2.5</td>
</tr>
<tr>
<td>Ice Skating</td>
<td>Ice skate</td>
<td>5.29</td>
</tr>
<tr>
<td>Lacrosse</td>
<td>Lacrosse</td>
<td>7.43</td>
</tr>
<tr>
<td>Roller Skating</td>
<td>Roller skate</td>
<td>3.32</td>
</tr>
<tr>
<td>Running for Exercise</td>
<td>Cross country</td>
<td>3.81</td>
</tr>
<tr>
<td>Skateboarding</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Snow Skiing</td>
<td>Snow ski</td>
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</tr>
<tr>
<td>Activity</td>
<td>Calorie Cost</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------</td>
<td></td>
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<tr>
<td>Soccer</td>
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<td></td>
</tr>
<tr>
<td>Softball</td>
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</tr>
<tr>
<td>Street or Floor Hockey</td>
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<td></td>
</tr>
<tr>
<td>Swimming Laps</td>
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<td></td>
</tr>
<tr>
<td>Swimming for Recreation</td>
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<td></td>
</tr>
<tr>
<td>Tennis</td>
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<tr>
<td>Volleyball</td>
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<td>Water Skiing</td>
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<tr>
<td>Weight Training</td>
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</tr>
<tr>
<td>Yoga*</td>
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</tr>
<tr>
<td>Martial Arts</td>
<td>8</td>
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</tr>
<tr>
<td>Barn Work</td>
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<td></td>
</tr>
<tr>
<td>Rock Climbing</td>
<td>4.38</td>
<td></td>
</tr>
<tr>
<td>Kayaking*</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Elliptical *</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* = values not available from reference paper; values derived from comparative analysis of similar activities based on perceived weight-bearing capacity
Nationwide Children’s Hospital Institutional Review Board Approval

* Please describe the tests or analyses to be performed on the study specimens:
Body Composition Measurements (iDXA) to include total body, hip, lumbar spine and forearm scans: The primary purpose of the study is to estimate the training response in these female athletes in terms of bone density, lean mass and fat mass. The iDXA is the perfect tool (compared with other body composition methods) to evaluate these three body compartments.
Resting metabolic rate: The ReeVue indirect calorimeter will be used to estimate resting metabolic rate.
Eating attitudes: Athletes will be asked to fill out a questionnaire to document their attitudes about food and their bodies.

* Please identify and describe the specimens to be analyzed and/or the data to be collected:
A urine sample will be collected prior to the body composition procedures (iDXA) in order to perform a pregnancy screen using hcg dipstick testing to ensure each athlete is not pregnant (safety for low dose radiation). The urine will also be used to document hydration status of the subject.

* Outline the major steps and methodologies in the clinical protocol(s).
If necessary, include a description of any procedures being performed already for diagnostic or treatment purposes.
Clearly differentiate between these procedures:
Research Procedures: Body Compositions Assessment: Height, weight will be measured using a standard Healthometer digital scale and stadiometer. Changes in body composition will be evaluated using the GE Lunar iDXA, specifically as fat mass (FM), lean mass (LM), and bone mineral density (BMD) for the total body as well as non-dominant hip, lumbar spine and non-dominant forearm. All DXA measures will be taken by a trained and licensed GXMO while applying ALARA principles. The use of iDXA slightly increases radiation exposure to subjects. However, this exposure is minimal (<3 mrem for the set of scans) and is comparable to the radiation that is received during a transcontinental flight and is about 1/10th that received with a standard x-ray. In adults, excellent in vivo precision has been established for the Lunar iDXA for measurements of body composition including lean tissue mass (CV 0.50%), total fat mass (CV 0.82%) and total bone mineral content (CV 0.60%).(Hind 2011) The iDXA can be utilized for a wide range of subject types including children and the extremely large or obese individuals (up to 450 pounds).(Williams 2006;Breithaupt 2011) Numerous normative databases exist for the pediatric population using dual x-ray absorptiometry.(Binkovitz 2008) In preparation for the iDXA scan, subjects shall wear clothing with no metal, remove all jewelry, empty pockets, and take off glasses and shoes. Prior to the scan subjects will be asked to urinate into a cup to perform
a pregnancy screen and to assess hydration status with a hand-held refractometer and the status and specific gravity values will be documented. Once a negative screen is determined, the subject will lay on the iDXA table in the position instructed by the technologist and will remain still until the scan is completed. The total body scan time per subject will vary between 7 or 13 minutes depending upon the size of the subject. The hip, spine and wrist scans take less than one minute each. The resting metabolic rate will be estimated for each athlete immediately following the iDXA scan using ReeVue technology. This indirect calorimeter is able to estimate the body’s use of oxygen over a ten to fifteen minute period while the subject lies still breathing through her mouth into a tube connected to the machine.

Describe any risks that may result from the procedures to be completed in order to obtain the samples.
There is risk of increased radiation with the use of the iDXA. This radiation dose is small (<3.0 mrem) and is less than a standard x-ray and comparable to 3 hours of flight time in an airplane. Prior to the iDXA scan, a pregnancy screen will be performed to ensure no radiation to an undiscovered fetus. Knowing one’s body composition may also be considered a psychological risk. Subjects will be asked if they would like to know their results.

* Please discuss your rationale for the classification you have chosen above in Question 1:
The use of the iDXA may be considered greater than minimal risk. However, the radiation exposure with the use of the iDXA is less than standard x-rays and comparable to flying in an airplane for 3 hours.

* Risk Minimization - Describe procedures for protection against or minimizing potential risks, and assess their likely effectiveness. All proposals should describe steps to be taken to ensure confidentiality regarding the collection, analysis, and storage of data, specimens, etc.:
Performance of the iDXA scans will be conducted by a licensed and trained GXMO with ALARA principles in mind. Data will be collected and stored by subject number without names. The master list of subject names and athlete consents will kept in a locked drawer in the laboratory designed for storage of such documents. Risk of breach of confidentiality is well-controlled in this manner.

* Describe any risks involved to healthy subjects, and the investigator's justification of the use of such subjects:
There is risk of increased radiation with the use of the iDXA. The radiation dose is small (3.0 mrem) for the total body, hip, spine and wrist scans. This is less than a standard chest x-ray and comparable to the radiation received in 3 hours of flight. For further comparison, everyone receives a small amount of unavoidable (background) radiation each year. Some of this radiation comes from space and some from naturally occurring radioactive forms of water and minerals. This research increases the equivalent of about two extra days' worth of this natural radiation (ie, like living two extra days). The justification for use of the iDXA is to be able to accurately evaluate changes in bone mass and body composition.

If this research involves more than minimal risk to participants, provide a detailed description of provisions for monitoring data to ensure the safety of participants. Include: what are the qualifications of those who will monitor the data, what data will be monitored, how often will data be monitored and what endpoints will be monitored.
The PI's (Stacey Fischer and Jackie Buell) will be in charge of overall monitoring the data to
ensure the safety of participants. Jackie Buell PhD, RD will be in charge of running and monitoring the iDXA and its output. Her lab, which is located within the biomechanics lab at Ohio State, utilizes the iDXA for both research and clinical purposes on a daily basis. Jackie Buell will conduct the iDXA scans on all subjects to ensure safety, consistency, and technique.

* Summarize any pre-specified criteria for stopping or changing the study protocol due to safety concerns:
A positive pregnancy screen will exclude subjects from participating in the iDXA scan and the study.

* Please complete the following table for all procedures involving exposure to radiation for research purposes, beyond what is required as part of standard care:

<table>
<thead>
<tr>
<th>Procedure Involving Radiation</th>
<th>Critical Organs Involved</th>
<th>Dose</th>
<th>Number of Times</th>
<th>If isotope is used, is this new use for isotope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual x-ray absorptiometry (iDXA)</td>
<td>all</td>
<td>3 mrem</td>
<td>3</td>
<td>No</td>
</tr>
</tbody>
</table>
Describing the Components of the Female Athlete Triad and Resting Metabolic Rate in a Cohort of Middle-to-Upper Class Adolescent Female Athletes: A Cross-Sectional Study

Kelsey A. Conrad, Jackie L. Buell, Diane L. Habash, and Anastasia N. Fischer

The female athlete triad is defined as “a spectrum of abnormalities in energy availability, menstrual function, and bone mineral density”.

The objective of this study was to describe the components of the female athlete triad and resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes. A convenience sample of adolescent female participants was recruited. Bone mineral density (BMD), body composition, and resting metabolic rate (RMR) were measured. A Food Frequency Questionnaire® was used to estimate daily energy intake. A compendium of validated questionnaires was generated using the Research Electronic Data Capture (REDCap) application and was used to estimate exercise energy expenditure and categorize participants by menstrual function. Nineteen participants were recruited. Nine participants (56.3%) exhibited low energy availability (N=16), 2 participants (14.3%) exhibited menstrual dysfunction (N=14), and 4 participants (21.1%) exhibited low lumbar spine BMD z-scores (N=19). One participant exhibited all three components of the female athlete triad (N=17). Data analysis revealed a significant correlation between TBLH BMD z-score and lumbar spine BMD z-score. Both total mass and lean body mass were significantly correlated with TBLH z-score and lumbar spine z-score. Dysmenorrheic participants trended toward lower energy availability than eumenorrheic participants in EA, however this trend was not significant. No significant correlations were observed between RMR and other variables. Additional research is needed to more thoroughly describe the prevalence and severity of the components of the female athlete triad and resting metabolic rate in the adolescent population.

Keywords: energy availability, menstrual function, bone mineral density, female athlete triad, adolescents

Since the 1970s, the opportunity for adolescent females to participate in sports has grown exponentially. With an increase in opportunity came an increase in participation, with almost 3 million girls participating in high school athletics by the year 2006. As participation rates have continued to increase, a grouping of interrelated conditions has presented among this population of athletes. The female athlete triad is defined as a spectrum of abnormalities in energy availability, menstrual function, and bone mineral density. Several other conditions have since been associated with the triad, including decreased resting metabolic rate. Sport participation reaps incredible benefits among adolescent females including the optimization of muscular strength and flexibility; maintenance of a healthy body weight; attainment of peak bone mass; development of neuromuscular awareness; optimization of cognition, mental health, mood, sleep, and academic performance; and the improvement of overall wellness and social behavior.

In light of these benefits, the answer to preventing the development of the female athlete triad and its associated conditions is not to discourage sport participation among this population. Rather, it is necessary to investigate the disorder itself and determine how to best screen for, prevent, and treat each of the triad components and associated conditions.

Components of the Female Athlete Triad

Energy availability to operationally defined as EA=(Energy Intake–Exercise Energy Expenditure)/LBM expressed in units of kcal/kg LBM/day. EA ranges from optimal EA to low EA with or without an eating disorder. Among adult women, a decline in bone mineralization and disruption of menstrual function have been reported below a threshold of 30 kcal/kg LBM/day. Such a threshold has not been
tested in the adolescent population. Optimal energy availability has therefore not been proposed for adolescent athletes.

Menstrual dysfunction ranges from eumenorrhea to functional hypothalamic amenorrhea. Subclinical menstrual disturbances, such as luteal phase defects, anovulation, and oligomenorrhea fall between these two extremes. Clinical and subclinical menstrual disturbances are prevalent among female athletes. Low energy availability has been associated with menstrual dysfunction in this population. It is unclear, however, at what degree of energy restriction menstrual disturbances begin to occur among adolescent female athlete. Additionally, it is unclear whether the degree of energy restriction is related to the severity of menstrual dysfunction in this population.

The spectrum of bone mineral density ranges from optimal bone health to osteoporosis. Dual-energy x-ray absorptiometry (DXA) is considered the gold standard for conducting BMD measurements. In premenopausal women and children, BMD measurements are expressed as z-scores, which compare individuals to age and sex-matched controls. Historically, chronic hypoestrogenism in the context of menstrual dysfunction has been accepted as the major cause of bone loss in women. However, more recent work has shown bone loss to occur through both an estrogen-dependent pathway and an estrogen-independent pathway, the latter of which is described by the changes in metabolic hormones and bone trophic factors that result from an energy deficiency and resulting hypometabolic state. These mechanisms are believed to work in tandem in adult women. Additional research is needed to tease out the differential effects of hypoestrogenism and energy deficiency in adult women, and to identify these mechanisms in an adolescent population.

Complications Beyond the Triad

Beyond the triad itself, decreases in resting metabolic rate have been observed in the presence of menstrual dysfunction and low energy availability. In 2014, the International Olympic Committee (IOC) introduced the term “relative energy deficiency in sport (RED-S)” as “a broader and more comprehensive term for the condition previously known as female athlete triad.” This new model included decreased metabolic function as a consequence of energy deficiency. Though there is speculation whether the RED-S model is an appropriate representation of the triad and its associated conditions, the literature does support a decrease in RMR in the presence of an energy deficiency, and the proposed RED-S model opens the door for more investigative research in this area.

The literature clearly demonstrates the interrelationships between low energy availability, menstrual dysfunction, and poor bone mineral density among female athletes. However, literature demonstrating the simultaneous occurrence of all three triad components among adolescent female athletes is currently lacking and the reported prevalence of each triad component varies between studies. Additionally, the far-reaching effects of an energy deficiency beyond the triad are difficult to study, thus not well supported or constructed. In light of these gaps in the literature, there is a need for additional descriptive studies to gain a better understanding of the triad components individually and synergistically, as well as the effects of an energy deficiency beyond the triad itself. In doing so, the concept of the female athlete triad can continue to be shaped and expanded upon. Evidence-based implications and recommendations can then be made for screening, preventing, and treating the female athlete triad and its associated conditions. Because of the incredible benefits of sport participation among adolescent females, it is necessary to gain a greater understanding of the triad and its associated conditions, not to discourage adolescent girls from competing in sports, but rather to protect the health and well being of these girls in the midst of greater participation and competition.

The objective of this study was to describe the components of the female athlete triad and resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes.

Methods

A convenience sample of adolescent female participants was recruited from a middle-to-upper class suburban high school in Ohio during the autumn semester of the 2014-2015 academic school year. To be included in this study, participants must have been female student athletes at the designated high school between 14-18 years of age at the time of recruitment. Participants were excluded from the study if they competed in a winter sport at the designated high school.
In December 2014 and January 2015, each participant attended one individual, 1-hour laboratory visit at The Ohio State University Sports Nutrition Laboratory at Martha Morehouse Medical Plaza. Height and weight were measured to the nearest tenth centimeter and kilogram respectively on a standardized and calibrated Health-O-Meter stadiometer scale (Model 500KL). Body composition and bone mineral density were estimated using a General Electric densitometer (Lunar iDXA, Encore 14.0 GE) following manufacturer protocol for total body, non-dominant hip, lumbar spine, and non-dominant radius BMD. Prior to testing, participants were required to provide a urine sample to ensure urine was negative for human chorionic gonadotropin (hCG). Negative results were required for testing to proceed.

Two questionnaires were sent to participants prior to the laboratory visit. A Vioscreen Food Frequency Questionnaire® was used to assess foods frequently consumed, consumption frequency of each food, and the typical serving size of each food consumed in the preceding 3 months. From this data, an estimate of daily energy intake for each participant was calculated. A compendium of validated questionnaires was generated using the Research Electronic Data Capture (REDCap) application. This application was used to deliver, collect, and store data in a confidential manner. This questionnaire will be referred to as the “REDCap questionnaire” throughout this report. The REDCap questionnaire was used to collect information regarding physical activity and menstrual function. This questionnaire assessed frequency of physical activity and sport participation. Metabolic equivalents (METS) of activities reported were retrieved from The 2011 Compendium of Physical Activities. Frequency data was multiplied by respective MET values to estimate total annual MET minutes of exercise and average daily exercise energy expenditure. The REDCap questionnaire was also used to categorize participants by past and present self-reported menstrual status. The questionnaire asked participants “Have you started your period?”, “How old were you when you started your period?”, “Have you missed any periods in the last three to four months?”, and “Have you been taking the pill (oral contraceptives)?”. It also asked participants to estimate how many periods they had in the past 12 months.

Describing Components of the Female Athlete Triad and Resting Metabolic Rate

Energy availability, operationally defined as “(energy intake – exercise energy expenditure) / kilograms lean body mass” was estimated using data collected in the laboratory and via questionnaire. The Vioscreen Food Frequency Questionnaire® was used to estimate energy intake. The REDCap questionnaire was used to estimate exercise energy expenditure. Lean body mass was estimated by the Lunar iDXA. Using the equation above, a value of energy availability was estimated for each participant.

Data from the REDCap Questionnaire were used to categorize participants by menstrual function into one of two categories: eumenorrheic or dysmenorrheic (oligomenorrheic and secondary amenorrheic); no participants self-reported primary amenorrhea, thus this category was not considered. Participants were classified as eumenorrheic if they reported having 9 or more menstrual periods in the previous 12 months. Participants were classified as dysmenorrheic if they reported 0-8 menstrual periods in the previous 12 months.

Bone mineral density was estimated using a General Electric densitometer (Lunar iDXA, Encore 14.0 GE) following manufacturer protocol for total body, non-dominant hip, lumbar spine, and non-dominant radius BMD. One investigator conducted and analyzed all bone mineral density measurements. Data were exported from the machine for statistical analysis.

To capture the osteogenic response to the weight bearing capacity and impact of each activity reported by the participants, responses to the REDCap questionnaire were quantified in terms of the probable osteogenic potential (Ost). Participants were asked to list which sports and activities they had participated in in the previous 12 months and the amount of time spent participating in each of these activities individually. Total annual hours of each activity were multiplied by the Ost value specific to that activity. Annual Ost scores for each activity were calculated and summed to produce an annual Ost score. This annual Ost score was then normalized to body weight.

The KORR Medical Technologies ReeVue indirect calorimeter was used to estimate resting metabolic rate. Participants were instructed to arrive to the laboratory fasted, having not eaten since midnight the previous night. A 10-minute rest period in a reclined state was required prior to testing. The machine was calibrated following manufacturer protocol. Two investigators and several trained laboratory
assistants conducted RMR measurements. Data were hand-recorded into the computer and double-checked for accuracy by two separate laboratory staff.

Statistical Analysis
Data were collated to a master excel spreadsheet and imported into IBM® SPSS Statistics Version 23.0 (International Business Machines Corp., Armonk, New York) for analysis. Descriptive analysis was performed to include mean, standard deviation, and range of variables. Spearman’s correlations were used to describe correlations between variables; correlations were significant if $p \leq 0.05$. The Mann-Whitney $U$ test was used to assess differences between the two independent menstrual function categories.

Results
Nineteen participants were recruited for this descriptive study. All 19 participants completed the anthropometric, bone mineral density, and RMR tests. Two of the participants failed to complete the questionnaires, leaving seventeen participants with estimates of energy intake, exercise energy expenditure, energy availability, and menstrual function. Anthropometric characteristics are presented in Table 1. Data analysis revealed a significant correlation between weight and lean body mass ($p \leq 0.01$).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>16.3 (1.19)</td>
<td>14.5</td>
<td>18.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.7 (5.7)</td>
<td>151.1</td>
<td>171.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.8 (8.6)</td>
<td>43.8</td>
<td>81.9</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>40.3 (4.2)</td>
<td>35.5</td>
<td>52.1</td>
</tr>
</tbody>
</table>

Table 1: Anthropometric Characteristics of Adolescent Female Athletes (N=19)

Descriptive analysis of annual exercise revealed a single outlier. This participant was removed from analysis of annual exercise as well as osteogenic potential, exercise energy expenditure, and energy availability as the reported annual exercise was used in the calculation of these other variables. Self-reported annual exercise and osteogenic potential are presented in Table 2.

Table 2: Self-Reported Physical Activity (min/year) and Osteogenic Potential of Adolescent Female Athletes (N=16)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Exercise (min/year)</td>
<td>29137 (17703)</td>
<td>7805</td>
<td>73659</td>
</tr>
<tr>
<td>Osteogenic Potential (Ost/kg/year)</td>
<td>55.6 (31.2)</td>
<td>13.7</td>
<td>109.56</td>
</tr>
</tbody>
</table>

Energy availability data are presented in Table 3. The mean energy availability estimated for this population of adolescent female athletes was 30.7 kcal/kg LBM/day. Excluding the outlier, approximately 56% of the sample fell below the energy availability threshold of 30 kcal/kg LBM/day proposed by Loucks.
et al. among adult female athletes. A significant correlation was observed between EA and daily energy intake (p≤0.01). Because energy availability is dependent on both EI and EEE, this significant correlation suggests daily energy intake and EA are more closely correlated than exercise energy expenditure and EA (p=0.362).

Table 3: Estimated Energy Availability of Adolescent Female Athletes (N=16)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI (kcal/day)</td>
<td>1736.2 (546.9)</td>
<td>1084.7</td>
<td>2734.0</td>
</tr>
<tr>
<td>EEE (kcal/day)</td>
<td>516.4 (321.6)</td>
<td>130</td>
<td>1288</td>
</tr>
<tr>
<td>EA (kcal/kg LBM/day)</td>
<td>30.7 (14.8)</td>
<td>4.8</td>
<td>51.7</td>
</tr>
</tbody>
</table>

None of the participants reported a late onset of menarche. Three participants were either currently taking or had previously taken oral contraceptives. These three participants were excluded from analysis due to this confounding variable. Two (14.3%) participants were categorized as “dysmenorrheic” and the remaining 12 participants were categorized as “eumenorrheic”.

After excluding the three participants who reported oral contraceptive use and the outlier, a Mann-Whitney U test was conducted to assess difference between menstrual function categories. This test revealed no significant differences between menstrual function groups in terms of weight, LBM, exercise energy expenditure, daily energy intake, energy availability, osteogenic potential, RMR, lumbar spine BMD z-score, or TBLH BMD z-score. Figure 1 and Figure 2 display the results of the Mann-Whitney U test, testing the null hypothesis concerning energy availability and energy intake respectively. Though the difference in energy availability between menstrual function groups was not statistically significant (p=0.154), it is noteworthy that the only dysmenorrheic participant included in this test exhibited the lowest energy intake (1106 kcal/day) and the lowest energy availability (20 kcal/kg LBM/day) of the 13 participants.

Figure 1: Energy Availability by Menstrual Function

![Figure 1: Energy Availability by Menstrual Function](image-url)
Bone mineral density measurements for this sample are presented in Table 4. Z-scores are reported for total body less head (TBLH) and lumbar spine. Of the 19 participants, 4 participants (21.1%) exhibited lumbar spine z-scores indicative of low BMD. A significant correlation was observed between TBLH z-score and lumbar spine z-score (p≤0.01). Total mass and lean body mass were significantly correlated with TBLH z-score (p=0.001 and p≤0.01) and lumbar spine z-score (p=0.003 and p=0.24). No significant correlations were observed between EA and BMD z-scores. After excluding the outlier, a significant correlation was observed between lumbar spine z-score and Ost score (p=0.047).

Table 4: Bone Mineral Density and BMD Z-scores of Adolescent Female Athletes (N=19)

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean BMD (SD)</th>
<th>Minimum Z-score</th>
<th>Maximum Z-score</th>
<th>“Low BMD” n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBLH</td>
<td>1.03 (0.09)</td>
<td>-0.29</td>
<td>2.93</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>0.73 (0.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral Neck</td>
<td>1.06 (0.11)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar Spine</td>
<td>1.15 (0.12)</td>
<td>-1.22</td>
<td>2.31</td>
<td>4 (21.1)</td>
</tr>
<tr>
<td></td>
<td>-0.03 (0.99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius UD</td>
<td>0.39 (0.05)</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* represents only z-score diagnostic criteria for “low BMD”; to be diagnosed with low BMD, participants would also require a history of nutritional deficiencies, hypoestrogenism, stress fractures, and/or secondary risk factors for fracture.

** z-scores cannot be used to express femoral neck and radius UD BMD; normative data for the adolescent population does not exist.
Descriptive analysis of resting metabolic rate is presented in Table 5. All participants reported a 12-hour fast prior to indirect calorimetry measurement. No significant relationships were observed between RMR and other variables in the study.

### Table 5: Measured and Predicted Resting Metabolic Rate of Adolescent Female Athletes (N=19)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured RMR (kcal/day)</td>
<td>1402.8 (180.0)</td>
<td>1022</td>
<td>1714</td>
</tr>
<tr>
<td>Cunningham Predicted RMR*</td>
<td>1386.0 (92.9)</td>
<td>1279</td>
<td>1645</td>
</tr>
<tr>
<td>HB Predicted RMR**</td>
<td>1168.4 (84.9)</td>
<td>1036</td>
<td>1414</td>
</tr>
</tbody>
</table>

*Cunningham equation (1980): RMR (kcal/day) = 500 + 22 (LBM)\(^{97}\)

**Harris-Benedict equation (1919): RMR (kcal/day) = 655.1 + 9.56 (wt) + 1.85 (ht) – 4.68 (age)\(^{97}\)
Ht: cm; Wt: kg; LBM: kg; Age: years

### Discussion

The purpose of this descriptive, cross-sectional study was to describe the components of the female athlete triad, namely energy availability, menstrual function, and bone mineral density, as well as resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes. The study explored the severity of each triad component and the prevalence of pathologic triad factors in this cohort of adolescents. Additionally, we explored the relationships between individual triad components as well as the relationship between resting metabolic rate and each triad component individually. Upon analysis of physical activity data, one participant was excluded as an outlier, reporting statistically greater levels of physical activity than the other participants. Of the 16 participants with usable data sets, 9 (56.3%) exhibited low energy availability (less than 30 kcal/kg LBM/day). After excluding the three participants reporting oral contraceptive use, 2 (14.3%) participants reported dysmenorrhea. Of the 19 participants with BMD data, 4 (21.1%) exhibited low lumbar spine BMD z-scores. One participant exhibited all three components of the female athlete triad, however it should be noted that she reported previously or currently taking an oral contraceptive. Using the 2007 definition of the female athlete triad, which requires only one pathologic triad component for diagnosis, 11 of the participants in this study would be diagnosed, however it can be hypothesized that number may be greater if complete and reliable data sets were provided by all 19 participants. Data analysis revealed a significant correlation between TBLH BMD z-score and lumbar spine BMD z-score. After excluding the outlier, a significant correlation was observed between lumbar spine z-score and Ost score. Total mass and lean body mass were significantly correlated with both TBLH z-score and lumbar spine z-score. Differences in energy intake and energy availability were observed between eumenorrheic and dysmenorrheic groups, however this difference was not significant. Other
significant correlations were observed between exercise energy expenditure and Ost score, exercise energy expenditure and annual minutes of exercise, annual minutes of exercise and Ost score, and energy availability and daily energy intake.

**Energy Availability**

Few studies have evaluated energy availability in adolescents. Of the studies that have been conducted, disordered eating has been assessed as a marker of low energy availability. This study, however, estimated energy availability for each participant using questionnaires to assess physical activity and energy intake. Though a threshold of energy availability has not been proposed for adolescents, these calculated EA values can be compared to the energy availability threshold of 30 kcal/kg LBM/day for adult women proposed by Loucks and Thuma. In this study, 56.3% of participants fell below this threshold. According to Loucks and Thuma, menstrual dysfunction presents below this threshold in adult women. Both participants reporting menstrual dysfunction fell below this threshold, however a total of 9 participants fell below the threshold, all of whom should theoretically experience menstrual dysfunction. This suggests several possibilities: the self-reported physical activity or menstrual function from this group of adolescent female athletes is inaccurate, the threshold is different in an adolescent population, or menstrual dysfunction occurs at a different EA for each individual and the threshold is therefore not applicable. If the threshold is different for an adolescent population, one could intuitively suggest the EA threshold is greater than 30 kcal/kg LBM/day due to the increased energy needs for growth and development in this population of athletes. Additional research is needed to evaluate a potential threshold of energy availability in an adolescent population.

**Menstrual Function**

The 14.3% prevalence of menstrual dysfunction in this cohort of adolescent female athletes is lower than that reported in the literature. It is more comparable to a prevalence of 15.9% reported by Fischer et al. among a sample of 491 adolescent female athletes presenting to a pediatric primary care sports medicine clinic in the same geographical location as the present study. A range of 18.8 – 54% is discernable among similar populations.

The accuracy of the self-reported menstrual function must be considered. In a recent manuscript titled “Challenges of Menstrual Dysfunction Screening Using Pre-Participation Physical Exam”, Fischer and her colleagues sought to determine if the pre-participation physical exam (PPE) was sufficient to screen for menstrual dysfunction among female high school and middle school athletes when compared to validated research protocols. In this observational, cross-sectional study, researchers screened for discrepancies in reported menstrual function between the PPE and the Healthy Wisconsin High School Female Athletes Survey (HWHSFAS), each of which was completed by participants or parents before the annual pre-participation physical. Of the 15 girls recruited for this study, 47% had at least one discrepancy between the answers on the PPE form and HWHSFAS regarding oral contraceptive use and menstrual function. The results of this study led the authors to conclude that menstrual dysfunction reported using the PPE form may be underreported and therefore undertreated. The authors suggest the use of menstrual cycle tracking devices such as calendars or phone applications to better identify menstrual dysfunction and risk for the female athlete triad among adolescent female athletes. The discrepancies reported in Fischer’s study suggest that discrepancies may exist between the true menstrual function and that reported using the REDCap questionnaire in the present study. As in Fischer’s study, it is possible that menstrual function was underreported among this cohort of adolescent female athletes. This becomes more of a possibility when considering the prevalence reported in this study falls below the prevalence range reported in the literature. Tools such as those recommended by Fischer and her colleagues could help to better identify menstrual dysfunction among this population.

Assuming the self-reported data of the present study is accurate, the results support the energy availability hypothesis and are consistent with the adult literature in that the dysmenorrheic participant ranked below eumenorrheic participants in EA. Because the present study did not evaluate differences in clinical and subclinical menstrual disturbances, conclusions cannot be made about the severity of menstrual function relative to EA. In adult studies, energy availability discriminated clinical menstrual status, however the magnitude of energy deficit did not predict the severity of the menstrual...
disturbances. A more comprehensive study is needed to evaluate the relationship between EA and the severity of menstrual dysfunction in this population.

Noteworthy is the oral contraceptive use among the participants. Three participants reported previously or currently taking oral contraceptives, however the questionnaire did not ask for the specific oral contraceptive used by each of these participants. Because the type of oral contraceptive used by each of these participants was unknown, conclusions could not be drawn regarding the effect of oral contraceptive use on menstrual function. In light of the discrepancies reported by Fischer et al., the possibility of discrepancies between actual and reported contraceptive use among this sample must also be considered.

**Bone Mineral Density**

The prevalence of low BMD among this cohort of adolescent female athletes is comparable to that reported by Rauh et al. In the 2010 study, 36 of the total 163 female high school athletes (28.3%) exhibited low total body or lumbar spine BMD z-scores (z ≤ -1). The present study reported a prevalence of 21.1%; all participants included in this percentage exhibited low lumbar spine BMD z-scores. It is possible that this value inaccurately represents the prevalence of low BMD. The GE Lunar iDXA produces a two-dimensional image which does not incorporate the depth or shape of the bone. A small bone may therefore appear to have a lower areal BMD, and a lower z-score, compared to a larger bone with a similar volumetric BMD. It has been suggested that z-scores be height-adjusted to correct for these differences in bone length and discrepancies in z-scores, however no validated method of height-adjusting BMD z-scores in the adolescent population exists. Therefore, z-scores in this present study were not height-adjusted. Further validation of height-adjustment techniques is needed before this is possible.

As was predicted, a significant correlation was observed between lumbar spine BMD z-scores and Ost score. BMD z-scores would be expected to increase with greater scores of osteogenic potential, as observed. These results lend support to the recommendation of weight-bearing physical activity in the development and maintenance of bone mass. More comprehensive measures of physical activity should be used to gain a greater understanding of the relationship between physical activity and osteogenic potential.

Surprisingly, a significant correlation was not observed between BMD z-scores and menstrual function. The self-report questionnaire used to assess menstrual function may not be the most reliable tool. With additional resources, urinary concentrations of E1G, PdG, LH, and other markers of menstrual status could be measured. These biological markers would improve the accuracy of menstrual function data and generate more significant results. More rigorous testing is needed to accurately evaluate the relationship between BMD and menstrual function.

The significant correlations between BMD z-scores and body composition measures are consistent with the results reported by Lima et al. The authors of this 2001 study reported significant correlations between weight and total BMD (p < 0.001), femoral neck BMD (p < 0.001), and lumbar spine BMD (p < 0.001). The present study also demonstrated a significant correlation between weight and TBLH BMD (p = 0.001), TBLH BMD z-score (p = 0.001), total body BMD (p = 0.006), total body BMD z-score (p < 0.001), lumbar spine BMD (p = 0.003), and lumbar spine BMD z-score (p = 0.003). A significant correlation was also observed between LBM and all these BMD measures and corresponding z-scores. This is intuitive in light of the correlation between weight and LBM; as lean body mass increases, total mass will also increase.

This correlation between lean body mass and total mass presents a challenge in the context of the female athlete triad. Often, female athletes engage in behaviors to decrease body weight for appearance or performance. This weight loss occurs through a reduction in EA. Based on the correlation between weight and LBM, a reduction in weight will concurrently reduce LBM. This reduction in weight and LBM will then lead to a reduction in BMD. This reduction in EA to decrease weight and the resulting decrease in LBM and BMD present as the female athlete triad; in these circumstances, menstrual dysfunction would also be expected. The idea of gaining weight to prevent such dramatic consequences is certainly a barrier to preventing or recovering from the female athlete triad, especially in an adolescent population. Because of this, education of athletes, parents, coaches, and sports medicine professionals is critical.
Female Athlete Triad

One participant exhibited all three components of the female athlete triad. It is remarkable that a study of 17 participants identified an athlete presenting with all three female athlete triad components, given that other studies estimate the prevalence of the full triad to be 1% of the athletic population.26

Resting Metabolic Rate

Interestingly, no significant correlations were observed between RMR and other variables. Additionally, significant differences in RMR were not observed between menstrual function groups. In 1991, Myerson et al. reported a significantly lower RMR in amenorrheic runners compared to eumenorrheic runners and controls.80 Similar results were reported by Lebenstedt et al. in 1998 and Myburgh in 1999.81,82 Correlations between RMR and EA have also been reported, yet were not observed in this study.66 All these studies, however, were conducted in adult women and cannot be generalized to include the adolescent female athletes of the present study. Additionally, it must be noted that menstrual function and data used to estimate energy availability in the present study were self-reported. More research and more reliable tools to assess menstrual status and energy availability are needed to properly assess the relationship between RMR and these variables.

Relative Energy Deficiency in Sport

The results of this small study were not able to support the RED-S paradigm. Though correlations were observed between EA and menstrual function, no significant correlations were observed between triad components or RMR. This study did not address the additional components of the RED-S model and therefore cannot speak to the relationships beyond the triad and RMR. There remains, however, the opportunity to develop a more comprehensive, well-supported definition of the female athlete triad and RED-S as more rigorous research is conducted.

Limitations

Several limitations exist in this study. With 19 participants recruited, 17 participants providing complete data sets for analysis, and the exclusion of one participant as an outlier, the statistical power of the study is low. Additionally, much of the data (menstrual status, energy intake, and physical activity) was self-reported. The REDCap questionnaire inquired about months per year, days per week, and minutes per day of participation in each sport and activity, but failed to ask how many weeks per month each sport or activity was performed. Because of this, it was assumed that the sport or activity was performed all weeks of the indicated months (i.e. 4.5 weeks/month). This could have led to an over-estimation of exercise energy expenditure. Menstrual status was also self-reported via the REDCap questionnaire. From this data, it was possible to distinguish between amenorrhea and eumenorrhea, but it was not possible to differentiate sub-clinical menstrual disturbances. This inability prevented researchers from estimating where on the spectrum of menstrual dysfunction the dysmenorrheic participants fell.

The socioeconomic status of the population studied serves as another limitation in this study. The adolescent female athletes were recruited from a middle-to-upper class high school in Central Ohio. It can be assumed that this population is food secure, having both physical and economic access to food that meets dietary needs as well as food preferences.4 In theory, these participants have the ability to consume adequate energy to maintain optimal energy availability and adequate dietary calcium to promote bone health. They also have the access and opportunity to participate in weight-bearing and impact sports and activities to promote bone mineralization. Lesser socioeconomic populations may not be food secure and therefore may not have access to adequate and nutrient-dense foods. These individuals may not be able to maintain optimal energy availability and consume recommended levels of dietary calcium due to a lack of resources. These populations may not have the access or opportunity to participate in weight-bearing and impact sports and activities. Because of this, the prevalence of the female athlete triad may be greater in these lower socioeconomic populations than in this sample of adolescent female athletes.

Furthermore, the lack of normative BMD data in the adolescent population prevented the researchers from describing femoral neck BMD, radius UD BMD, total femur BMD, and total radius BMD in terms of Z-scores. This lack of normative data prevented the comparison of these BMD measurements from this sample to a larger population of adolescent females. Additionally, the z-scores of this study were
not height adjusted for bone length. There is currently no validated method to height-adjust BMD z-scores in the adolescent population; this validation is needed before these adjustments can accurately be made.

Finally, the exclusion criterion of this study, which excluded winter sport athletes from participation, decreased the athletic diversity among the participants. By excluding winter sport athletes, several groups of athletes were not represented in the sample, namely basketball players and swimmers. This limits the generalizability of the results.

**Future Research**

Though a high prevalence of the female athlete triad was not observed in this cohort of adolescent female athletes, individual components of the female athlete triad were observed across the spectrum of severity, and significant relationships were observed among several variables. In light of the existing literature concerning the female athlete triad, these observations evoke the need for additional research. As a pilot study, funding and resources were limited. With greater funding and resources, future research should consider a greater population of adolescent female athletes, including athletes from all sports and activities to produce a diverse and all-encompassing sample of adolescent female athletes. More comprehensive tools should be used to collect diet and physical activity data; diet analysis and fitness software, heart rate monitors, pedometers, and the like may be considered. Blood samples should be used to measure reproductive and metabolic hormones as well as markers of bone formation and resorption. Other hormones, such as cortisol and leptin, may also be measured to further evaluate the complex relationships of the triad and RED-S. Future research should also investigate differences between adolescent female athletes and adolescent controls in terms of energy availability, disordered eating, menstrual function, bone mineral density, and RMR. Differences in triad components and RMR between lean and non-lean sports in the adolescent population should also be studied. The effect of weight bearing and impact loading exercise on BMD over time should be considered. The effect of “time since menarche” on BMD should also be evaluated. Finally, the eating attitudes and behaviors studied but not evaluated by the present study should be explored.

**Conclusion**

This study described the components of the female athlete triad and resting metabolic rate among a cohort of middle-to-upper class adolescent female athletes. Results identify the presence of all three female athlete triad components among this small sample of adolescent females and add to the literature more evidence of this ever-present condition. In light of the serious health consequences associated with the female athlete triad, it is crucial that athletes, parents, coaches, and sports medicine practitioners are informed about the triad and take the necessary steps to screen, prevent, and treat low energy availability, menstrual dysfunction, and low bone mineral density. Sport participation among adolescent females reaps incredible benefits. However, in order to support these females in their athletic endeavors, an educational curriculum and protocol to address screening, prevention, and treatment of the female athlete triad must be developed. This descriptive study lends support to the need for these programs and resources. As additional research is conducted, these programs and resources can be developed. With the support and dedication of the entire team of parents, coaches, and sports medicine professionals, adolescent female athletes will have the tools and resources they need to thrive physically, mentally, and athletically.