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Correlation Between Female Athlete Screening Tool (FAST) Scores and Biomarkers to Identify Female Athlete Triad Among Collegiate Athletes and to Evaluate the Validity of the Instrument

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CORRELATION BETWEEN FEMALE ATHLETE SCREENING TOOL (FAST)
SCORES AND BIOMARKERS TO IDENTIFY FEMALE ATHLETE TRIAD
AMONG COLLEGIATE ATHLETES AND TO EVALUATE THE VALIDITY OF
THE INSTRUMENT

A Thesis
Presented to
The Faculty of the Department of Psychology
Western Kentucky University
Bowling Green, Kentucky


In Partial Fulfillment
Of the Requirements for the Degree
Master of Arts

By
Lindsey Elise Hinken

May 2018

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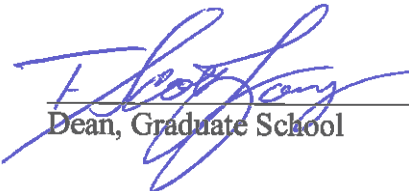
Dr. Frederick Grieve, Director of Thesis



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Date

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CONTENTS

Introduction.....	1
Literature Review.....	6
Methods.....	13
Results.....	18
Discussion.....	23
References.....	28
Appendix A: Demographics.....	32
Appendix B: Menstrual Status Questionnaire.....	34
Appendix C: Female Athlete Screening Tool.....	35
Appendix D: Blood Sampling Protocol.....	36
Appendix E: ELISA Protocol.....	37
Appendix F: Informed Consent.....	39
Appendix G: Debriefing.....	41

LIST OF FIGURES

Figure 1. Prealbumin levels of varsity versus recreational athletes.....	21
Figure 2. CTx levels of varsity versus recreational athletes.....	22

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The purpose of this study was to determine if the Female Athlete Screening Tool (FAST) is a valid indicator of the three physical components of Female Athlete Triad (FAT), malnutrition, amenorrhea, and low bone mineral density, and if administration of the FAST can be used as a time- and cost-effective way to identify FAT. Participants completed the FAST, the Menstrual Status Questionnaire, and had a blood sample drawn. Interpretation of the total FAST score placed participants in one of three conditions: normal, subclinical disordered eating, or clinical eating disorder. The presence of the three biomarkers of FAT was determined by participants' responses on the Menstrual Status Questionnaire and protein analyses of their blood samples, in which plasma Prealbumin and CTx levels were tested. Results indicated that there were not significant differences between total FAST scores of participants in each of the groups for any of the three biomarkers (i.e., low versus normal Prealbumin levels; amenorrhea versus normal menstruation; high versus normal CTx levels). More research is needed before it can be determined if the FAST is an appropriate diagnostic tool for FAT. An overwhelming majority of the sample (79.5%) received total FAST scores that fell in the subclinical disordered eating or clinical eating disorder ranges, which supports previous findings that female athletes are at a high risk of developing disordered eating habits or eating disorders due to their unique characteristics and lifestyles.

Introduction

A dangerous health condition is on the rise among today's female athletes, especially adolescents and young adults who participate in endurance and aesthetically-judged sports. Female Athlete Triad (FAT) is characterized by the presence of three independent, yet intricately related, conditions: malnutrition, amenorrhea, and low bone mineral density (BMD; Yeager, Agostini, Nattiv, & Drinkwater, 1993). The concurrence of these three symptoms leads to severe consequences for athletes' short- and long-term health (Knapp, Aerni, & Anderson, 2014). It is estimated that up to 16% of female athletes experience all three components of FAT, and that 60% of athletes in particular sports are affected by two of the three components of FAT (Barrack, Ackerman, & Gibbs, 2013). Collegiate endurance athletes may be among the most susceptible to developing FAT (Bonci et al., 2008; Knapp et al., 2014; Sherman & Thompson, n.d.). Due to its prevalence and severe consequences, FAT deserves substantial research attention.

Past Findings and Future Directions of FAT Research

Previous literature has detailed the three components of FAT, as well as investigated risk factors and causes, prevalence, prevention, long term health consequences, and treatments of FAT (International Olympic Committee Medical Commission Working Group Women in Sport [IOC], 2005). The first component of FAT is malnutrition due to disordered eating. Athletes' disordered eating can be unintentional (i.e., athletes mistakenly underestimate their caloric needs) or intentional (i.e., athletes engage in dieting or have clinical eating disorders; Sherman & Thompson, n.d.). Disordered eating may arise from athletes' intention to perform better in their

sport; however, it does not usually occur as a direct result of sport participation, and affected athletes would most likely practice disordered eating under other circumstances (Sherman & Thompson, n.d.). Disordered eating is often considered the precipitating factor of FAT, as malnutrition in young females can lead to amenorrhea, which then causes low BMD (Sherman & Thompson, n.d.)

The second component of FAT, amenorrhea, is the cessation of menstruation for at least three consecutive months. Amenorrhea can occur as a result of various circumstances, including pregnancy, abnormal hormone levels, tumors of the pituitary gland, and steroid use (Sherman & Thompson, n.d.). In the case of FAT, athletes experience amenorrhea due to low energy availability, as a result of disordered eating. Chronic energy deficiency, caused by inadequate dietary intake, excessive exercise, or both, interferes with hormones that are involved in reproductive development and functioning (IOC, 2005).

Low BMD, the third FAT component, is a result of the two preceding factors. Bone minerals, such as calcium and phosphate, deteriorate quickly in the absence of adequate nutrition and estrogen, which are both essential for healthy bone growth (Sherman & Thompson, n.d.). Low BMD occurs when the rate of bone growth is exceeded by that of bone resorption. It is particularly problematic when FAT affects pubescent athletes, during the time when they should be accumulating peak bone mass (IOC, 2005). Bone mass degenerates quickly in amenorrheic athletes; low BMD increases the risk of fractures, and is potentially irreversible (Yeager et al., 1993).

A variety of risk factors contribute to the development of FAT, including dieting, social pressure, belief that low body weight contributes to athletic success, and desire to

gain competitive advantage (Kransdorf, Vegunta, & Files, 2013; Thompson, 2007). Low energy balance due to inadequate caloric intake, amenorrhea, and low BMD have physiological repercussions that impact current athletic performance and lifelong health (Marquez & Molinero, 2013; Zeigler, n.d.). Athletes with FAT experience muscle loss, fatigue, stress fractures, anemia, depression, and infertility (IOC, 2005). Treatment is most successful when implemented by a team of experts, and when intervention occurs early (Lassiter & Watt, 2007; Zeigler, n.d.). However, treatment is difficult; initial prevention of FAT, by means of screenings and pre-participation exams, is the best approach. Nonetheless, research indicates that few athletic institutions implement such assessments. The insufficient use of appropriate prevention and screening techniques highlights the importance of the present topic, and this study took steps to investigate and fill gaps in the current body of knowledge.

FAT Identification Dilemmas

One issue of particular interest is the ability to recognize athletes who are at high risk of developing FAT. Self-report questionnaires are commonly employed as screening tools to identify those at risk of developing eating disorders. However, measures of this sort typically do not consider the unique characteristics of the female athlete population, who may be predisposed to pathological eating attitudes and behaviors and driven by motivation for athletic success (McNulty, Adams, Anderson, & Affenito, 2001). It can be difficult to diagnose eating disorders in athletes because many signs of such pathology can be explained by characteristics of “good athletes,” such as self-discipline, persistence, dedication to training, and high expectations (Kransdorf et al., 2013; Thompson, 2007). McNulty and colleagues (2001) developed a screening tool specifically to identify eating

disorders in female athletes. They demonstrated the discriminant validity of the Female Athlete Screening Tool (FAST) by showing that it accurately differentiates between athletes and non-athletes with and without eating disorders (McNulty et al., 2001). However, to date, there is no evidence that the FAST is a valid indicator of the presence of the three components of FAT.

A Proposition for More Accurate FAT Screening

Accurate detection of FAT using biomarkers is time consuming and expensive. It is not realistic to implement such intensive screening for all athletes. Administering the FAST to athletes could be an ideal solution to this dilemma. In the present study, the manifestation of the symptoms of FAT was compared to outcomes of the FAST. If the FAT components occurred in correlation with above-threshold FAST scores, then there would be evidence that the FAST is an accurate tool for predicting FAT among athletes. The FAST takes a relatively short time to complete and interpret, and it is available at no cost to the public. This protocol would facilitate the FAT screening process. In turn, more efficient detection of FAT would allow early intervention, which is critical in the recovery process. This study carries significant implications for individual athletes and sports institutions at large; perhaps the validation of such a screening tool would lead to more frequent utilization of FAT assessments and pre-participation exams.

In summary, the purpose of this study was to investigate the co-occurrence of FAT physical symptoms and above-threshold scores on the FAST. The independent variables were the three biomarkers of FAT, including malnutrition, which was measured via plasma levels of Prealbumin; amenorrhea, which was measured via self-reported menstrual history; and low BMD, which was measured via plasma levels of collagen C-

terminal telopeptide (CTx). The dependent variable was the value of participants' FAST scores; a score of 79 or higher is considered above-threshold and suggests the occurrence of disordered eating patterns. Data, including FAST scores and physiological conditions, were collected from 40 athletic females from one National Collegiate Athletic Association (NCAA) Division I university in the Southcentral United States to determine the FAST's efficacy in accurately indicating the presence of FAT in female athletes.

The goal of this research was to determine if the FAST is a valid indicator of malnutrition, amenorrhea, and low BMD, which are key, and often "silent," symptoms of FAT. The researcher hypothesized that FAST scores would positively correlate with the three components of FAT. It was postulated that abnormalities in the three biomarkers under study (plasma Prealbumin and CTx levels and menstrual history) would predict higher FAST scores.

Literature Review

Female Athlete Triad (FAT) is a complicated condition that arises from interactions between biological, psychological, and social factors (Bonci et al., 2008); therefore, relevant research has been conducted in a variety of academic domains, including Psychology, Nutrition, and Exercise Science, among others. FAT is characterized by the presence of three physiological conditions: low energy availability, amenorrhea, and low bone mineral density (BMD). Low energy availability is often the triggering factor, which consequently leads to amenorrhea, and finally low BMD (Sherman & Thompson, n.d.). FAT is an important topic to explore because it affects many young female athletes and has the potential to cause dramatic, and sometimes irreversible, long-term health consequences.

Previous Research

Preceding literature reveals that the components of FAT are more rampant among athletes than non-athletes, particularly athletes who are involved in thin-build, aesthetic, or endurance sports (Barrack et al., 2013; Thompson, 2007). The true prevalence of FAT is unknown, but it is estimated that disordered eating affects up to 35.4% of athletes, menstrual dysfunction affects up to 54% of athletes, and low BMD affects up to 22% of athletes (Barrack et al., 2013). The occurrence of amenorrhea during adolescence may have implications for future bone and reproductive health. Lack of normal menstruation may prevent females from reaching peak bone mass, which can cause increased susceptibility to bone injuries, osteopenia, or osteoporosis (IOC, 2005). Further, even after the resumption of normal menstruation, bone mass may not fully recover (Barrack et al., 2013; Ziegler, n.d.). In addition, a persistent absence of progesterone, due to

amenorrhea, may cause females to experience infertility in the future (IOC, 2005). The significant number of female athletes affected, and the severity of its long-term effects, demonstrate the importance of continuing to study FAT.

Each of the FAT variables exist on a continuum of severity that ranges from health to disease (Nattiv, Loucks, Manore, Sanborn, Sundgot-Borgen, & Warren, 2007). Low energy availability may be unintentional, deliberate, or pathological (Nattiv et al., 2007). Intentional low energy intake can be a result of abnormal eating behaviors, subclinical disordered eating, or clinical eating disorders, such as Anorexia Nervosa, Bulimia Nervosa, or eating disorders not otherwise specified (EDNOS; IOC, 2005). Prolonged low energy availability can lead to a state of malnutrition, during which physiological functions that are needed for development and health maintenance, such as menstruation and bone growth, are suppressed (IOC, 2005; Nattiv et al., 2007). Various physiological conditions can be determined by examining levels of particular proteins in the blood. Specifically, Prealbumin, has been identified as the earliest bio-indicator of nutritional status, and is considered the primary marker for malnutrition (Beck & Rosenthal, 2002). Low levels of Prealbumin, a plasma protein produced by the liver, are indicative of compromised visceral protein production, which occurs in states of nutrient deficiency (Smith, 2017). Prealbumin levels <15 mg/dL fall below the “normal” range and may indicate an increased risk of poor nutritional status (Beck & Rosenthal, 2002).

The existing literature suggests that the second component of FAT, amenorrhea, occurs when insufficient energy availability disrupts the secretion of luteinizing hormone (LH), which is a critical component in the process of normal follicular development (IOC, 2005). In contrast to previous beliefs, LH levels, and, consequently, menstrual

cycles are not disrupted by exercise alone; rather, abnormal menstruation is a result of the negative energy balance that occurs when there is not an increase in nutritional intake to compensate for exercise energy expenditure (IOC, 2005). Like low energy availability, abnormal menstruation also exists on a continuum of severity, which ranges from irregular menstrual cycles (oligomenorrhea) to persistent absence of cycles for at least three months (amenorrhea; IOC, 2005).

The third component of FAT, low BMD, is generally seen in postmenopausal elderly women. However, it is increasingly seen among young female athletes due to low energy availability and hindered bone development, resulting from inadequate nutrition and irregular menstrual cycles, which disrupt bone growth and maintenance (IOC, 2005). Calcium and phosphate, two minerals stored within bones, are essential for developing and supporting bone strength (“What Causes,” 2016). During nutrition deficits and states of low energy availability, these minerals are reabsorbed from the bones into the blood; they are utilized for other functions elsewhere in the body, and, consequently, bone integrity is compromised (“What Causes,” 2016). Athletes with low BMD are at an increased risk for fractures and other musculoskeletal injuries (IOC, 2005).

Like the two preceding FAT variables, BMD exists on a continuum. Age-appropriate bone growth and development constitute the healthy end of this range; osteopenia and osteoporosis, which are bone loss diseases of increasing severity, respectively, lie at the disease end of this continuum (Nattiv et al., 2007). Osteopenia and osteoporosis occur as a result of increased bone resorption in the absence of compensatory bone formation (Mayo Medical Laboratories, n.d.). In the process of bone resorption, the major component of bone matrix, type I collagen, is degraded into

molecular fragments by proteases that are secreted by osteoclasts; subsequently, the carboxyl end of the collagen peptide, CTx, is secreted into the bloodstream (ClinLabNavigator, 2017; Mayo Medical Laboratories, n.d.). High plasma levels of CTx function as a biomarker for increased bone resorption (i.e., bone loss), and have been used to monitor bone breakdown in patients with low BMD (Lab Tests Online, 2017; Mayo Medical Laboratories, n.d.; Wynn, n.d.). Increased levels of CTx are correlated with osteopenia and osteoporosis (Mayo Medical Laboratories, n.d.). The reference range for “normal” plasma CTx concentration among premenopausal women is 25 to 573pg/mL; levels above the expected range have been seen in patients with osteopenia and osteoporosis (Mayo Medical Laboratories, n.d.).

The importance of detecting FAT has been well-established; however, screening for this condition can be difficult. Prior research reveals that there is considerable overlap between characteristics of “good” athletes, those with disordered eating habits, and those with clinical eating disorders (IOC, 2005; Thompson, 2007). Personality traits of successful athletes, including high self-motivation, high achievement expectations, competitiveness, compulsiveness, perfectionism, and over-compliance are risk factors for eating disorders (IOC, 2005; Macleod, 1998).

The desirability of these traits differs between domains of sport and health. Coaches often expect and celebrate such characteristics of dedicated, hardworking athletes, rather than recognize them as potential signs of pathological behavior that are associated with disordered eating (IOC, 2005). For example, obsessive-compulsive tendencies and high achievement standards are typically seen among individuals with eating disorders; these characteristics may also significantly contribute to successful

athletic performance (IOC, 2005). Additionally, eating disorder patients may engage in excessive exercise, while dedicated athletes may train longer and harder than their teammates and competitors (Sherman & Thompson, n.d.). Eating disorder patients often feel compelled to be perfect; likewise, good athletes strive for excellence in their performances (Sherman & Thompson, n.d.). Furthermore, eating disorder patients often go to great lengths to please others and win their approval; similarly, good, “coachable” athletes are compliant to coaches’ wishes, and are willing to sacrifice their own wellbeing for that of their team (Sherman & Thompson, n.d.). In addition to these predisposing personality traits, female athletes with eating disorder pathology also commonly encounter other contributing factors, including environmental pressures to perform well and maintain an idealized appearance (Bonci et al., 2008; IOC, 2005; Sherman & Thompson, n.d.).

Screening for eating disorders among athletes differs greatly from screening the general population because of these extraneous factors (Knapp et al., 2014). Consequently, female athlete-specific screening tools have been developed to more accurately identify eating disorder pathology among this susceptible population. These specific screening tools are designed to distinguish between female athletes who are practicing typical athletic training behaviors from those who are at risk of disordered eating (Knapp et al., 2014). The Athletic Milieu Direct Questionnaire (AMDQ; Nagel, Black, Leverenz, & Coster, 2000), Brief Eating Disorder in Athletes Questionnaire (BEDA-Q; Martinsen, Holme, Pensgaard, Torstveit, & Sundgot-Borgen, 2014), and Female Athlete Screening Tool (FAST; McNulty et al., 2001) are measures that have

been validated for use with female athletes (Knapp et al., 2014). The FAST is the only tool that can identify subclinical eating disorders (Knapp et al., 2014).

The FAST is a questionnaire that consists of 33 questions and takes roughly 15 minutes to complete. It has been validated in a population of NCAA Division I and Division III female collegiate athletes. It has demonstrated the ability to accurately distinguish among athletes and non-athletes with and without pathological eating behavior (McNulty et al., 2001). Reliability analysis of this measure revealed that it has a high internal consistency (Cronbach's $\alpha = .87$). Correlation analyses revealed that the FAST correlates strongly with the Eating Disorder Examination- Questionnaire (EDE-Q; $r = .60$) and the Eating Disorder Inventory (EDI; $r = .89$; McNulty et al., 2001).

Limitations of Previous Research

Existing literature provides evidence that the FAST is a valid screening tool to use with female athletes to identify eating disorder pathology. However, gaps in the literature fail to address whether or not the FAST is a valid indicator of the three physiological components of FAT. No prior research has investigated whether the FAST could be used as a more time- and cost-effective replacement for thorough physical examination to detect FAT among female athletes.

The Current Study

The present study sought to fill this void in the existing body of knowledge. The purpose of this study was to further demonstrate the validity of the FAST by comparing athletes' scores on this measure to their objective, physiological manifestations of the three components of FAT. An additional objective was to provide evidence that the FAST is a measure capable of accurately identifying disordered eating among varsity and

recreational female collegiate athletes, which could be used as a more efficient substitute for extensive physical examinations.

Hypotheses

Based on previous literature, it was predicted that athletes with one or more of the three physiological manifestations of FAT would produce higher scores on the FAST, which are indicative of subclinical disordered eating (scores of 79 to 94 points) or a clinical eating disorder (scores above 94 points), than their healthy peers. The following hypotheses were evaluated:

1. Participants with insufficient caloric intake (defined by abnormally low levels, <15.0mg/dL, of plasma Prealbumin, a reliable biomarker of malnutrition) will score higher on the FAST than their peers.
2. Participants with amenorrhea (defined by the self-reported absence of normal menstruation for at least three consecutive months) will score higher on the FAST than their peers.
3. Participants with low bone mineral density (defined by abnormally high levels, >573pg/mL, of plasma CTx) will score higher on the FAST than their peers.

Method

Participants

The presents study's population of interest consisted of varsity and recreational female collegiate athletes, who may be at increased risk of developing FAT. A power analysis was conducted to determine how many participants were needed. Four studies (Aerni, Knapp, Anderson, & Trojian, 2014; Folscher, Grant, Fletcher, & Janse van Rensberg, 2015; McNulty et al., 2001; Quatromoni, 2008) were assessed. The studies included 10 comparisons that yielded effect sizes. The mean effect size was $d = 2.75$. With this effect size, having 20 participants per group yields a power of $> .99$, which means there is $>99\%$ chance of detecting a true difference if it exists.

This study was conducted at an NCAA Division I university in the Southcentral United States. A total of 40 female college students, who either participate in varsity intercollegiate athletics ($n = 20, 50\%$) or voluntarily engage in habitual exercise ($n = 20, 50\%$), participated in this study. Of the varsity athletes, there were 9 (22.5%) soccer players, 5 (12.5%) track and field athletes, 2 (5%) track and field and cross country runners, 2 (5%) cheerleaders, 1 (2.5%) tennis player, and 1 (2.5%) volleyball player. Length of participation in the aforementioned sports ranged from 1 to 18 years ($M = 11.45, SD = 4.883$). Participants' ages ranged from 18 to 31 years ($M = 20.28, SD = 2.44$). Participants' Body Mass Index (BMI) ranged from 18.70 to 33.90 kg/m^2 ($M = 23.02, SD = 3.05$). According to established BMI classification standards, the BMIs of all participants fell within the healthy (18-24 kg/m^2), overweight (25-29 kg/m^2), or obese (30-39 kg/m^2) ranges. Participants' body fat percentages ranged from 17.30% to 37.10% ($M = 22.82, SD = 4.51$).

Of the participants, 33 (82.5%) were white, non-Hispanic, 3 (7.5%) were African American; 2 (5%) were Asian American, and 2 (5%) were white, Hispanic. There were 14 (35%) first-year college students, 6 (15%) second-year college students, 7 (17.5%) third-year college students, 10 (25%) fourth-year college students, and 3 (7.5%) fifth (or higher)-year college students. Among the participants, there were 14 (35%) Exercise Science majors, and three (7.5%) Biology majors; the remaining 23 (57.5%) were majors of other fields. All participants identified as heterosexual. There were 19 (47.5%) participants who estimated their total annual family income to be over \$100,000; 7 (17.5%) who estimated between \$75,000 and \$99,999, 7 (17.5%) who estimated between \$50,000 and \$74,999, 5 (12.5%) who estimated between \$35,000-\$49,999, and 1 (2.5%) who estimated less than \$20,000. There was one participant who did not answer this question. Of the participants, 30 (75%) indicated that their socioeconomic status was average, and 10 (25%) indicated that their socioeconomic status was above average.

Measures

The participants completed a Demographics Questionnaire to provide personal background information. Relevant anthropometric data of the participants (i.e., height and weight) was collected at the time of the study by the researcher. Current menstrual status and history was self-reported by participants using a questionnaire. Athlete-specific eating disorder attitudes and behaviors were assessed by the Female Athlete Screening Tool (FAST). The participants' plasma Prealbumin levels were assessed through blood protein analyses as a measure of current nutritional status. Plasma CTx levels were evaluated via blood protein analyses to assess the bone mineral density (BMD) of the participants.

Demographics. Participants were asked to report their age, race/ethnicity, education level, field of study, sexual orientation, socioeconomic status, university sport in which they participate, and length of time they have participated in that sport at any level of competition. See Appendix A.

Anthropometric Data. The researcher measured participants' heights (to the nearest 0.25 inch) with a height rod, and their weights (to the nearest 0.1 pound) with a digital scale. The researcher used the participants' heights and weight measurements to calculate Body Mass Index (BMI). A handheld bioelectrical impedance analysis device was used to obtain participants' body fat percentages.

Menstrual Status Questionnaire. Participants answered three questions pertaining to their current and past menstrual cycles. A sample question of this questionnaire includes, "Have you missed three or more consecutive menstrual cycles within the last year?" See Appendix B for Menstrual Status Questionnaire.

Female Athlete Screening Tool (FAST; McNulty et al., 2001). Participants were asked to complete the FAST. The FAST consists of 33 questions that assess eating disorder attitudes and behaviors. Recognizing that athletes are particularly susceptible to eating disorders because of actions they take to be competitive in their sport, the questions on this measure are "athlete-specific." An example question consists of, "If I were to be injured, I would still exercise even if I was instructed not to do so by my athletic trainer or physician." Questions are rated on a four-point Likert-type scale, from 1 (*Strongly Agree; Frequently*) to 4 (*Strongly Agree; Never*). The FAST demonstrates high internal consistency ($\alpha = .87$; McNulty et al., 2001). The FAST also expresses discriminant validity, in that it distinguishes between athletes with and without eating

disorders, as well as between athletes with eating disorders and non-athletes with eating disorders (McNulty et al., 2001). FAST scores are typically significantly higher among athletes with eating disorders than athletes without eating disorders and non-athletes with eating disorders (McNulty et al., 2001). The FAST demonstrates concurrent validity, as it has a strong association with the total scores of the Eating Disorder Inventory-2 (EDI-2; $r = .60, p < .05$) and the Eating Disorder Examination Questionnaire (EDE-Q; $r = .89, p < .001$; McNulty et al., 2001). In addition, the FAST shows significant correlations with specific subscales of the EDI-2, including drive for thinness ($r = .64$), body dissatisfaction ($r = .76$), ineffectiveness ($r = .66$), interpersonal distrust ($r = .63$), asceticism ($r = .79$), and social insecurity ($r = .71$; McNulty et al., 2001). See Appendix C for the FAST.

Plasma Prealbumin and CTx Blood Assay. Approximately 10mL of venous blood was drawn from each participant using Universal Precautions (Centers for Disease Control and Prevention [CDC], 1996). Blood samples were taken from the cubital or cephalic vein of each participant's arm. See Appendix D for blood sampling protocol.

Following the collection of blood samples, the researcher performed Human Prealbumin and CTx Enzyme-linked immunosorbent assays (ELISA), also known as enzyme immunoassays (EIA), biochemical techniques used mainly in immunology to detect the presence of Prealbumin and CTx, respectively, within samples. See Appendix E for ELISA protocol (Abcam, 2017).

Participation Incentive

Participants were given \$10 cash at the research site after they completed the aforementioned procedures.

Procedures

The study was advertised to students of the university's women's varsity track and field and soccer teams, introductory Biology classes, and the Exercise Science department. Individuals who were interested in volunteering contacted the researcher to enroll in the study. Upon arrival to the university's Exercise Physiology Laboratory, participants were properly consented using an Institutional Review Board (IRB) approved informed consent document (Appendix F). After obtaining consent, participants' heights and weights were measured and recorded. These measurements were used to calculate Body Mass Index (BMI). Participants' body fat percentages were obtained using a handheld bioelectrical impedance analysis device.

Participants completed the Demographics Questionnaire, Menstrual Status Questionnaire, and FAST. The questionnaires took each participant approximately 10 minutes to complete. The top corner of each packet was labeled with a different number between 1 and 40. The researcher drew approximately 10ml of venous blood from each participant's arm into a tube that was labeled with a number that corresponded to the number on the individual's packet to maintain anonymity. Participants were given a debriefing form (Appendix G) and \$10 cash after they completed the aforementioned protocol and before they left the lab.

Following data collection, the researcher performed Human Prealbumin and CTx Enzyme-linked immunosorbent assays (ELISAs) to detect the abundance of Prealbumin and CTx, respectively, within the blood samples. Protein analysis of the blood samples, using the ELISA experimental procedure, occurred in the Exercise Biochemistry lab. The data set was kept in password protected Excel and SPSS files on a secure drive.

Results

Preliminary Analyses

Data was analyzed and interpreted using IBM's SPSS 24 software. Cronbach's alpha of the FAST was calculated to be .91, which indicates that the instrument has excellent internal reliability. To calculate participants' scores on the FAST, all the items answered were summed to create a total score, using the following point system:

Frequently = 4 points; *Sometimes* = 3 points; *Rarely* = 2 points; *Never* = 1 point. Reverse scoring was used for numbers 15, 28, and 32. The mean and standard deviation of the total FAST scores were 91.49 and 14.67, respectively. The scores ranged from 58 to 124, and were interpreted using the following criteria: < 79 = normal; 79 to 94 points = subclinical disordered eating; > 94 points = clinical eating disorder. Using this categorization, 8 participants (20.5%) were in the normal eating condition, 15 (38.5%) were in the subclinical disordered eating condition, and 16 (41%) were in the clinical eating disorder condition.

The results of a One-Way Analysis of Variance, which examined FAST scores based on the type of eating for a manipulation check, indicated that there were differences among the groups, $F(2, 38) = 58.90, p < .001$. Follow-up Scheffe tests indicated that all three groups differed from each other. That is, participants in the normal eating condition ($M = 71.13, SD = 6.66$) scored lower on the FAST than participants in the subclinical disordered eating condition ($M = 88.27, SD = 5.09$) and the participants in the clinical eating disorder condition ($M = 104.69, SD = 9.09$). In addition, participants in the sub-clinical disordered eating condition scored lower on the FAST than participants in the clinical eating disorder condition.

Operational definitions of the three biomarkers under investigation were established. Insufficient caloric intake was objectively identified via abnormally low levels of plasma Prealbumin, a reliable biomarker of malnutrition. Levels < 15.0 mg/dL were considered subthreshold. Amenorrhea was defined by the self-reported absence of normal menstruation for at least three consecutive months without taking birth control medication. Low BMD was defined by abnormally elevated levels (> 570 pg/mL) of plasma CTx, a reliable biomarker of increased bone resorption and low BMD.

Hypotheses Testing

Hypothesis one states that athletes with insufficient caloric intake, manifested by low levels of plasma Prealbumin, will score higher on the FAST than their peers with sufficient caloric intake. To test this hypothesis, the relationship between Prealbumin levels and FAST scores was investigated by comparing the FAST scores of participants with subthreshold Prealbumin levels ($n = 2$, $M = 94.00$, $SD = 4.24$) to those of participants with above-threshold Prealbumin levels ($n = 37$, $M = 86.46$, $SD = 14.80$). This comparison was done with an independent samples t -test. Results indicated that there were not significant differences between FAST scores of the two groups, $t(37) = -.711$, $p = .48$.

Hypothesis two states that athletes with amenorrhea will score higher on the FAST than their peers with regular menstruation. To test this hypothesis, the relationship between amenorrhea and FAST scores was investigated by comparing the FAST scores of participants with amenorrhea ($n = 3$, $M = 81.33$, $SD = 7.64$) to those of participants with normal menstruation ($n = 15$, $M = 89.07$, $SD = 15.98$). This comparison was done

with an independent samples *t*-test. Results indicated that there were not significant differences between FAST scores of the two groups, $t(16) = .805, p = .43$.

Hypothesis three states that athletes with low BMD, manifested by high levels of plasma CTx, will score higher on the FAST than their peers with normal BMD. To test this hypothesis, the relationship between plasma CTx levels and FAST scores were investigated by comparing the FAST scores of participants with increased CTx levels ($n = 9, M = 86.00, SD = 15.36$) to those of participants with normal CTx levels ($n = 30, M = 87.10, SD = 14.53$). This comparison was done with an independent samples *t*-test. Results indicated that there were not significant differences between FAST scores of the two groups, $t(37) = .197, p = .85$.

Exploratory Analyses

After testing the hypotheses, additional statistical analyses were performed to further investigate the data. Pearson correlations were used to determine if there were relationships between participants' total FAST scores and the presence of each of the three biomarkers. Results of the Pearson correlations indicated that there were not significant correlations between total FAST scores and Prealbumin levels ($r[39] = .11, p = .52$), amenorrhea ($r[18] = -.20, p = .43$), or CTx levels ($r[39] = -.06, p = .72$).

Pearson correlations were also used to investigate whether relationships existed between participants' total FAST scores and their anthropometric measurements. Results of the Pearson correlations indicated that there were not significant correlations between total FAST scores and BMI ($r[39] = -.26, p = .12$) or body fat percentage ($r[39] = -.18, p = .26$).

Additionally, differences in biomarker presentation between the two levels of athletic competition (i.e., varsity versus recreational) were investigated. Plasma Prealbumin levels of varsity athletes ($n = 20$, $M = 43.10$, $SD = 19.32$) were compared to those of recreational athletes ($n = 20$, $M = 33.76$, $SD = 14.49$), as shown in Figure 1. This comparison was analyzed with an independent samples t -test. Results indicated that there were not significant differences between plasma Prealbumin levels of the two groups, $t(38) = -1.730$, $p = .09$. Plasma CTx levels of varsity athletes ($n = 20$, $M = 505.77$, $SD = 121.16$) were compared to those of recreational athletes ($n = 20$, $M = 534.29$, $SD = 182.69$), as shown in Figure 2. This comparison was analyzed with an independent samples t -test. Results indicated that there were not significant differences between plasma CTx levels of the two groups, $t(38) = .582$, $p = .564$.

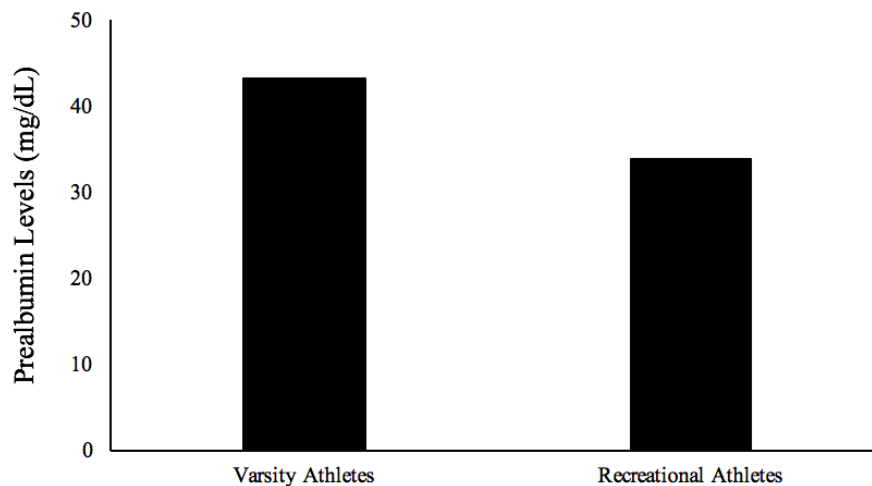


Figure 1: Prealbumin levels of varsity versus recreational athletes

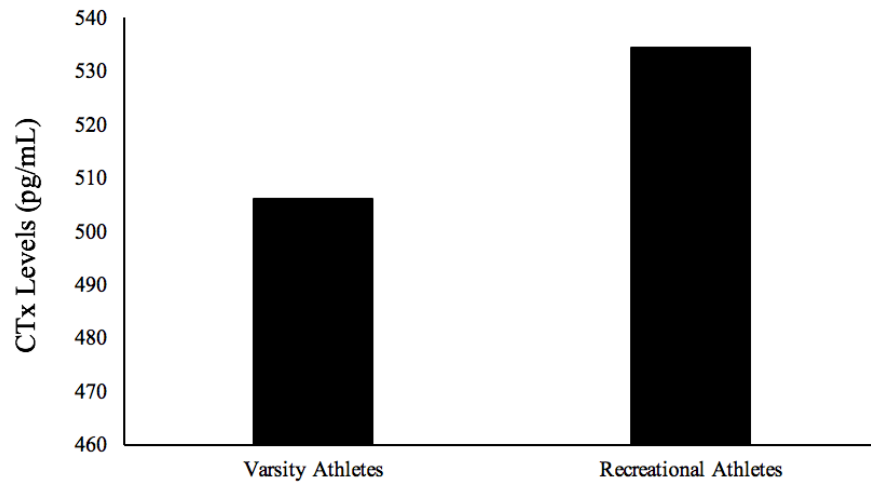


Figure 2: CTx levels of varsity versus recreational athletes

Discussion

Previous literature demonstrates that the athletic females are at an increased risk of developing unhealthy eating and exercise attitudes and behaviors compared to the general population. This information, coupled with knowledge of the negative health consequences that often result from disordered eating such as malnutrition, amenorrhea, and low BMD (the three biomarkers of FAT), makes this area of research critical to restoring, maintaining, and improving the health of current and future female athletes. Some barriers to adequately addressing these issues in the past have included lack of instruments that screen for pathological eating while also accounting for the unique characteristics and lifestyles of athletes, as well as the cost and time constraints that prevent thorough physical examinations to identify the biomarkers of disordered eating. The purpose of the present study was to investigate whether scores on the FAST, a valid measure that was developed specifically for the population under study, are indicative of the presence of the biomarkers that constitute FAT (McNulty et al., 2001). If so, the FAST could be used as a preliminary diagnostic tool that does not require substantial temporal or monetary investments. Such findings would have significant implications because early diagnosis and immediate intervention are crucial to athletes' recovery from FAT and maintenance of their athletic performance and long-term health (IOC, 2005).

The first hypothesis states that athletes with insufficient caloric intake, manifested by low levels of plasma Prealbumin, will score higher on the FAST than their peers with sufficient caloric intake. The second hypothesis states that athletes with amenorrhea will score higher on the FAST than their peers with regular menstruation. The third hypothesis states that athletes with low BMD will score higher on the FAST than their

peers with normal BMD. The three hypotheses were not supported by the results of this study. The results revealed that there were not significant differences between the total FAST scores of participants in each of the groups for any of the three biomarkers (i.e., low versus normal Prealbumin levels; amenorrhea versus normal menstruation; high versus normal CTx levels). The results of this study indicate that there was not a significant correlation between the participants' total FAST scores and the presence of the three biomarkers. This suggests that the FAST is not a valid indicator of the physical components of FAT. Further, there was no relationship found between total FAST scores and body mass and composition.

The current results were unexpected, based on past literature about the factors that contribute to and/or maintain FAT, as well as the content of the FAST (Kransdorf et al., 2013; McNulty et al., 2001; Thompson, 2007). Certain behaviors (e.g., food restriction, unhealthy compensatory behaviors, excessive exercise) and attitudes (e.g., belief that low body weight leads to athletic success, perfectionism, and desire to obtain the ideal “athlete” body) are endorsed by individuals with FAT (Kransdorf et al., 2013; Thompson, 2007). Since these same behaviors and attitudes are included in the contents of the questions on the FAST, it was expected that individuals who endorsed those items, and had a corresponding high total FAST score, would also exhibit the physical symptoms of FAT. It is possible that some individuals in the present study have recently adopted the problematic attitudes and behaviors that they endorsed on the FAST; in this scenario, their bodies may not yet reflect the three biomarkers, because physical deterioration can be delayed in some individuals.

Despite the aforementioned discrepancies, some of the data obtained through this study aligns with, and further strengthens, findings from previous literature. For example, it has been suggested that athletic females constitute a unique population that is at a high risk of developing disordered eating habits due to their unique characteristics (IOC, 2005; Thompson, 2007). The present study implies similar conclusions, since an overwhelming majority of the sample (79.5%) received total FAST scores that fell in the subclinical disordered eating or clinical eating disorder ranges. This suggests that women involved in competitive and/or recreational sports are at a higher risk of developing disordered eating habits or eating disorders than the general population. Additionally, results of this research confirmed that the FAST has excellent internal consistency, and that it is a measure that is appropriate for use among its intended population.

The results of this research further contribute to the existing body of knowledge regarding the development of an effective screening protocol for FAT. Based on the findings from the current study, the null hypothesis was accepted: the FAST does not appear to be a valid stand-alone diagnostic measure for showing the physiological characteristics of FAT. This suggests that the FAST should not be used in lieu of physical examinations to diagnose FAT. If this study's results had been significant, there could have been noteworthy implications for female athletes, especially those involved in institutions such as collegiate athletics, where screening procedures and pre-participation exams are largely ignored (Barrack et al., 2013).

Some limitations of the current study were identified. The sample size was relatively small, and the presence of the three biomarkers was extremely limited among the sample, which is something that could not have been predicted a priori. More

specifically, only two participants had low Prealbumin levels, three had amenorrhea, and nine had elevated CTx levels. Too few participants met criteria for the diagnostic groups, which limited the statistical power. Additionally, in the examination of amenorrhea, data from 22 participants had to be discarded because they reported taking birth control medication to regulate their menstrual cycle. Birth control negates the possibility of amenorrhea; therefore, it is possible that some of these excluded individuals would have met criteria for amenorrhea without the interference of birth control. Furthermore, plasma levels of Prealbumin and CTx can fluctuate throughout the day, and may be affected by exercise. If some of the participants exercised immediately before completing the study, the protein levels in their blood sample may have slightly differed from their normal values. Without these limitations, it is possible that the study would have yielded different results.

Future researchers of this topic may consider the following suggestions to counteract such limitations. A larger sample size should be used to increase the chances of including a greater number of individuals with low Prealbumin levels, amenorrhea, and elevated CTx levels. Future studies may consider targeting a more specific sample of female athletes, who may be more susceptible to developing FAT, such as those who participate in thin-build, aesthetic, or endurance sports (Barrack et al., 2013; Thompson, 2007).

To address the amenorrhea dilemma, researchers could make taking birth control medication a participation exclusion criterion; alternatively, they could modify the Menstrual Status Questionnaire to include additional questions, such as purpose of taking birth control and/or menstrual history prior to taking birth control. Lastly, other methods

could be used to examine biological markers of malnutrition and BMD to account for potential discrepancies in blood protein values. If it is feasible to acquire the resources, a dual-energy X-ray absorptiometry (DXA) machine could be used to perform a more precise assessment of athletes' BMD. Future research may also consider examining other athlete-specific eating disorder screening tools to see if those scores are related to the manifestation of the three components of FAT.

In conclusion, the results of this study demonstrated that there were not significant differences between total FAST scores of participants in each of the groups for any of the three biomarkers (i.e., low versus normal Prealbumin levels; amenorrhea versus normal menstruation; high versus normal CTx levels). Since there was not a significant correlation between the presence of the biomarkers of FAT and total FAST scores, additional research should be conducted before the FAST is used as an FAT diagnostic tool in place of physical examinations. Future research should focus on creating new measures that are capable of simultaneously identifying disordered eating attitudes and behaviors and the presence of FAT. If this can be accomplished, it would be possible to implement a quick, inexpensive FAT screening protocol, diagnose the condition more readily, and provide immediate intervention to those in need. Such a system could prevent long-term negative health consequences and improve the overall future health of female athletes.

References

- Abcam. (2017). *Sandwich ELISA protocol*. Retrieved from <http://www.abcam.com/protocols/sandwich-elisa-protocol-1>
- Aerni, G.A., Knapp, J., Colletto, M., Anderson, J., & Trojian, T. (2014). The effect of a peer-led intervention, the Female Athlete Body Project (FABP), on collegiate Division I female student-athletes, as evaluated by the Female Athlete Screening Tool (FAST) [Abstract]. *Clinics in Sports Medicine*, 24, 168.
- Barrack, M. T., Ackerman, K. E., Gibbs, J. C. (2013). Update on the female athlete triad. *Current Reviews in Musculoskeletal Medicine*, 6, 195-204.
<http://doi.org/10.1007/s12178-013-9168-9>
- Beck, F., & Rosenthal, T. (2002). Prealbumin: A marker for nutritional evaluation. *American Family Physician*, 65, 1575-1579.
- Bonci, C. M., Bonci, L. J., Granger, L. R., Johnson, C. L., Malina, R. M., Milne, L. W., ... Vanderbunt, E. M. (2008). National Athletic Trainers' Association position statement: Preventing, detecting, and managing disordered eating in athletes. *Journal of Athletic Training*, 43, 80–108. doi: 10.4085/1062-6050-43.1.80.
- Centers for Disease Control and Prevention. (1996). *Universal precautions for prevention of transmission of HIV and other bloodborne infections*. Retrieved from <http://www.nbcht.org/Resources/CDC%20doc%20%20Universal%20precautions%20for%20Prevention%20of%20Transmission%20of%20HIV.pdf>
- ClinLabNavigator. (2017). *C terminal telopeptide measures bone resorption*. Retrieved from <http://www.clinlabnavigator.com/c-terminal-telopeptide.html>

- Folscher, L., Grant, C. C., Fletcher, L., & Janse van Rensburg, D. C. (2015). Ultra-marathon athletes at risk for the Female Athlete Triad. *Sports Medicine - Open, 1*, 29. doi: 10.1186/s40798-015-0027-7
- International Olympic Committee Medical Commission Working Group Women in Sport (2005). *Medical commission position stand on the Female Athlete Triad*. Retrieved from http://www.olympic.org/documents/reports/en/en_report_917.pdf
- Knapp, J., Aerni, G., & Anderson, J. (2014). Eating disorders in female athletes: Use of screening tools. *Current Sports Medicine Reports, 13*, 214-218. doi: 10.1249/JSR.0000000000000074.
- Kransdorf, L. N., Vegunta, S., & Files, J. A. (2013). Everything in moderation: What the Female Athlete Triad teaches us about energy balance. *Journal of Women's Health, 22*, 790-792. doi:10.1089/jwh.2013.4515
- Lab Tests Online. (2017). *Bone markers*. Retrieved from <https://labtestsonline.org/understanding/analytes/bone-markers/tab/all/>
- Lassiter, J. W., & Watt, C. A. (2007). Student coaches' knowledge, attitudes, skills, and behaviors regarding the Female Athlete Triad. *Physical Educator, 64*, 142-151.
- Macleod, A. D. (1998). Sport psychiatry. *Australian & New Zealand Journal of Psychiatry, 32*, 860-866. <https://doi.org/10.3109/00048679809073876>
- Márquez, S., & Molinero, O. (2013). Energy availability, menstrual dysfunction and bone health in sports; An overview of the Female Athlete Triad. *Nutrición Hospitalaria, 28*, 1010-1017. doi:10.3305/nh.2013.28.4.6542.

Martinsen, M., Holme, I., Pensgaard, A., Torstveit, M., & Sundgot-Borgen, J. (2014).

The development of the Brief Eating Disorder in Athletes Questionnaire (BEDA-Q). *Medicine & Science in Sports & Exercise*, *46*, 1666-1675. doi:

10.1249/MSS.0000000000000276.

Mayo Medical Laboratories (n.d.). *Test ID: CTX*. Retrieved from

<https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/83175>

McNulty, K.Y., Adams, C. H., Anderson, J. M., & Affenito, S. G. (2001). Development and validation of a screening tool to identify eating disorders in female athletes.

Journal of the American Dietetic Association, *101*, 886-892.

[https://doi.org/10.1016/S0002-8223\(01\)00218-8](https://doi.org/10.1016/S0002-8223(01)00218-8)

Nagel, D., Black, D., Leverenz, L., & Coster, D. (2000). Evaluation of a screening test

for female college athletes with eating disorders and disordered eating. *Journal of Athletic Training*, *35*, 431-440.

Nattiv, A., Loucks, A. B., Manore, M. M., Sanborn, C. F., Sundgot-Borgen, J., &

Warren, M. P. (2007). American College of Sports Medicine position stand: The Female Athlete Triad. *Medicine & Science in Sports & Exercise*, *39*, 1867-1882.

<https://doi.org/10.1249/mss.0b013e318149f111>

Quatromoni, P. A. (2008). Clinical observations from nutrition services in college

athletics. *Journal of The American Dietetic Association*, *108*, 689-694.

<https://doi.org/10.1016/j.jada.2008.01.008>

- Sherman, R., & Thompson, R. (n.d.). *Managing the Female Athlete Triad*. Retrieved from <http://www.femaleathletetriad.org/wp-content/uploads/2008/10/NCAA-Managing-the-Female-Athlete-Triad.pdf>
- Smith, S. H. (2017). Using albumin and prealbumin to assess nutritional status. *Nursing*, 47, 65-66. doi: 10.1097/01.NURSE.0000511805.83334.df
- Thompson, S. (2007). Characteristics of the female athletic triad in collegiate cross-country runners. *Journal of American College Health*, 56, 129-136. <https://doi.org/10.3200/JACH.56.2.129-136>
- What Causes Bone Loss? (2016). In *MedlinePlus Medical Encyclopedia online*. Retrieved from <https://medlineplus.gov/ency/patientinstructions/000506.htm>
- Wynn, R. L. (n.d.). *Recent data on the value of the CTX test as predictive of osteonecrosis of the jaw bone in patients exposed to bisphosphonates*. Retrieved from https://www.lexi.com/individuals/dentistry/newsletters.jsp?id=april_10
- Yeager, K., Agostini, R., Nattiv, A., & Drinkwater, B. (1993). The Female Athlete Triad: Disordered eating, amenorrhea, osteoporosis. *Medicine & Science in Sports & Exercise*, 25, 775-777. <http://psycnet.apa.org/doi/10.1249/00005768-199307000-00003>
- Zeigler, T. (n.d). *The Female Athlete Triad*. Retrieved from http://www.sportsmd.com/SportsMD_Articles/cid/12/id/367/n/the_female_athlete_triad.aspx#sthash.kgtGk7VT.K81ixjfH.dpbs

Appendix A: Demographics

1. Age: _____
2. Race/Ethnicity:
 - a. African American
 - b. Asian American
 - c. White, non-Hispanic
 - d. White, Hispanic
 - e. Middle Eastern
 - f. Other: _____
3. Current Academic Status:
 - a. First-year college student
 - b. Second-year college student
 - c. Third-year college student
 - d. Fourth-year college student
 - e. Fifth (or higher)-year college student
4. College Academic Major: _____
5. Sexual Orientation:
 - a. Heterosexual
 - b. Homosexual
 - c. Bisexual
 - d. Other: _____
6. Estimate your family's combined yearly total income:
 - a. Less than \$20,000

- b. \$20,000 - \$34,999
- c. \$35,000 - \$49,999
- d. \$50,000 - \$74,999
- e. \$75,000 - \$99,999
- f. Over \$100,000

7. How would you describe your current socioeconomic status?

- a. Above average
- b. Average
- c. Below average

8. Western Kentucky University sport in which you participate: _____

9. Years of participation in the abovementioned sport (at any competition level): _____

Appendix B: Menstrual Status Questionnaire:

1. At what age did you have your first menstrual period?
2. Have you missed three or more consecutive menstrual cycles within the last year?
3. Do you (or have you previously) taken birth control to regulate your menstrual cycles?

Appendix C: Female Athlete Screening Tool

RESEARCH

Please answer as completely as possibly:

Key: Exercise = Physical activity \geq 20 minutes
 Practice = Scheduled time allotted by coach to work as a team or individually in order to improve performance.
 Training = Intense physical activity. The goal is to improve fitness level in order to perform optimally.

1. I participate in additional physical activity \geq 20 minutes in length on days that I have practice or competition.
1)Frequently 2)Sometimes 3)Rarely 4)Never
2. If I cannot exercise, I find myself worrying that I will gain weight.
1)Frequently 2)Sometimes 3)Rarely 4)Never
3. I believe that most female athletes have some form of disordered eating habits.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
4. During training, I control my fat and calorie intake carefully.
1)Frequently 2)Sometimes 3)Rarely 4)Never
5. I do not eat foods that have more than 3 grams of fat.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
6. My performance would improve if I lost weight.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
7. If I got on the scale tomorrow and gained 2 pounds, I would practice or exercise harder or longer than usual.
1)Frequently 2)Sometimes 3)Rarely 4)Never
8. I weigh myself _____.
1)Daily 2)2 or more times a week
3)Weekly 4)Monthly or less
9. If I chose to exercise on the day of competition (game/meet), I exercise for
1)2 or more hours 2)45 minutes to 1 hour
3)30 to 45 minutes 4)Less than 30 minutes
10. If I know that I will be consuming alcoholic beverages, I will skip meals on that day or the following day.
1)Frequently 2)Sometimes 3)Rarely 4)Never
11. I feel guilty if I choose fried foods for a meal.
1)Frequently 2)Sometimes 3)Rarely 4)Never
12. If I were to be injured, I would still exercise even if I was instructed not to do so by my athletic trainer or physician.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
13. I take dietary or herbal supplements in order to increase my metabolism and/or to assist in burning fat.
1)Frequently 2)Sometimes 3)Rarely 4)Never
14. I am concerned about my percent body fat.
1)Frequently 2)Sometimes 3)Rarely 4)Never
15. Being an athlete, I am very conscious about consuming adequate calories and nutrients on a daily basis.
1)Frequently 2)Sometimes 3)Rarely 4)Never
16. I am worried that if I were to gain weight, my performance would decrease.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
17. I think that being thin is associated with winning.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
18. I train intensely for my sport so I will not gain weight.
1)Frequently 2)Sometimes 3)Rarely 4)Never
19. During season, I choose to exercise on my one day off from practice or competition.
1)Frequently 2)Sometimes 3)Rarely 4)Never
20. My friends tell me that I am thin but I feel fat.
1)Frequently 2)Sometimes 3)Rarely 4)Never
21. I feel uncomfortable eating around others.
1)Frequently 2)Sometimes 3)Rarely 4)Never
22. I limit the amount of carbohydrates that I eat.
1)Frequently 2)Sometimes 3)Rarely 4)Never
23. I try to lose weight to please others.
1)Frequently 2)Sometimes 3)Rarely 4)Never
24. If I were unable to compete in my sport, I would not feel good about myself.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
25. If I were injured and unable to exercise, I would restrict my calorie intake.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
26. In the past 2 years I have been unable to compete due to an injury
1)7 or more times 2)4 to 6 times
3)1 to 3 times 4)No significant injuries
27. During practice I have trouble concentrating due to feelings of guilt about what I have eaten that day.
1)Frequently 2)Sometimes 3)Rarely 4)Never
28. I feel that I have a lot of good qualities.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
29. At times I feel that I am no good at all.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
30. I strive for perfection in all aspects of my life.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
31. I avoid eating meat in order to stay thin.
1)Strongly Agree 2)Agree 3)Disagree 4)Strongly Disagree
32. I am happy with my present weight.
1)Yes 2)No
33. I have done things to keep my weight down that I believe are unhealthy.
1)Frequently 2)Sometimes 3)Rarely 4)Never

FIG. Female Athlete Assessment Tool (FAST)[®]

Appendix D: Blood Sampling Protocol

1. Wrap a tourniquet around the patient's upper arm to stop blood flow.
2. Sterilize the puncture site with alcohol
3. Insert the needle into the vein with the bevel up
4. Attach the appropriate test tube to the needle. Allow the blood to fill the test tube.
5. Remove the tourniquet to restore blood flow
6. Place a gauze pad over the site while withdrawing the needle
7. Apply firm pressure to the site until bleeding has stopped.

Appendix E: ELISA Protocol

Coating with capture antibody

1. Coat the wells of a PVC microtiter plate with the capture antibody at 1–10 µg/mL concentration in carbonate/bicarbonate buffer (pH 9.6).

Unpurified antibodies (eg ascites fluid or antiserum) may require increased concentration of the sample protein (try 10 µg/mL) to compensate for the lower concentration of specific antibody.

2. Cover the plate with adhesive plastic and incubate overnight at 4°C.
3. Remove the coating solution and wash the plate twice by filling the wells with 200 µL PBS. The solutions or washes are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

Blocking and adding samples

1. Block the remaining protein-binding sites in the coated wells by adding 200 µL blocking buffer (5% non-fat dry milk/PBS) per well.
2. Cover the plate with adhesive plastic and incubate for at least 1–2 h at room temperature or overnight at 4°C.
3. Wash the plate twice with 200 µL PBS.
4. Add 100 µL of diluted samples to each well. Always compare signal of unknown samples against those of a standard curve. Run standards (duplicates or triplicates) and blank with each plate. Incubate for 90 min at 37°C.

Ensure concentration of standards spans the most dynamic detection range of antibody binding. You may need to optimize the concentration range to obtain a suitable standard curve. Always run samples and standards in duplicate or triplicate.

5. Remove samples and wash the plate twice with 200 μ L PBS.

Incubation with detection and secondary antibody

1. Add 100 μ L of diluted detection antibody to each well.

Check that the detection antibody recognizes a different epitope on the target protein to the capture antibody. This prevents interference with antibody binding. Use a tested matched pair whenever possible.

2. Cover the plate with adhesive plastic and incubate for 2 h at room temperature.

3. Wash the plate four times with PBS.

4. Add 100 μ L of conjugated secondary antibody, diluted in blocking buffer immediately before use.

5. Cover the plate with adhesive plastic and incubate for 1–2 h at room temperature.

6. Wash the plate four times with PBS.

Detection

1. Dispense 100 μ L of the substrate solution per well with a multichannel pipette.

2. Add 100 μ L of stop solution (either Horse radish peroxidase [HRP] or alkaline phosphatase [ALP]) to the wells.

3. Read the absorbance of each well with a plate reader.

Data Analysis

1. With concentration (log scale) on the X-axis and Absorbance (linear) on the Y-axis, create a standard curve using the data obtained from the serial dilutions.

2. Interpolate the concentration of the sample from the curve.

Appendix F: Informed Consent Document



INFORMED CONSENT DOCUMENT

Project Title: Correlation Between Female Athlete Screening Tool (FAST) Scores and Biomarkers to Identify Female Athlete Triad Among Collegiate Athletes and to Evaluate the Validity of the Instrument
Investigator: Lindsey Hinken, B. S.; Western Kentucky University Psychology
lindsey.hinken301@topper.wku.edu

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

You must be 18 years old or older to participate in this research study.

The investigator will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you may have.

If you then decide to participate in the project, please sign this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

1. **Nature and Purpose of the Project:** The purpose of this project is to evaluate whether the Female Athlete Screening Tool (FAST) is a valid indicator of malnutrition, amenorrhea, and low Bone mineral density, and determine if it can be reliably used for FAST among female collegiate athletes.

2. **Explanation of Procedures:** Following consent for participation, you will be asked to attend one session to complete the study that will take approximately 45 minutes. During the session, you will be asked to complete three short questionnaires, and a researcher will measure your height and weight and draw a small amount of blood from your arm. After you complete all of the aforementioned requirements, you will receive \$10 cash for your participation before you leave the session.

3. **Discomfort and Risks:** The venipuncture blood collection process can cause minor bruising around the sample area and slight discomfort during the sampling procedure. You will be monitored during and after testing, and testing will be terminated if you exhibit adverse signs/symptoms such as the onset of chest pains, lightheadedness, confusion, pallor, nausea, or cold, clammy skin, or if you feel for any other reason you need/want to stop. In case of accident or illness, a CPR certified individual will provide proper care, until emergency medical services personnel arrive.

WKU IRB# 18-160 Approval - 11/9/2017 End Date - 5/10/2018 Expedited Original - 11/9/2017
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4. **Benefits:** Information obtained from this study could benefit you and your peers by promoting the implementation of more efficient and effective Female Athlete Triad screening protocols within collegiate athletics. Additionally, you will receive \$10 for your participation in this study.

5. **Confidentiality:** Any of your personal information that is obtained during this research will be kept confidential. All records related to your involvement in this study will be stored in a locked file cabinet. Your information will be associated with a randomly assigned number rather than your name. The information connecting your number with your identity will be stored apart from the research records. Individuals names will not be used in any publications that result from this research.

6. **Refusal/Withdrawal:** Refusal to participate in this study will have no effect on any future services you may be entitled to from the University. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks.

Signature of Participant

Date

Witness

Date

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT
THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY
THE WESTERN KENTUCKY UNIVERSITY INSTITUTIONAL REVIEW BOARD
Paul Mooney, Human Protections Administrator
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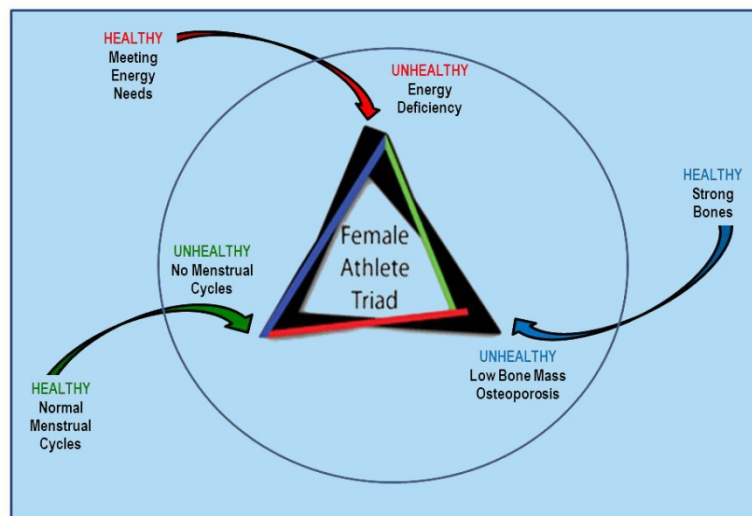
Appendix G: Debriefing

Thank you for participating in this study. All of the information received from this survey will be kept confidential. The purpose of this project is to evaluate whether the FAST is a valid indicator of the three symptoms of Female Athlete Triad, and determine if it can be reliably used for screening of this condition among female collegiate athletes. This study could benefit female collegiate athletes by providing university athletic departments with a cost and time-effective screening method. This could reduce the development and subsequent negative health consequences that occur as a result of this condition.

Female Athlete Triad is a serious condition effectively treated through a multidisciplinary team of health care professionals, i.e., medical doctor, psychologist, and nutritionist; and the support of family, teammates, and coaches. If treated in a timely manner, most athletes can successfully restore their health and establish new healthy eating and exercise habits. With restored energy and strength, many athletes experience improved performance.

The occurrence of Female Athlete Triad is not uncommon, especially among competitive athletes at the high school and collegiate level. If you believe that Female Athlete Triad currently affects you or someone you know, please find more information at the resources provided below and seek medical attention as soon as possible.

If you would like more information about this study or have additional questions, please contact Lindsey Hinken (lindsey.hinken301@topper.wku.edu) or her advisor Dr. Rick Grieve (rick.grieve@wku.edu).



More information can be found at the following link:
<https://www.acsm.org/docs/brochures/the-female-athlete-triad.pdf>