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Assessment and identification of health problems of female athletes

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ABSTRACT

Female athletes tend to be susceptible to health problems related to the physical stress of exercise and suboptimal nutrition habits. In 1992, The American College of Sport Medicine identified the Female Athlete Triad (disordered eating, amenorrhea, osteoporosis) as an increasing health problem among female athletes.

Weight-bearing exercise stimulates bone formation, which in a healthy state exceeds bone breakdown and increases the bone mass density and bone mineral content. However, bone breakdown from excessive exercise may exceed bone formation, thus contributing to bone loss. Bone loss may also be accelerated by the physical impact of sports; higher impact sports tend to have greater bone turnover and risk for bone loss. In addition to the triad, many female athletes suffer from iron deficiency. Iron is an essential mineral for iron-dependent enzymes in collagen formation, thus may play a role in bone health. Decreased bone mass can result in stress fractures and place the athlete at risk for osteoporosis later in life.

This study examined sport impact and bone status in 117 collegiate female athletes while developing a model to predict stress fracture risk. Secondly, it examined the relationship between iron status and bone health in collegiate female athletes.

Data from this study suggest that bone resorption is greater ($P<0.05$) in high impact sports than low impact sports. It also introduces a model, which suggests that high impact sports together with a low bone formation to breakdown ratio (<8) are predictive of increased probability of stress fractures ($P<0.05$).

The data also suggest that iron status is negatively associated with bone formation ($P<0.05$) and positively associated with bone breakdown ($P<0.05$). Subjects with iron depletion have significantly higher bone breakdown and subjects with iron deficiency have significantly

higher bone formation than other categories of iron status. Thus, this study contradicts a previously postulated theory that adequate iron promotes bone formation.

It is recommended that sport impact be included when assessing bone status and stress fractures in female athletes. The negative relationship between tissue iron status indicators and bone turnover warrants further research. Finally, future research needs to identify bone markers that are both valid and reliable with standardized reference values to facilitate the comparison of bone status among studies.

CHAPTER 1. INTRODUCTION

Until the early 19th century, athletic activities were believed to compromise a woman's ability to reproduce. In fact, athleticism for women was socially unacceptable because it was believed that it compromised her ability to serve as a good housewife (Vertinsky, 1994). Societal beliefs have evolved and women are participating in athletics in growing numbers (Costa & Guthrie, 1994). A significant catalyst for this evolution was World War II, where women served in roles that were typically considered male roles (Hult, 1994). The health benefits of hard, laborious work were observed, and facilitated a change in attitudes.

Exercise programs, including athletic teams for women, grew from evolution and women's athletics began its ascent. Women participating in the Olympic games comprised 2% of all athletes in 1900 compared to 38% in 2000 (International Olympic Committee, 2005). Athleticism among women is now a respected and promoted activity that fosters female health.

While the upsurge in the number of women that are active in some form of athletic activity is positive, there are potential health implications. The physical impact of intense and rigorous exercise on women needs to be monitored, particularly on pubertal maturation (De Souza et al., 1994). In 1992, the American College of Sports Medicine affirmed this by defining a series of interrelated health considerations for female athletes called the female athlete triad (disordered eating, amenorrhea, osteoporosis) (Yeager et al., 1993).

The Female Athlete Triad

The female athlete triad is a health concern consisting of three interrelated problems including, eating disorders/disordered eating, amenorrhea, and osteoporosis (Otis et al., 1997). The triad negatively impacts short term and long-term morbidity, performance, and even mortality (Eichner, 1992; Otis et al., 1997). While more research is needed for an accurate assessment of the prevalence, nutrition researchers believe that the prevalence is high and must be a priority for continued research (Otis et al., 1997). In addition to the triad, researchers believe iron deficiency also should be monitored because of its relationship with

disordered eating, bone health, and physical performance (Brownlie et al., 2004; Eichner, 1992; Harris et al., 2003).

Disordered Eating

Disordered eating is defined as abnormal food and eating behavior, which can involve starving, bingeing, vomiting, laxative abuse, or excessive exercise in combination with poor nutrition, distorted body image, and psychological or developmental abnormalities (Spear, 2004). Disordered eating can develop in female athletes exposed to pressure from parents and coaches to maximize performance. Another significant contributor to disordered eating is societal and peer pressure stressing a thin physical appearance. These pressures distort the athlete's perception of a healthy body image and initiate disordered eating (Otis et al., 1997).

While more accurate measures are needed to assess the prevalence of eating disorders, current literature suggests that 2 to 20% of women aged 15 to 39 are impacted by eating disorders (Beals & Manore, 2002; Sundgot-Borgen & Torstveit, 2004). Disordered eating includes anorexia nervosa, bulimia nervosa, and eating disorders not otherwise specified (EDNOS) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 1994). Eating disorders have a negative impact on health and can result in unhealthy weight fluctuations and/or body composition (Otis et al., 1997). Loss of essential body fat and/or inadequate caloric intake can cause dysfunction of the pituitary gland, ovaries, uterus, hypothalamus, and cause hypoestrogenemia analogous to menopause known as amenorrhea (Calabrese et al., 1983; Drinkwater et al., 1984; Nelson et al., 1986; Yen, 1986).

Amenorrhea

Amenorrhea is typically caused by delayed menarche (De Souza et al., 1994), caloric restriction (Calabrese et al., 1983), anorexia nervosa, and/or excessive exercise (Malina, 1983). There are two categories of amenorrhea, primary and secondary. Primary amenorrhea is defined by the absence of menstruation by the age of 16. Secondary amenorrhea is defined as the lack of 3 or more consecutive menstrual cycles following menarche (Sakala, 1999). Although the prevalence of all female athletes is unknown, research indicates that 6-79% of female athletes suffer from menstrual dysfunction (Abraham et al., 1982; Beals & Manore,

2002; Cohen et al., 1982). Estrogen is an important hormone of the reproductive system that is decreased as a result of decreased gonadotropin releasing hormone (GnRH) from the hypothalamus. Decreased GnRH depresses luteinizing hormone (LH) stimulus on the ovaries, which can result in hypothalamic amenorrhea. Estrogen plays an important role in bone formation, thus hypoestrogenemia places the athlete at risk for osteoporosis (De Souza et al., 1994).

Osteoporosis

Osteoporosis is a serious disease that affects 10 million Americans each year and an additional 34 million are at risk (National Osteoporosis Foundation, 2004). The disease results from structural deterioration of bone, which weakens the bone and increases the risk for bone fractures. In athletes, a combination of excessive exercise, inadequate dietary intakes, and amenorrhea can impede the development of peak bone mineral density in adolescents and/or increase bone loss in adults. The decrease in bone mineral density places female athletes at greater risk for stress fractures (Warren et al., 1986) and long-term osteoporosis (Otis et al., 1997). Bone consists of living cells known as osteoblasts and osteoclasts, which are responsible for bone formation and breakdown, respectively. If breakdown exceeds formation, bone mineral density decreases. Decreased bone mineral density has been reported in women with iron deficiency, thus relating iron status to bone health (Harris et al., 2003).

Iron Deficiency

Iron deficiency is the most prevalent nutritional deficiency in the world and affects 31% of female athletes (Risser et al., 1988). Iron deficiency in female athletes is predominantly caused by inadequate dietary intake, increased blood loss (menstruation, gastrointestinal bleeding, sweat, and foot-strike hemolysis with exercise), and iron malabsorption (Cowell et al., 2003).

Iron is an essential micronutrient found in the protein structure of hemoglobin (Hb), myoglobin, iron-dependent enzymes, and respiratory chain proteins (Haas & Brownlie, 2001). Thus, iron plays an important role in binding and transporting oxygen to tissues and

oxidative energy production, both of which impact physical performance (Davies et al., 1984; Brownlie et al., 2002; Zhu et al., 1997; Haas et al., 2001). In addition, iron plays a role in the prevention of aging, reduction of neurological damage and dysfunction (Atamna et al., 2002), and immune function (Ahluwalia et al., 2004).

Conclusion

Considering the consequences of the female triad and iron deficiency, it is critical that screening tools for identification of the triad are identified. Predictors for psychological disorders, menstrual dysfunction, and stress fractures in female athletes are needed to intervene and prevent the development of the female athlete triad. While screening tools are used to assess the prevalence of eating disorders, and biochemical indices are readily used to assess iron and hormonal status and bone turnover, they diagnose an existing problem and are inadequate for preventive measures. The goal of this research was to identify a screening tool to detect those who were at risk for health problems associated with the triad and the relationship between sport impact, iron status, and bone health. The objectives were:

Research Objectives

- To examine and describe anthropometric indices, biochemical indices, and psychological profiles related to the components of the female athlete triad among collegiate female athletes.
- To examine and describe injuries related to components of the female athlete triad among collegiate female athletes.

Thesis Organization

This thesis includes an introduction, a review of the literature, methods, and two manuscripts, followed by a conclusion and references for the three first chapters.

CHAPTER 2. LITERATURE REVIEW

EATING DISORDERS

Female athletes are exposed to psychological pressures from society and the athletic world (Sundgot-Borgen et al., 2004). Society emphasizes thinness and competitive sport emphasizes optimal performance, which places female athletes at considerable risk for disordered eating (Otis et al., 1997; Sundgot-Borgen et al., 2004). People with a higher body weight range are exposed to social pressures to lose weight and follow a thinner societal norm (Sundgot-Borgen et al., 2004). However, Polivy and Herman (1983) contend that each individual has his/her own healthy body weight range, which is regulated by homeostasis.

Although, the prevalence of eating disorders among all female athletes is unknown, it has been estimated that 2.3% to 3.3% of collegiate female athletes (Beals & Manore, 2002) and 20% of Olympic or elite female athletes suffer from disordered eating/eating disorders (Sundgot-Borgen & Klungland Torstveit, 2004). However, self-reported data and large numbers of athletes meeting some but not all of the criteria for eating disorders, make accurate identification difficult, thus reported prevalence may under represent the actual number of cases of eating disorders.

Although the true number of athletes suffering from eating disorders/disordered eating is unknown, the prevalence is greater in athletes than non-athletes, and is more common among women than men (Sundgot-Borgen, 2004). Numerous female athletes feel the need to lose weight and up to 62% of female athletes engage in weight-control behavior (Black & Burkes-Miller, 1988; Burckes-Miller & Black, 1988).

It has been suggested that the type of competitive sport impacts the development of disordered eating (Byme & McLean, 2002). Disordered eating is particularly common among women who participate in aesthetic sports and sports benefiting from low body weight such as, gymnastics, ballet dancing, and cross country running (Cobb et al., 2003; Beals & Manore, 2002). In athletes who participate in strenuous exercise at a very young age, an eating disorder can be detrimental to growth, and pubertal maturation (Weimann, 2002).

Eating disorders impact over 5% of adolescents, making it the third most common chronic illness in this age group (Fisher et al., 1995; Croll et al., 2002; Leichner, 2002). The

earlier the age of onset of the eating disorder, the greater the health risks associated with eating disorder (Table 1). If not detected and treated, health problems can persist through adolescence and early adulthood (Biller et al., 1989; Mehler, 2002).

Table 1. Health implications related to eating disorders

Health concerns
• Micronutrient deficiency due to purging or starvation • Energy deficiency suppressing mood, endocrine status, growth, reproductive function, bone health, and causing fatigue, anxiety, anger, and irritability. • Stunted growth in adolescents and poor bone development. • Inhibition of gonadotropic hormone (lutenizing hormone, follicle-stimulating hormone) production and ovarian production of the sex hormones estrogen and progesterone. • Menstrual irregularities causing amenorrhea resulting in infertility. • Predisposition to osteoporosis due to low estrogen levels. Athletes with eating disorders have decreased spinal vertebral bone mineral densities compared to normal values, but higher densities than non-athletes with eating disorders (Bennell et al., 1999; Golden, 2002) • The mortality rate of athletes with eating disorders is unknown, however the mortality rate of patients in the U.S. with anorexia nervosa is 1% to 18% (Thompson & Trattner-Sherman, 1993). This could be a result of fluid and electrolyte imbalance, lack of energy, suicide, and/or the abuse of drugs (Sundgot-Borgen & Larsen, 1993)

Definition

Eating disorders are defined by the American Psychiatric Association (Table 2) as abnormal food and eating behavior, which can involve starving, bingeing, vomiting, laxative abuse, or excessive exercise in combination with poor nutrition, distorted body image, and psychological or developmental abnormalities (Spear, 2004). Eating disorders are divided into three major subgroups: anorexia nervosa, bulimia nervosa, and eating disorders not otherwise specified (EDNOS). In 1993, Sundgot-Borgen introduced an additional classification of EDNOS specific to athletes called anorexia athletica.

Anorexia nervosa is recognized as restrictive eating behavior where the individual perceives itself as overweight even though he/she may be 15% below ideal body weight. Whereas, bulimia nervosa is characterized by binge eating followed by purging. Purging often involves vomiting and the use of diuretics, laxatives, enemas, and/or excessive exercise (American Psychiatric Association, 1994).

Table 2. Definitions of Eating Disorders

- **Eating disorders**

Anorexia nervosa

- The refusal of maintaining normal body weight at or above a minimally normal weight for age and height (e.g. weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
- Intense fear of gaining weight, or becoming fat, even though underweight.
- Disturbance in the way in which one's body weight or shape is experienced; undue influence of body weight or shape on self-evaluation; or denial of the seriousness of the current low body weight.
- In postmenarcheal females, amenorrhea, i.e. the absence of at least three consecutive menstrual cycles.
    1. Restricting type: During the current episode of anorexia nervosa, the person has not regularly engaged in binge eating or purging behavior.
    2. Binge eating/purging type: During the current episode of anorexia nervosa, the person has regularly engaged in binge eating and purging behavior.

Bulimia Nervosa

- Repeated episodes of binge eating. An episode of binge eating is characterized by both of the following:
    1. Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances.
    2. A sense of lack of control over eating during the episodes (e.g., a feeling that one cannot stop eating or control what or how much one is eating).
- Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting, misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
- The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.
- Self-evaluation is unduly influenced by body shape and weight.
- The disturbance does not occur exclusively during episodes of anorexia nervosa.
    1. Purging type: During the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.
    2. Nonpurging type: During the current episode of bulimia nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics of enemas.

Eating Disorder not Otherwise Specified (EDNOS)

- EDNOS is the criteria for eating disorders that do not meet the criteria of anorexia nervosa or bulimia nervosa. These are also referred to as disordered eating. EDNOS can include the following examples:
  1. For females, all of the criteria for anorexia nervosa are met except that the individual has regular menses.
  2. All of the criteria for anorexia nervosa are met except that, despite significant weight loss, the individual's current weight is in the normal range.
  3. All of the criteria for bulimia nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur at a frequency of less than twice a week or for a duration of less than 3 months.
  4. The regular use of inappropriate compensatory behavior by an individual of normal body weight after eating small amounts of food.
  5. Repeatedly chewing and spitting out, but not swallowing, large amounts of food.
  6. Binge Eating Disorder (BED): Recurrent episodes of binge eating in the absence of the regular use of inappropriate compensatory behaviors characteristic of bulimia nervosa.

(American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text revision). Washington, DC: APA Press.)

Anorexia Athletica

Competitive athletes are subject to additional stressors related to training and eating patterns, which place them at risk for disordered eating. In addition, athletes are more likely to have a competitive, perfectionistic personality, which places them at risk for anorexia athletica (Sundgot-Borgen, 1993).

Anorexia athletica falls under the category of EDNOS (Fairburn & Harrison, 2003) and has characteristics similar to anorexia nervosa. Characteristics of anorexia athletica include: loss of fat and/or muscle mass for performance, not physical appearance, where calorie reduction is voluntary or as a result of trainers' and coaches' recommendations; weight loss results in lean physique, as a result of increased level of exercise rather than calorie restriction with common occurrences of weight cycling; and eating behavior returns to normal at the end of the athletic career. The characteristics of anorexia athletica may occur infrequently or simultaneously, making it difficult to identify the existence of the disorder.

Most EDNOS (except anorexia athletica) are similar to anorexia nervosa and bulimia nervosa (Fairburn & Harrison, 2003); however, the odds of recovering from an EDNOS are

greater than from anorexia nervosa and bulimia nervosa (Fairburn & Harrison, 2003). All EDNOS can, and often do, develop into clinical eating disorders (Sudi et al., 2004). Therefore, early detection and prevention is imperative.

Identifying an Eating Disorder

Identifying an eating disorder during its early stages of development is critical to treat the problem and prevent the development of the serious health implications it is associated with. Currently, valid and reliable screening tools in the form of questionnaires are used to identify eating disorders/disordered eating in athletes and non-athletes (McNulty et al., 2001). Questionnaires that have been tested for reliability and validity include: the Eating Attitude Test (EAT) (Garner & Garfinkle, 1979), Eating Disorder Examination Questionnaire (Fairburn & Beglin, 1994), Bulimia Test Revised (BULIT-R) (Brelsford et al., 1992), Eating Disorder Inventory-2 (Garner, 1991), Athletic Milieu Direct Questionnaire (Nagel et al., 2000), Physiologic Screening Test (Black et al., 2003), Restraint Eating (Herman & Polivy, 1975), The Dutch Eating Behavior Questionnaire (DEBQ) (van Strien et al., 1986), and Female Athlete Screening Tool (FAST) (McNulty et al., 2001).

Eating Attitude Test (EAT)

The EAT is an index of the symptoms of anorexia nervosa (Garner & Garfinkel, 1979). The survey consists of 40 items presented in a self-reported format and scored on a 6 point Likert scale. The survey has been validated in patients with anorexia nervosa and a control group, where the survey scores were significantly correlated within each group. The reliability coefficient for subjects with anorexia nervosa was α=0.79 and concurrent validity of α=0.87 ($P<0.001$).

Eating Disorder Examination Questionnaire (EDE-Q)

The EDE-Q is a self-report questionnaire based on the Eating Disorder Examination (EDE) interview (Fairburn & Beglin, 1994). The EDE interview focuses on the assessment of core attitudinal and behavioral characteristics of patients with eating disorders. Due to the complexity and length of the EDE interview the EDE-Q was developed. The 36-item questionnaire focuses on behavioral features of eating disorders and subscales, including restraint eating, body shape, and weight concern, which strongly correlated to the EDE interview (Fairburn & Beglin., 1994). The correlations between the EDE-Q and the EDE

subscales range from 0.68 to 0.78 for Eating Concern and Shape Concern, respectively (Mond et al., 2004). The validity coefficients (sensitivity = 0.83, specificity = 0.96, and positive predictive value = 0.56) from the assessment of episodes of bulimia and/or use of exercise to control weight, indicate that the EDE-Q has concurrent validity and can be used in epidemiological studies (Mond et al., 2004).

Bulimia Test Revised (BULIT-R)

The bulimia test survey was developed to measure attitudes and behaviors associated with bulimia nervosa (Welch et al., 1993). The survey consists of 35 items targeting the severity of bulimic behaviors, including body shape and weight preoccupation, feelings, food, laxative/diuretic abuse, and bingeing and purging (Black et al., 2003). Each item is rated on a 5-point Likert scale with scores ranging from 28 to 140. A score of $\geq$102 is indicative of bulimia nervosa (Brelsford et al., 1992). The test is valid with an internal consistency of α=0.92 and a correlation of r = 0.83 between 2 test occasions (Brelsford et al., 1992); however, the external validity is not based on college-aged female athletes (Black et al., 2003).

Eating Disorder Inventory-2 (EDI-2)

The eating disorder inventory was designed to measure cognitive and behavioral characteristics of anorexia nervosa and bulimia nervosa in accordance with the criteria established by the American Psychiatric Association (Garner, 1991). The 91 item-screening test is designed to assess feelings, attitudes, and behaviors common to bulimia and anorexia nervosa. The questionnaire provides 11 subscales pertaining to clinically relevant dimensions. The three most emphasized subscales focus on the drive for thinness, bulimia, and body dissatisfaction (Black et al., 2003). The questionnaire has been found to be reliable for the general population with an internal consistency of α=0.44 to α=0.93, and is widely used by health professionals (Garner, 1991). Unfortunately, the external validity is not based on college-aged female athletes (Black et al., 2003).

Athletic Milieu Direct Questionnaire (AMDQ)

The AMDQ was designed to reflect psychosocial and athletic milieu behavior relevant to weight management, diet, and exercise among female college athletes. The 119-item survey was tested against the EDI-2 and BULIT-R. The AMDQ is unique in its

assessment of EDNOS, whereas the other surveys assess only anorexia nervosa and bulimia nervosa. Five statistic criteria were established to increase the validity and sensitivity of the survey; for example a Cronbach α of at least 0.85 for each item. Of the 119 items, 51 items met the five criteria, which resulted in a sensitivity of 80% compared to the EDI-2 (64%) and BULIT-R (27%) (Nagel et al., 2000). However, validity of the survey in assessing athletes needs further research.

Physiologic Screening Test

The physiologic screening test is a physical screening specifically designed to detect eating disorders/disordered eating in collegiate female athletes engaged in athletic competition or highly athletic performances (Black et al, 2003). The test consists of 18 items, including 8 interview items, 4 physiologic measurements (percent body fat, waist-to-hip ratio, standing diastolic blood pressure, and enlarged parotid gland), and 6 self-reported questionnaire items. The physiologic screening test has been tested against EDI-2 and BULIT-R, and suggests greater sensitivity (87%) versus (27%, and 62%, respectively). The test most closely resembles the AMDQ test; however, physical signs and symptoms used in the physiologic screening test may reduce response bias (Black et al, 2003).

Restraint Eating

Intense dieting is often the consequence of emotional (emotions trigger eating) and external (eating triggered by food stimuli regardless of hunger) eating, which are the components of the Restraint Eating theory (Herman et al, 1975). The Restraint Eating survey was developed to assess the extent to which individuals exhibit behavioral and attitudinal concern about dieting and weight control (Herman & Polivy, 1975). The self-report questionnaire consists of 11 items divided into two subgroups: diet and weight history, and concern with food and eating. The questions are scored with a total sum of ≥ 17 representing restraint eating. Reliability of the survey has been established with an internal consistency of $\alpha = 0.75$, and confirmed concurrent validity has been verified (Herman & Polivy, 1979).

The Dutch Eating Behavior Questionnaire (DEBQ)

The DEBQ was developed to assess restrained, emotional, and external eating behavior to examine the etiology of obesity (van Strien et al., 1986). The DEBQ further

divides emotional eating into eating due to diffused emotions, and eating due to clearly labeled emotions. The DEBQ has a high internal consistency and factorial validity with Cronbach's alpha values for the three scales (restrained, emotional, and external eating) ranging from $\alpha = 0.81$ to $\alpha = 0.95$. Although the internal validity is high, the external validity is uncertain (van Strien et al., 1986). Thus, the accuracy of the DEBQ in an athletic population is questionable.

Female Athlete Screening Tool (FAST)

The FAST survey was developed specifically for use in female athletes (McNulty et al., 2001). Because an excessive exercise regimen can be due to the desire to improve performance and not to lose weight, many self-report questionnaires are insensitive to accurately identify eating disorders in female athletes (McNulty et al., 2001). In contrast, the FAST distinguishes athletes with eating disorders from non-athletes with eating disorders. This self-report survey consists of 32 questions scored on a 4-point Likert scale. A score of 77-94 is classified as subclinical eating disorder and a score >94 is classified as clinical eating disorder. Reliability of the survey has been established with an internal consistency of α=0.88. The survey has been correlated with BULIT-R [concurrent validity (r=0.69, $p<0.0001$)], and 4 related EDI-2 subscales, [bulimia (r=0.35, $p<0.04$), drive for thinness (r=0.70, $p<0.0001$), body dissatisfaction (r=0.48, $p<0.006$), and perfectionism (r=0.48, $p<0.006$)].

Screening tools used in this study

Female athletes require adequate caloric consumption to support the high-energy demands of exercise. Because the motivation for weight loss may be desired for either, or both, physical appearance and athletic performance, identification of behavioral and attitudinal concern about dieting and the desire to obtain a minimal weight for performance is important. For these reasons, the Restraint Eating survey and the FAST were chosen for this study. Restrained eaters may fail to meet their energy requirements due to an eating disorder, which needs to be identified to prevent health implications. The FAST survey was selected to target female athletes who exercise excessively to improve performance and lose weight, and are at risk for developing eating disorders.

AMENORRHEA

Eating disorders often involve low caloric intake, excessive exercise and/or weight loss, which can result in the second component of the female athlete triad, amenorrhea (Costa & Guthrie, 1994). Amenorrhea is the absence or abnormal cessation of the menstrual cycle, which can result from polycystic ovary syndrome, hypothalamic dysfunction, hyperprolactinemia, or ovarian failure (Stedman's Medical Dictionary, 2000). Exercise-induced amenorrhea seen in female athletes is usually the result of hypothalamic dysfunction (Loucks et al., 1989).

Competitive female athletes are three times more likely to suffer from amenorrhea than non-athletes (Warren & Goodman, 2003). Although the prevalence of amenorrhea among all female athletes is unknown, research reports a range from 3% to 66% (Yeager et al., 1993). This broad range may be due to variations in the definition of amenorrhea, error in data collection caused by self-reported data, and/or the age of menarche of the subjects studied (Costa & Guthrie, 1994). To recognize the health risks of exercise-induced amenorrhea it is important to understand the definition of menstrual regularity, hypothalamic function, and the contributing factors including weight loss, delayed menarche, caloric intake, as well as volume and intensity of exercise (Costa & Guthrie, 1994).

Definition of menstrual regularity

Eumenorrhea, oligomenorrhea, and amenorrhea are terms defining menstrual regularity. Eumenorrhea is characterized by a consistent and cyclic menstrual cycle of 25 to 39 days. Oligomenorrhea refers to irregular or inconsistent menstrual cycles of 39 to 90 days. Finally, amenorrhea is defined as menstrual cycles occurring at intervals of greater than 90 days (Loucks & Horvath, 1985).

Amenorrhea can be characterized as primary or secondary. Primary amenorrhea is the absence of menstruation by the age of 14 with absence of secondary sex characteristics (i.e. breast and pubic hair development), or age 16 with presence of secondary sex characteristics. Secondary amenorrhea is the absence of menstruation for three months where previous cycles were regular, or six months where previous cycles were irregular (Sakala, 1999).

Hypothalamic amenorrhea

Reproductive function is moderated by a positive feedback system of the hypothalamus, pituitary, and ovary, also known as the H-P-O axis (Yen, 1986). The hypothalamus releases a gonadotropin response hormone (GnRH), which stimulates the pituitary gland (in the brain) to secrete the follicular stimulating hormone (FSH) and luteinizing hormone (LH). These hormones, (FSH and LH) stimulate the ovary inducing follicular maturation, ovulation, and production of the sex steroid hormones, estradiol and progesterone. Estradiol and progesterone stimulate the development of secondary sex characteristics (i.e. breast development and female fat distribution), optimize bone mineral deposition, and control the release of GnRH, FSH, and LH. A disruption in the hypothalamus, pituitary, and/or the ovary can result in amenorrhea (Yen, 1986).

The etiology of exercise-induced amenorrhea resides in the hypothalamus or higher brain centers that modulate hypothalamic activity. Amenorrheic athletes can experience reduced production of the GnRH. This impacts the pituitary gland and results in reduced LH production and to a lesser extent reduced FSH production (Costa & Guthrie, 1994). Reduced FSH and LH hormone production suppresses the ovarian function and lowers the levels of ovarian steroid production. Reduced ovarian steroid production has been documented in amenorrheic athletes (Loucks et al., 1989; Bonen et al., 1989) and indicates inadequate follicular and luteal development.

Decreased body fat, weight loss and delayed menarche

Body weight and percent body fat may influence the onset of menarche, where a low body weight is associated with a delayed menarche (Malina, 1983). In addition, strenuous exercise (Abraham et al., 1982), and/or genetics (Kaprio et al., 1995) may contribute to menstrual disorders. Yet, some research studies report that caloric intake, rather than percent body fat, is a better predictor of amenorrhea (Calabrese et al., 1983; Drinkwater et al., 1984; Nelson et al., 1986). To better understand the etiology of amenorrhea and describe the late onset of menarche in physically active females, two models have been introduced.

The first model, by Malina et al. (1983), excludes physical activity as a factor and focuses on inherited physique and socialization. Inherited physique implies that female

athletes with late menarche are genetically predisposed to mature later. The socialization process suggests that those with late physiological maturation are socially involved in sports.

The second model suggests that intense exercise delays menarche by decreasing body fat and the fat/lean ratio (Frisch & McArthur, 1974; Frisch et al., 1981). Some suggest that the onset of menarche is dependent upon energy stores and occurs at a minimum mean body weight of 48 kg, or 17 percent body fat (Frisch & McArthur, 1974; Frisch & Revelle, 1970). A body weight near this critical weight alters the metabolic rate, which reduces the hypothalamic sensitivity to estrogen and consequently changes the ovarian-hypothalamic feedback system.

Whether either of the models accurately reflects the cause of late menarche is uncertain (Costa & Guthrie, 1994). The research procedure and statistical analysis by Frisch and colleagues has been criticized because the percent body fat was indirectly estimated using equations of total body water and weight rather than direct body fat measurements, and ethnic differences in body composition were not considered (Johnston et al., 1971; Trussell, 1978). Regular menstrual cycles have been identified in athletes with less than 17 % body fat, which also contradict the model by Frisch and colleagues. The percent body fat at which menarche occur remains uncertain.

Nevertheless, several studies support the hypothesis that weight loss from excessive exercise is correlated with an increased risk of amenorrhea (Bullen et al., 1985; Klentrou & Plyley, 2003; Loucks et al., 1995). The prevalence of amenorrhea is higher in female athletes participating in aesthetic (ballet dancing, gymnastics) and endurance (long-distance running) sports where low body weight benefits performance. Amenorrhea among ballet dancers ranges from 37% to 44% (Abraham et al., 1982; Calabrese et al., 1983; Cohen et al., 1982) and 6% to 26% among runners (Cummin et al., 1985; Dale et al., 1979; Sanborn et al., 1982; Shangold & Levine, 1982; Wakat et al., 1982). Whether, this high prevalence is caused by low percent body fat is uncertain. Further research is required to determine the exact relationship between increased body weight, percent body fat, and the development of amenorrhea (Costa & Guthrie, 1994).

Caloric intake and exercise

Athletes on a low caloric diet can experience changes in the endocrine status, which result in menstrual dysfunction (Loucks et al., 1998). Low caloric consumption and excessive exercise decrease LH pulse frequency, thus contributing to the development of amenorrhea (Calabrese et al., 1983; Carlberg et al., 1983; Drinkwater et al., 1984; Loosli et al., 1985; Loucks et al., 1995; Nelson et al., 1986; Wilmore et al., 1992). Prolonged exercise indirectly suppresses LH pulsatility by increasing energy cost and decreasing energy availability; however, energy restriction causes a greater suppression in LH pulsatility than exercise energy expenditure (Loucks et al., 1998)

Amenorrheic athletes consume fewer calories than their energy demands (Drinkwater et al., 1984; Deuster et al., 1986; Loosli et al., 1985; Marcus et al., 1985; Nelson et al., 1986; Wilmore et al., 1992) and eumenorrheic athletes. Consequently, amenorrheic athletes have lower resting metabolic rates, possibly related to a biological response to preserve energy (Wilmore et al., 1992). The lower caloric consumption and low energy availability can result from psychological (Costa & Guthrie, 1994) and physiological stress (Chrousos, 2000), respectively. Psychological stress, such as eating disorders, affects menstrual function by a hypothalamic mechanism similar to that occurring in exercise-induced amenorrhea (De Souza & Metzger, 1991). Physiological stress such as injury, surgery, burn, and infection (Chrousos, 2000) increases the energy demand and decreases the energy availability. In addition, prolonged physiological stress and psychological stress result in elevated cortisol levels (Chrousos, 2000; De Souza & Metzger, 1991; Kennedy et al., 1991).

Cortisol is a hormone that is indicative of catabolism characterized by protein degradation and glugoneogenesis (Lindholm et al., 1993). Low calorie consumption increases the resting cortisol levels in amenorrheic women (Loucks et al., 1998; Meczekalski et al., 2000). Elevated cortisol levels, inhibit GnRH and LH secretion, ovarian estrogen and progesterone biosynthesis, and the actions of estrogen (Kalantaridou et al., 2004). This suggests a possible correlation between amenorrhea and hypercortisolism (De Souza & Metzger, 1991; Loucks et al., 1989).

Although, a negative energy balance is strongly correlated with the risk of amenorrhea, exercise alone can induce the problem (Keizer et al., 1989). Exercise induced

amenorrhea often occurs when the individual has menstrual irregularities prior to beginning an exercise program, (Frish et al., 1981; Shangold et al., 1982) and/or as a result of increased exercise intensity (Bullen et al., 1985). In addition, high intensity exercise alters the metabolism of estrogen, forming catecholestrogens, which inhibit the GnRH release (De Crée et al., 1997; Russel et al., 1984; Snow et al., 1989).

Health risks

The initial health risk of amenorrhea is reproductive dysfunction (Bullen et al. 1985). However, decreased estradiol production resulting from hypothalamic amenorrhea can contribute to decreased bone mass. Estradiol plays an important role in collagen formation, which comprises the structure of bone and different tissues. Therefore, hypoestrogenemia increases the risk for low peak bone mass in adolescents and increased bone loss in adults (Compston, 2001). Consequently, athletes with irregular menstruation cycles are more prone to develop multiple stress fractures (Warren et al., 1999) and are at greater risk for developing osteoporosis long-term (Miller & Klibanski, 1999).

OSTEOPOROSIS

Eight million women in the United States suffer from osteoporosis each year, and an additional 34 million are at risk (NOF, 2004). Osteoporosis is characterized by structural breakdown of bone tissue, which results in decreased bone mineral density and increased fragility of bone (Dalsky et al., 1990; Drinkwater et al., 1994). The disease is generally asymptomatic until a fracture occurs, then can cause morbidity and mortality (Mehler, 2003). Osteoporosis is responsible for more than 1.5 million fractures annually and half of all women over the age of 50 will suffer from an osteoporosis-related fracture in their lifetime (NOF, 2004).

Although the disease is most commonly found in postmenopausal women, decreased bone mass of 0.9% to 20% has been reported in amenorrheic athletes (Bennell et al., 1997; Drinkwater et al., 1984; Marcus et al., 1985), which mimics that seen in menopause (Biller et al., 1991; Grinspoon et al., 2003; Drinkwater et al., 1984). In fact, amenorrheic athletes (24.9 ± 1.3 years of age) with bone mineral densities equivalent to 51 year-old women have

been reported (Riggs et al., 1982). Decreased bone mass places female athletes at greater risk for developing stress fractures (Warren et al., 1986) and osteoporosis (Otis et al., 1997).

Definition of osteoporosis

Bone mineral density and bone mineral content decrease with age at a rate determined by genetics, life-style, and hormonal factors [World Health Organization (WHO), 1994]. The severity of the decrease is classified into three groups, normal, osteopenia and osteoporosis (WHO, 1994).

Normal decrease in bone mass is characterized by <1 standard deviation below normal peak bone mass (WHO, 1994). A decrease in bone mineral density of 1 to 2.5 standard deviations below normal peak bone mass is defined as osteopenia. Osteopenia precedes osteoporosis and is more commonly seen in young female athletes than osteoporosis (Khan et al., 2002). Osteoporosis is defined as the bone mineral density 2.5 standard deviations below normal peak bone mass (WHO, 1994).

The development of osteoporosis

The risk of developing osteoporosis can be predicted as early as adolescence when peak bone mass is formed. A low peak bone mass during adolescence may increase the risk for developing osteoporosis later in life (Loro et al., 2000).

Bone mineral density increases rapidly during childhood and adolescence, but slows at the onset of menarche in females to reach a peak bone mineral density by the age of twenty (Bonjour et al., 1991; Bonjour et al., 1996; Compston, 2001). Normal bone mineral content (BMC) and bone mineral density (BMD) of 17 year-old female adolescents are 2173 g and 1.04 g/kg, respectively and do not significantly change until after the age of 21 years (Faulkner et al., 1996). Adequate nutrition and weight bearing activity during adolescence is critical for optimal bone development (Otis et al., 1997).

Unfortunately, the onset of eating disorders is common and the prevalence is high in adolescents when peak bone mass should be attained (Crisp & Toms, 1972; Croll J et al., 2002; Fisher et al., 1995; Gilsanz et al., 1986; Leichner, 2002). Young women who suffer from eating disorders and amenorrhea before the age of 18 years have significantly lower BMDs than those who develop amenorrhea later in life (Biller et al., 1989) and cases of osteoporosis have been reported (Brotman et al., 1985; Lucas et al., 1991).

After peak bone mass is attained and maintained for approximately two decades, a negative bone turnover (bone breakdown exceeds bone formation) decreases bone mineral density (Ihle & Loucks, 2004). Negative bone turnover in combination with low peak BMD, poor nutrition (Bronner, 1994; Compston, 2001), imbalanced hormonal status (Compston, 2001; Otis et al., 1997.), and/or lack of weight-bearing exercise (Compston, 2001; Todd et al., 2003) contribute to osteoporosis.

Bone turnover

Bone is a living tissue primarily consisting of osteocytes, osteoblasts, and osteoclasts. Osteocyte cells are osteoblasts that have ceased synthesis and are embedded in the bone matrix. Osteoblasts and osteoclasts are cells responsible for bone formation and bone resorption respectively, and make up the structure and turnover of bone (Compston, 2001).

Bone turnover is a coupling between bone formation and bone resorption. Bone formation is a process of type I collagen deposition, where crosslinked bonds are formed between collagen fibrils to form the mineralized structure of bone (Bernardi et al., 2004). When bone is formed, procollagen type I N- and C-terminal propeptides (PICP) and ostesocalcin (OC) are released into the blood stream (Risteli et al., 1993). These proteins can be quantified to assess the rate of bone formation. Similarly, during bone resorption bone markers including N-terminal cross-linking telopeptides (NTx), or C-terminal cross-linking telopeptides (CTx), of type I collagen, and pyridinoline (PYD) are released into the blood stream and can be quantified to estimate the rate of bone resorption (Ihle & Loucks, 2004).

During growth (childhood and adolescence) bone formation and bone resorption rates are high. However, at the third decade of life when peak bone mass has been reached, the rate slows to maintain BMD. Peak bone mass is maintained until the fifth decade of life when age-related bone loss begins. The onset of age-related bone loss has not been well defined, but bone loss results from a decreased rate of bone formation, an increased rate of bone resorption, or a combination of both (Ihle & Loucks, 2004). It appears that genetic, hormonal, physical activity, and nutritional factors all play a role (Compston, 2001).

Dietary intake

Nutrition plays an essential role in bone health. Adequate caloric consumption is imperative to meet the requirements for vitamins and minerals and to maintain hormonal

balance essential for bone health (Braam et al., 2003; Otis et al., 1997). Low caloric intake can result in inadequate consumption of essential nutrients important for bone health, including calcium, vitamin D (Bronner, 1994), vitamin K (Karsenty, 1998), and iron (Harris et al., 2003).

Inadequate caloric consumption is common among female athletes and commonly results in calcium deficiency (Hinton et al., 2004). Individuals with low calcium intakes have lower BMD, more fragile bones, and develop fractures at an earlier age (Matkovic et al., 1979). This concept is particularly important during growth when children and adolescents require adequate calcium for the development of peak bone mass (Kalkwarf et al., 2003). Calcium is essential for the structure of bone and vitamin D facilitates its absorption in the small intestine (Bronner, 1994). Ultimately, vitamin D and calcium metabolism impact bone formation (Miller et al., 1999). As a cofactor in the synthesis of calcium binding proteins, vitamin K is an important nutrient in bone turnover (Karsenty, 1998). Low dietary intake of vitamin K has been associated with an increased risk for osteoporotic fractures (Booth et al., 2000). Finally, iron is another important micronutrient for optimal bone health (Propckop, 1971). It impacts the function of osteoblasts (Propckop, 1971), converts vitamin D to its active form (DeLuca, 1976), and plays a role in collagen formation (Propckop, 1971). Iron deficiency and iron overload have been associated with low BMD in rat models (Malecki et al., 2000; Medeiros et al., 2002) and humans (Diamond et al., 1991; Sinigaglia et al., 1997). However, further research is imperative to better understand iron's impact on bone health in humans.

Inadequate caloric consumption contributes to the development of amenorrhea, which disrupts the hormonal balance of the reproductive system (Otis et al., 1997). As a result, hypoestrogenemia negatively impacts bone turnover by increasing bone breakdown (Miller et al., 1999; Warren et al., 1986). In addition to estrogen, insulin growth factor-1 (IGF-1), leptin, and cortisol play a role bone health (Mehler, 2003).

Hormonal regulation of bone health

Hormonal regulation is a significant factor to optimal bone health. Estrogen is a steroid hormone that plays a significant role in bone health. The hormone is produced and secreted by the ovary and to some extent the adrenals in women (Compston, 2001;

Drinkwater et al., 1984; Ettinger et al., 1998; Zanker et al., 1998). Estrogen exists in three forms, estrone (E1), estradiol (E2), and estriol (E3). The major and most commonly analyzed form is estradiol (E2), which has a normal serum range of 10-400 pg · ml^{-1} (Martin, 1978).

Estrogen increases the expression of receptors for bone regulating hormones (Compston, 2001). More importantly, it inhibits the cytokines that are responsible for the activation of osteoclasts. Thus, a decrease in estrogen levels stimulates activation of osteoclasts and an increase in bone resorption (Mehler, 2003).

Hypoestrogenism is a serious problem commonly seen in postmenopausal women (Compston, 2001), patients with anorexia nervosa (Mehler, 2003), and females with hypothalamic amenorrhea (Biller et al., 1991; Drinkwater et al., 1984; Miller et al., 1999). Low estrogen levels have also been associated with intensive exercise and inadequate caloric consumption (Drinkwater et al., 1986).

Other hormones contributing to the development of osteoporosis include insulin growth factor-1 (IGF-1), leptin, and cortisol (Mehler, 2003). Insulin growth factor-1 positively impacts osteoblast function. Weight loss resulting from low caloric intake seen in patients with anorexia nervosa, and depressed leptin levels (Mundy et al., 1999), decreases IGF-1 levels and contributes to a net loss of bone mass (Mehler et al., 1999). Finally, hypercortisolism, together with other adrenal factors, negatively impacts reproductive function through the H-P-O axis (Loucks et al., 1989). Elevated cortisol levels have been found in amenorrheic athletes (Loucks et al., 1989); although mild, this may have a negative effect on BMD.

Weight bearing exercise

Formation and maintenance of BMD can be stimulated by weight-bearing exercise (Creighton et al., 2001; Fehling et al., 1995; Todd et al., 2003). Activity including running and jumping, stimulate osteogenesis (Creighton et al., 2001). Athletes participating in weight-bearing sports, such as volleyball (Fehling et al., 1995), running, and gymnastics (Bemben et al., 2004; Markou et al., 2004), have higher BMD than athletes participating in non-weight-bearing sports, such as swimming (Fehling et al., 1995).

Due to the osteogenic effect of weight-bearing exercise, it is important to include this type of activity during childhood and adolescence when the majority of BMD is formed

(Compston, 2001). Weight-bearing exercise also slows bone breakdown in exercising adults (Liu-Ambrose et al., 2004; Nakatsuka et al., 1994). Although weight-bearing exercise stimulates bone formation, excessive exercise in combination with inadequate nutrition can result in amenorrhea and decrease the bone formation/breakdown ratio, which leads to accelerated bone loss (Drinkwater et al., 1984).

Identifying bone turnover

Measuring bone markers in the serum or the urine for bone formation and resorption, can identify bone turnover and predict the development of osteoporosis. The rate of bone formation is a 2-3 month process, whereas bone resorption occurs in 7-10 days. As a result, bone resorption markers respond faster to change (2-12 weeks) than markers of bone formation (3-6 months) (Bernardi et al., 2004). The level of bone markers circulating in the blood is intra-variable and depends on age, exercise, fractures, immobility, and diet (Bernardi et al., 2004).

During childhood and adolescence the level of bone markers is very high. At this age, bone turnover is up to 10 times greater than later in life, with the exception of menopause (Bernardi et al., 2004). Although the turnover rate decreases in the second decade of life, it can be increased by 15% to 40% with exercise. Thus, bone turnover is high in female athletes and increases the risk for stress fractures (Orava et al. 1978). In addition, dietary intake may alter bone turnover, however its impact is controversial, ranging from no impact of energy consumption to great impact on NTx, PICP, and OC (Bernardi et al., 2004; Ihle and Loucks, 2004). An indirect estimation of bone turnover is the assessment of BMD and BMC using Dual-energy X-ray absorptiometry (DXA) (Prior et al., 1997; Testolin et al., 2000).

Assessment of body composition

DXA is a valid and reliable tool that uses radiation to assess fat mass (FM), fat-free mass (FFM), and bone mass. The method is independent of age, gender, race, athletic status, musculoskeletal development, body size, and body fatness, thus can be used in numerous populations (Prior et al., 1997). The DXA is non-invasive, quick, and easy to use. The subject lies on a table in supine position. An x-ray tube located under the table releases an x-ray beam. The photons in the beam pass upward through the body in a posterior-to-anterior direction to a detector. The photons pass through a K-edge filter (cerium), which generates

two energy peaks (~40 and 70-110 keV) (Ellis, 2000). The ratio of the low to high-energy peaks allow for the distinction between FM and FFM. Tissues have different densities and chemical structure, which determine their attenuation, or reduction in intensity, of the photons. The attenuation of high-density tissue (bone) is greater than that of soft tissue (FFM and FM). This allows for the distinction between soft tissue and bone mass (Pietrobelli et al., 1996; Wagner & Heyward, 1999). During a whole body scan, the exposure of radiation to the skin is 0.9 mRem, which is comparable to 2 days of environmental background radiation, or flying at 39 000 ft. for one hour (Lloyd et al., 1998).

The DXA is a valid and precise method for body composition assessment (Prior et al., 1997; Mazess et al., 1990; Van der Ploeg et al., 2002; Pietrobelli et al., 1998). The reported precision error of total bone mineral density (0.01 g/cm^2), percent body fat (%BF, 1.4%), FM (1.0 kg), and FFM (0.8 kg) is low (Mazess et al., 1990). Lohman et al. (1996) suggested that the precision of a body composition method should be based on the standard error of the estimate (SEE) and a SEE of <3% body mass (BM) indicates good accuracy of the assessment method. Prior et al. (1997), reported a SEE of 2.8% BM when comparing the DXA with a four-component model, which was similar to that reported (SEE=1.6 %BF) by van Der Ploeg et al. (2003).

The DXA does have limitations (Priory et al., 1997) including hydration status (Pietrobelli et al., 1998; Testolin et al., 2000). A 5% change in water content of FFM has resulted in a 1-2.5% change in the estimation of %BF (Lohman et al., 2000). There are contradictory studies suggesting that hydration is not a major factor if the error in estimating total body water is small (Lohman et al., 2000; Pietrobelli et al., 1998). DXA can also be impacted by body thickness and variation in fat distribution (Prior et al., 1997). In addition, very large individuals do not fit on the scanning table, thus cannot be accurately assessed (Roubenoff et al., 1993). Finally, different technologies and software for the different manufacturers (Hologic and Lunar) of dual-energy densitometers vary in precision (Tothill & Hannan, 2000).

Despite the limitations, DXA is one of the most critically tested and precise assessment methods for body composition (Lintsi et al., 2004; Pietrobelli, A., 1998; Prior et al., 1997; Sun et al., 2005; Tothill & Hannan, 2000; Wagner & Heyward, 1999). Therefore, it

is the preferred reference method for body composition assessment used in research (Lintsi et al., 2004; Pietrobelli, A., 1998; Prior et al., 1997; Sun et al., 2005; Wagner & Heyward, 1999).

IRON STATUS

Iron deficiency is the most prevalent dietary deficiency in the world (DeMaeyer & Adiels-Tegman, 1985) affecting nearly 66% to 80% of the world's population, of which 30% suffer from iron deficiency anemia (WHO, 2003). In the United States, 9% to 16% of all women 12 to 49 years old suffer from iron deficiency (Centers for Disease Control and Prevention, 2002). Although the prevalence of iron deficiency of female athletes is unknown, estimates are 26% to 60% (Dubov & Constantini, 2004; Malczewska et al., 2000; Peterson, 1998).

Exercising females and female athletes are highly susceptible to iron deficiency (Brutsaert et al., 2003; Weaver & Rajaram, 1992; Zhu & Haas, 1997). Mechanical stress of exercise destroys red blood cells and induces intravascular hemolysis resulting in iron loss. Furthermore, exercise is believed to increase the amount of plasma relative to the red cell mass, causing blood dilution (Hallberg & Magnusson, 1984). Exercise may also cause a negative iron balance through increased sweat (Brune et al., 1986), urinary (Siegel et al., 1979) and fecal losses, and decreased intestinal absorption (Stewart et al., 1984). Iron stores are reduced, particularly at the initiation of an exercise program. Normally, the decrease in iron stores plateaus (Weaver & Rajaram, 1992), but in highly trained endurance athletes the stores may continue to decrease with aerobic training (Beard & Tobin, 2000).

Role of iron

Iron is an essential micronutrient important for oxidative energy production (Bothwell et al., 1979). It binds oxygen in the blood and transports it to oxygen dependent tissues. Iron that binds oxygen is referred to as functional iron (Bothwell et al., 1979) and is found in the protein structure of hemoglobin (Hb), myoglobin (Mb), iron-dependent enzymes, and respiratory chain proteins (Table 1). Iron also plays a role in the prevention of aging, reduction of neurological damage and dysfunction (Atamna et al., 2002), immune function (Ahluwalia et al., 2004), and bone health (Harris et al., 2003). Related to its role in oxygen

carrying capacity (Hb and Mb), tissue oxidative capacity, and lactate production, iron status greatly impacts physical performance (Davies et al., 1984; Brownlie et al., 2002; Zhu & Haas, 1997; Haas & Brownlie, 2001).

Decreased oxygen carrying capacity (decreased VO_2max) impairs aerobic adaptation (Davies et al., 1982) and occurs with iron deficiency anemia, when Hb levels are decreased (Haas & Brownlie, 2001). Elevated lactate levels have been reported in individuals with iron deficiency anemia (Gardner et al., 1977). On the other hand, tissue oxidative capacity, which impacts the oxidative energy production in the mitochondria, is affected at all levels of iron deficiency (Hinton et al., 2000; Brownlie et al., 2004).

Table 1. Iron containing proteins

Protein	*Functional Site*	*Major biological function*
Hemoglobin	Red blood cell	Oxygen transport
Myoglobin	Cytoplasm of muscle cells	Facilitate diffusion of oxygen towards the mitochondria
Oxidative enzyme (e.g. Dehydrogenase)	Mitochondria inner membrane and matrix	Oxidation of substrate (acetyl-CoA) to produce NADH and $FADH_2$
Respiratory chain proteins (e.g. cytochromes)	Mitochondria inner membrane	Electron transfer from O_2 to NADH and $FADH_2$

(Haas. J.D., Brownlie IV, T. (2001). Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *Journal of Nutrition, 131*, 676S-690S.)

Iron deficiency

Iron deficiency is the result of inadequate dietary iron intake, inadequate intestinal absorption of iron, and/or increased iron losses (Haas & Brownlie, 1997). Iron deficiency can be defined into three stages, iron depletion, iron deficiency erythropoiesis, and iron deficiency anemia (Herbert, 1987) (Figure 1).

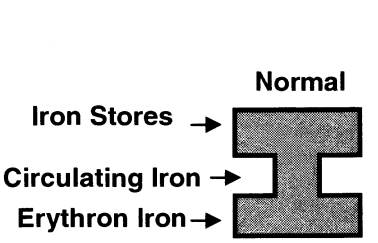
Iron depletion results from a drop in the iron stores and can be identified by a decrease in plasma ferritin to 20 μg/L (Herbert, 1987). This is the only parameter affected at this stage.

Iron deficiency erythropoiesis, the second stage of iron deficiency, results when there is insufficient iron to support erythropoiesis in the bone marrow. This stage is recognized by an elevation in red cell protoporphyrin and serum transferrin receptors, and a reduction in ferritin (<12 μg/L) and transferrin saturation (<16%) (Cook & Finch, 1979). At this stage,

exercise performance can be reduced due to increased lactate production as a result of insufficient oxygen to support aerobic metabolism (Schoene et al., 1983).

If iron levels continue to drop, iron stores are completely depleted, and the iron supply is minimized, iron deficiency anemia develops. Iron deficiency anemia is identified by a hemoglobin level of <120 g/L, microcytic/hypochromic red blood cells, and very high levels of transferrin receptors (Herbert, 1987).

Different definitions of iron deficiency and iron parameters associated with each stage, have been reported. Herbert (1987) defines plasma ferritin levels of iron depletion, iron deficiency erythropoiesis, and iron deficiency anemia as 20 µg/L, 10 µg/L, and < 10 µg/L respectively. Herbert's criterion for iron depletion (≤20 µg/L) has been used in several studies (Ahluwalia et al., 2003; Brutsaert et al., 2003; Dubnov & Constantini, 2004). However, the most commonly used ferritin level to identify iron deficiency erythropoiesis, and iron deficiency anemia is <12 µg/L (Cook & Finch, 1979; Cook et al., 1986; Blum et al., 1986; Flowers & Cook, 1999; Skikne et al., 1990).



	Normal	Iron Depletion	Iron Deficient Erythropoiesis	Iron Deficiency Anemia
RE marrow iron	2-3+	0-1+	0	0
TIBC (µg/dl)	330±30	360	390	410
Plasma ferritin (µg/L)	100±60	20	<12	<12
Plasma iron (µg/dl)	115±50	115	<60	<40
Transferrin saturation(%)	35±15	30	<16	<16
Erythrocyte protoporphyrin (µg/dl)	30	30	100	200
Erythrocytes	Normal	Normal	Normal	Microcytic hypochromic
Hemoglobin (g/dl)	>12	>12	>12	<12
Serum transferrin receptors	Normal	High	Very high	Very high

Figure 1. Sequence of change in iron status. The shaded areas represent the onset characteristics of each deficiency state. Adapted from Cook & Finch (1979), Herbert et al. (1996), and Herbert (1987) to represent the most commonly used definitions in scientific research (Ahluwalia et al., 2003; Blum et al., 1986; Brutsaert et al., 2003; Cook & Finch, 1979; Cook et al., 1986; Dubnov & Constantini, 2004; Flowers & Cook, 1999; Skikne et al., 1990).

Iron status assessment

There are several methods to assess iron status including Hb, hematocrit (Hct), mean red cell volume (MCV) serum iron, total iron binding capacity (TIBC), transferrin saturation, erythrocyte protoporphyrin, ferritin, serum transferrin receptor (sTFr), and total body iron. Because the methods assess iron deficiency at different stages and sites, a combination of two or more measurements is recommended for true assessment of iron (Cook & Skikne,1982).

Hemoglobin and Hematocrit

Quantification of Hb and Hct are commonly used to assess iron status (Haas & Brownlie, 2001). The normal levels of Hb and Hct found in women are 120-160 g/L and 37%-47%, respectively (Pagana & Pagana, 2003). Iron deficiency anemia is defined as Hb<120 g/L (Haas & Brownlie, 2001) and Hct<36% (Cook et al., 1974). However, in an iron-replete person, who previously suffered from iron deficiency but have replenished the stored to obtain normal iron status, a 20 g/L drop in Hb, regardless of the final Hb value can cause anemia. Therefore, anemia may be prevalent at other Hb levels (Cook et al.,1986). In addition, Hb can decrease several hundred milligrams without being detected in the blood and may not be identified until the stage of iron deficiency anemia (Cook et al., 1985). Because the normal Hb range is quite broad it causes an overlap between normal and anemic values, making it difficult to detect anemia (Cook et al., 1985). Therefore, Hb assessment is most appropriate to assess severe iron deficiency anemia (Cook et al., 1985).

Serum iron, TIBC, and Transferrin saturation

Serum iron, and to some extent TIBC, are used to examine bone marrow iron supply (Cook et al., 1985). Normal serum iron ranges between 60-160 µg/dl in women whereas <60 µg/dl suggests iron deficiency erythropoiesis. Normal TIBC ranges between 250-460 µg/dl and are elevated to ≥360 µg/dl with iron depletion (Pagana & Pagana, 2003). The ratio of serum iron to TIBC is the transferrin saturation. Although, widely used in studies, transferrin saturation is a labile measurement that can change within hours in response to mild inflammation (Cook et al., 1985). Normal transferrin saturation is 15% to 50% (percent of transferrin saturated with iron) (Pagana & Pagana, 2003). Transferrin saturation is not considered abnormal until the iron stores are depleted; however, low levels (<16%) can

suggest iron deficiency erythropoiesis. Hemoglobin production by the red blood cell will decrease when the transferrin saturation falls below 15% (Cook et al., 1985).

Erythrocyte Protoporphyrin

A more stable measurement of bone marrow iron supply is erythrocyte protoporphyrin. Protoporphyrin is a complex that binds iron to form hemoglobin. Normal levels are 30 µg/dl of red blood cells. When iron is deficient, the unbound protoporphyrin levels are elevated in the red blood cells to >100 µg/dl (Cook & Finch, 1979). This increase occurs weeks after the initial iron lack and slowly improves with iron supplementation, making it more stable than transferrin saturation. However, erythrocyte protoporphyrin assessment is only indicative of iron deficiency erythropoiesis when the iron stores have become depleted (Cook et al., 1985).

Ferritin

The human body uses approximately 70% of its iron, while the remaining 30% is stored in the form of ferritin (Baldwin, 2003). Ferritin can be found in the liver, bone marrow, and spleen. A small amount, proportional to the iron stores, circulates in the blood and can be measured in the serum (Cook & Skikne, 1982). Approximately 1 µg/L of serum ferritin corresponds to ~10 mg storage iron (Cook & Skikne, 1982; Worwood, 1980). The normal ferritin level in women is 10-150 µg/L (Pagana & Pagana, 2003); however, a serum ferritin level below 12 µg/L is indicative of iron deficiency (Cook & Skikne, 1982).

Ferritin has greater specificity than the transferrin saturation or erythrocyte protoporphyrin assessment because it distinguishes between iron deficiency anemia and the anemia of chronic disease (Cook and Finch, 1979). Ferritin assessment is also useful to evaluate changes in iron status with iron supplementation, and identifies developmental stages of iron overload. Although ferritin is a good assessment tool to estimate the iron stores, once the stores have been depleted it can no longer identify the severity of iron deficiency (Cook and Finch, 1979).

Ferritin is an acute phase protein that can be elevated approximately 20% for several days with exercise and chronic inflammation (Schumacher et al., 2002). Exercise induces an inflammatory-like response in the reticuloendothelial system, which increases ferritin synthesis. In addition, exercise causes cell membrane damage of the iron storage tissues,

which results in the release of ferritin (Pattini et al., 1990). Serum ferritin levels of 60 μg/L can be seen in patients with iron deficiency and inflammation (Cook & Skikne, 1982). Regardless of the effect inflammation has on ferritin, it is widely used to assess iron deficiency because it reflects changes in iron stores before complete depletion (Cook & Skikne, 1982).

Serum Transferrin Receptor

Currently the most commonly used marker for tissue iron deficiency in athletes is sTFr (Schumacher et al., 2002; Brownlie et al., 2002; Brownlie et al., 2004). Unlike ferritin, the sTFr is not impacted by inflammation or the inflammation-like response of exercise (Brownlie et al., 2004; Schumacher et al., 2002).

Transferrin receptors are located on the surface of erythroid cells. The receptors bind to transferrin, which carries iron in the blood, and transfers iron into the erythroid cell (Schumacher et al., 2002). Cells that are iron deficient increase the production of sTfr (Rouault et al., 1985) to bind more iron to support erythropoiesis, thus sTfr levels reflect erythropoietic activity (Schumacher et al., 2002). In addition, sTFr is a direct indicator of functional iron and total body iron stores (Schumacher et al., 2002), and indicates iron deficiency at the tissue level (Skikne et al., 1990; Brownlie et al., 2004).

Similar to ferritin, a small amount of transferrin receptors circulate in the serum and can be quantified. Due to the lack of international standards, the normal range of sTfr is not standardized (Beguin, 2003). However, research studies report mean levels of 5.0±1.0 mg/L (Beguin, 2003), and 5.6 mg/L with a range of 2.8-8.5 mg/L in females with normal iron status (Brownlie et al., 2002).

Total Body Iron

A new method assessing total body iron has been proposed by Cook et al. (2003). A strong linear relationship between the logarithm of the sTFr to ferritin ratio (R/F μg/L) and the body iron (mg/kg body weight) has been observed and resulted in a new equation for the assessment of total body iron [body iron (mg/kg) = - (log(R/F ratio) – 2.8229)/0.1207] (Cook et al. 2003). A positive body iron reflects adequate stores, whereas a negative value represents tissue iron deficiency. The equation can be used to assess iron deficiency, which is identified by a negative total body iron. Iron deficiency anemia is identified at a body iron

deficit of lower than -4 mg/kg (Cook et al., 2003). Because the method relies on accurate sTFr and ferritin assessment, its limitations are related to the influence of inflammation and liver disease, and variations in sTFr and ferritin independent of iron status (Cook et al., 2003). Because body weight is a factor, measuring body iron per kilogram of body weights allows for extrapolation to young individuals.

CHAPTER 3. METHODS

Subjects

Subjects for this study were 117 female freshmen collegiate athletes 18 $\pm$ 0.5 years recruited from the Iowa State University Athletic Program. The subjects included 9 basketball players, 4 golfers, 10 gymnasts, 22 soccer players, 15 swimmers, 21 softball players, 7 tennis players, 20 track and field athletes, and 9 volleyball players. The subjects participated in these sports during high school and were recruited to Iowa State University division 1 college program due to their expertise in the respective sports. All protocols were approved by the Institutional Review Board at Iowa State University and all subjects/guardians signed an informed consent form.

Study protocol

Athletes were categorized into three groups by approximate ground reaction force (GRF) of each sport as reported by previous studies (Creighton et al., 2001). Swimmers comprised the low-impact (Low) group (n = 15) (GRF <1 x body weight). Track and field (n = 20), golf (n = 4), soccer (n = 22), tennis (n = 7), and softball (n = 21) comprised the medium-impact (Med) group (n = 74) (GRF = 2-3 x body weight) (Cavanaugh & Lafortune, 1980). Finally, basketball (n = 9) (Steele & Milburn, 1987), gymnastics (n = 10) (Caine et al., 1996), and volleyball (n = 9) (Salci et al., 2004) comprised the high-impact (High) group (n = 28) (GRF = 3-9 x body weight). Tennis, softball, gymnastics, and golf were classified per personal communication with an expert in GRF (T. Derrick, personal communication). The subjects underwent a radiological assessment of bone density (DXA scan), blood collection for assessment of iron and estrogen status, bone formation, and bone breakdown, and completed a questionnaire assessing menstrual history and eating attitudes during the summer or early fall prior to beginning their collegiate athletic career. A physical exam was required of all female athletes entering the athletic program and was performed at the Student Health Center on campus. During the physical exam 7 ml of blood were drawn from the antecubital vein, stored on ice, and centrifuged at 10°C at 2500 rpm for 15 minutes. Serum was stored at -80°C within 1 hour of collection in separate aliquots for the above analysis.

Anthropometrics and Bone Mineral Density Measurements

Each subject's height was measured, using a set square to the nearest 0.1 cm, and weight was measured using a triple beam balance scale to the nearest 0.1 kg. Total body fat,

total body density, total BMD and BMC were estimated (N=117) by a trained certified professional with dual-energy x-ray absorptiometry (DXA; Hologic Delphi W bone densitometer, software version 11.2; Hologic, Inc.; Bedford, MA). BMC and BMD were compared to a peer non-athlete population recommended by Hologic, Inc. (Faulkner et al., 1996). Faulkner et al. (1996) reported no significant difference in BMD or BMC at any site between 17 and 21 year old females, thus this population served as a peer reference group for this study.

Estradiol status

Serum was analyzed for estradiol (N=112) by enzyme-linked immunosorbent assay (ELISA) (Cayman Chemical Company, Ann Arbor, MI) and by an independent clinical laboratory (Quest Diagnostic; Wooddale, IL). The ELISA analysis had an intraassay coefficient of variation (%CV) of 13%. Blood draw appointments were part of the athlete's physical exam, which was independent of the study and stage of menstrual cycle of each individual was not controlled. Therefore, subjects were classified as having normal ($\geq$32 pg/ml) or abnormal (<32 pg/ml) estradiol levels for statistical analyses [reference level for estradiol is 32-526 pg/ml (Quest Diagnostics; Wooddale, IL)].

Iron status

Hemoglobin (Hb), Hematocrit (Hct), serum ferritin, and serum transferrin receptor (sTfr) status of the athletes were examined using the blood samples. Serum ferritin (N=113) was assayed by Immunoradiometric (RIA) procedure (Ramco Laboratories Inc., Stafford) with an intraassay %CV range of 3.6%-10.8% and sTfr (N=74) by ELISA with an intraassay %CV range of 6.1%-6.9% [normal range 2.9-8.3 µg/ml (Ramco Laboratories Inc., Houston)]. Hb (N=107) and Hct (N=101) values were obtained from an independent laboratory as part of the physical exam. Iron status was examined using criteria from Cook & Finch (1979) and Herbert (1986), which classifies a ferritin level of 20-40 ng/ml as iron depletion, <12 ng/ml as iron deficiency erythropoiesis, and <12 ng/ml as iron deficiency anemia. Anemia is also defined as hemoglobin <12 g/dL, hematocrit <36% (Cook & Finch, 1979), and serum transferrin receptor >8.0 µg/ml (Skikne et al., 1990). Total body iron was calculated using the serum transferrin receptor to ferritin ratio (body iron (mg/kg) = -[log(R/F ratio) – 2.8229]/0.1207) (Cook et al., 2004), with a negative value indicating iron deficit in tissues.

Bone markers

Bone formation was assessed by ELISA for the measurement of the C-terminal propeptides of type I collagen (CICP) (N=80) in serum [normal range for 4-18 years of age, 110-966 ng/ml (Metra, Quidel)]. Bone resorption was assessed by ELISA for the estimate of cross-linked N-telopeptides of type I collagen (NTx) (N=80) in serum [normal range for 25-49 years of age, 6.2-19.0 nM BCE (Ostex International)]. The intra- and interassay %CV for CICP were 7.0% and 3.2%, and for NTx were 5.0% and 1.6% respectively.

Surveys

All athletes completed a Student-Athlete Sport Health Evaluation, which provides information on age of menarche, menstrual regularity, and use of birth control pills. Amenorrhea was classified as lack of the menstruation cycle for >3 months. Subjects also completed the FAST (McNulty et al., 2001) and Restraint Eating (DePalma et al., 2002) surveys. Thirty of the 33 questions of the FAST survey were scored on a Likert scale of 1-4 where higher probability correlated with a higher score; the remaining three questions were reverse scored. A total FAST score <77 represents normal, 77-94 represents a subclinical eating disorder and >94 represents a clinical eating disorder (McNulty et al., 2001). The 11 questions of the Restraint Eating survey were scored on scales of 0-4 and scales dependent on weight fluctuation. Individuals scoring $\geq$17 on the Restraint Eating survey were classified as restrained eaters (DePalma et al., 2002).

Statistical Analyses

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS, version 11.5) software (SPSS, Inc.; Chicago, IL). Descriptive statistics (frequencies, percentiles, minimum, maximum, means, and standard deviations) and a stepwise logistic regression were performed on the data. Group differences in descriptive data were evaluated using ANOVA with a Tukey's comparison test used for a post hoc analysis.

CHAPTER 4. STRESS FRACTURES IN FEMALE ATHLETES: ROLE OF BONE TURNOVER AND SPORT IMPACT.

A paper to be submitted to Journal of Applied Physiology

ABSTRACT

Female athletes are at risk for stress fractures related to sport impact and bone resorption associated with exercise. This study examined sport impact and bone status, and developed a model to predict stress fracture risk in 117 collegiate female athletes. Sport ground reaction force was used to categorize sports into Low (Swimming N=15), Medium (Med) (Track, Soccer, Tennis, Softball, Golf, N=74), and High (Volleyball, Gymnastics, Basketball, N=28) impact groups. Data collected included bone mineral density (BMD), bone mineral content (BMC), body composition [body mass index (BMI) and percent body fat (%BF)], bone formation (C-terminal propeptide of type I collagen), bone resorption (cross-linked N-telopeptides of type I collagen), bone formation to breakdown ratio (Bone F:B), estradiol, and menstrual history. Injury reports of stress fractures, musculoskeletal injuries, and acute infections were collected throughout the study. Athletes in the High group had lower ($P<0.05$) BMI and %BF ($P<0.01$) than the Low group. The High and Med groups had greater ($P<0.01$) BMD than Low and the High group had greater ($P<0.05$) bone breakdown than the Low group. No significant relationships were observed between BMD, BMC, estrogen status, and age of menarche. The percent of athletes suffering from injuries, including stress fractures, was greater in the High group than the Med group ($P<0.05$). Yet, high BMD did not appear to protect against stress fractures related to increased bone resorption with high sport impact. According to logistic regression model, all athletes with Bone F:B of < 8 participating in High impact sports are at greater risk for stress fractures.

female athletes, sport impact, bone turnover, stress fractures

INTRODUCTION

Bone health presents a significant health problem for many female athletes (Otis et al., 1997). Suboptimal bone health is a component of the female athlete triad, which also includes eating disorders and amenorrhea (menstrual cessation). Nutritional deficiencies, altered body composition, and delayed menarche or amenorrhea associated with the female athlete triad, can negatively impact bone health (Golden, 2002; Otis et al., 1997;Warren and Goodman, 2003). Amenorrhea is associated with depressed estrogen, which increases bone resorption (or bone breakdown) and accelerates bone loss. Bone loss can also be accelerated by the mechanical stress of exercise, compounding the risk for stress fractures and osteoporosis in female athletes (Bennell et al., 1999; Drinkwater et al., 1984; Mehler, 2003; Pettersson et al., 1998). Amenorrheic athletes have lower bone mineral densities (BMD) (Marcus et al., 1985) and a higher prevalence of stress fractures than their eumenorrheic counterparts (Warren et al., 1986).

Exercise impacts BMD negatively and positively. Weight-bearing exercise has an osteogenic effect on bone (Creighton et al., 2001); the mechanical stress of exercise induces microstructure breakdown of bone, which triggers bone formation in a healthy state. Bone formation exceeds breakdown, increasing BMD as an adaptation to the workload. Thus, athletes participating in weight-bearing sports, such as volleyball (Fehling et al., 1995), gymnastics, and running (Bemben et al., 2004; Markou et al., 2004), have higher BMD than athletes participating in non-weight-bearing sports, such as swimming (Creighton et al., 2001; Fehling et al., 1995). However, high impact sports where ground reaction force is great [i.e. volleyball (Salci et al., 2004), gymnastics (Caine et al., 1996), and basketball (Steele & Milburn, 1987)] have high rates of bone breakdown, and intensive training regimens may not allow for adequate bone formation. Negative bone turnover (breakdown exceeding formation) and repetitive loading can contribute to partial or complete stress fractures (Bennell et al., 1999). A high prevalence of stress fractures has been reported in athletes with lower BMD (Bennell et al., 1995; Myburgh et al., 1990), thus high BMD may protect against stress fractures.

Although high BMD and normal estrogen status exert a protective effect on bone, stress fractures have been reported in female athletes with these characteristics (Grimston et

al., 1991; Cline et al. 1998). This suggests that female athletes may require greater BMD than non-athletes related to the mechanical stress of exercise (Bennell et al., 1999). The impact of a sport may also play a role; high impact sports with greater mechanical stress increase bone resorption. Currently, there is limited research about the relationship between bone turnover and sport impact in female athletes (Creighton et al., 2001; Matsumoto et al., 1997). The purpose of this study was to examine the role of sport impact, assessed by ground reaction force, and its relationship with bone formation and bone resorption; and to develop a multiple regression model to predict stress fracture risk in collegiate female athletes.

METHODS

Subjects. Subjects were 117 female freshmen collegiate athletes 18 $\pm$ 0.5 years of age recruited during a three-year period from the Iowa State University Athletic Program. The athletes were recruited based on their expertise in a particular sport, which they participated in prior to college. Athletes were categorized into three groups by approximate ground reaction force (GRF) of each sport as reported by previous studies (Creighton et al., 2001). Swimmers comprised the low-impact (Low) group (n = 15) (GRF <1 x body weight). Track and field (n = 20), golf (n = 4), soccer (n = 22), tennis (n = 7), and softball (n = 21) comprised the medium-impact (Med) group (n = 74) (GRF = 2-3 x body weight) (Cavanaugh & Lafortune, 1980). Finally, basketball (n = 9) (Steele & Milburn, 1987), gymnastics (n = 10) (Caine et al., 1996), and volleyball (n = 9) (Salci et al., 2004) comprised the high-impact (High) group (n = 28) (GRF = 3-9 x body weight). Tennis, softball, gymnastics, and golf were classified per personal communication with an expert in GRF (T. Derrick, personal communication). All protocols were approved by the Institutional Review Board at Iowa State University and all subjects/guardians signed an informed consent form.

Study protocol. Subjects were contacted and recruited during the summer or early fall prior to their collegiate career. A physical exam, required of all female athletes entering the athletic program, was performed at the Student Health Center on campus. During the physical exam 7 ml of blood were drawn from the antecubital vein, stored on ice, and centrifuged at 10°C at 2500 rpm for 15 minutes. Serum was pipetted from the sample and stored in separate aliquots at -80°C within 1 hour of collection.

Anthropometrics and DXA. Each subject's height was measured, using a set square to the nearest 0.1 cm, and weight was measured using a triple beam balance scale to the nearest 0.1 kg. Total body fat, total lean body mass (LBM), total body density, total BMD and bone mineral content (BMC) were estimated (N=117), by a trained certified professional, with dual-energy x-ray absorptiometry (DXA; Hologic Delphi W bone densitometer, software version 11.2; Hologic, Inc.; Bedford, MA). BMC and BMD were compared to a peer non-athlete population recommended by Hologic, Inc. (Faulkner et al., 1996).

Biochemical Indices. Serum was analyzed for estradiol (N=112) by enzyme-linked immunosorbent assay (ELISA) (Cayman Chemical Company, Ann Arbor, MI) and by an independent clinical laboratory (Quest Diagnostic; Wooddale, IL). The ELISA analysis had an intra-assay coefficient of variation (%CV) of 13%. Stage of menstrual cycle of each individual was not controlled related to the physical exam taking place independent of the study. Subjects with estradiol $\geq$32 pg/ml were classified as normal and those <32 pg/ml were classified as low for statistical analyses [reference level for estradiol is 32-526 pg/ml (Quest Diagnostics; Wooddale, IL)].

Bone formation was assessed by ELISA for the C-terminal propeptide of type I collagen (CICP) (N=80) in serum [normal range 4-18 years of age, 110-966 ng/ml (Metra, Quidel)]. Bone resorption was assessed by ELISA for cross-linked N-telopeptides of type I collagen (NTx) (N=80) in serum [normal range 25-49 years of age, 6.2-19.0 nM BCE (Ostex International)]. The intra- and inter-assay %CV for CICP were 7.0% and 3.2%, and for NTx were 5.0% and 1.6% respectively. To provide an estimate of bone turnover a ratio of bone formation to bone breakdown (Bone F:B) was calculated.

Injury report. Injury reports of stress fractures, musculoskeletal injuries, and acute infections were collected from the medical records of each athlete during the course of the three-year study. All injuries were diagnosed by the team physician and categorized/recorded by the athletic trainers in the athletes' medical record maintained in the athletic department. The frequency and severity of the injuries were considered since most healthy individuals suffer from occasional infections or musculoskeletal injuries without underlying health problems. Athletes who suffered from one or more stress fractures, more than one

musculoskeletal injury or acute infection, or from a musculoskeletal injury or acute infection lasting more than 10 days, were noted.

Statistical Analysis. Descriptive statistics (frequencies, percentiles, minimum, maximum, means, and standard deviations) were performed on the data. Data were examined to verify normality of distribution. The bone marker analysis data showed a skewed distribution, thus statistical analysis was performed on logarithmically transformed data. Descriptive statistics are reported as means $\pm$ SD, all other statistical analysis are reported as means $\pm$ SE. BMC, BMD, bone turnover, injuries, and estradiol status were analyzed between sport impact groups using ANOVA followed by a Tukey's post hoc test. Comparison of age of menarche and estradiol status was examined by independent sample *t*-tests. Variables discriminative to stress fractures were used for multivariate stepwise logistic regression, using both forward and backward approaches. A p-value <0.05 was used for statistical significance. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS, version 11.5) software (SPSS, Inc.; Chicago, IL).

RESULTS

Subject Characteristics. Subject demographics (Table 1) reveal no significant difference in age, height, and weight between the sport impact groups. Athletes in the High group had significantly lower BMI than the Low group and significantly lower percent body fat (%BF) than the Low and Med groups. In addition, the LBM and the mean age of menarche in the High group were significantly greater than the Med group. Athletes participating in High and Med exercise had significantly greater BMD than the Low group and the BMC was significantly greater in the High group compared to the Low and Med groups (Figure 1).

Bone Turnover and Estradiol Status. Biochemical indices (Figure 2) reveal that the High group had greater ($P<0.05$) bone breakdown than the Low group; a similar trend ($P=0.09$) was seen between the High and Med groups. Bone formation and Bone F:B was not significantly different between the groups. Low estradiol levels were observed in 32% of the High group, compared to 17% and 21% for the Med and Low groups, respectively. Athletes with later menarche (>15 years of age) and low estradiol levels (<32 pg/ml) appear to have

lower BMD than athletes with earlier menarche and normal estradiol levels >32 pg/ml (Figure 3). A negative relationship between low estrogen levels and BMD may exist; however, no significant relationships were observed between BMD, estrogen status, and age of menarche.

Injury Report. The data suggest that injuries and acute infections are higher in athletes participating in high impact sports (Figure 4). Athletes suffering from fractures, musculoskeletal injuries, and acute infections were greater in the High group compared to the Med group ($P<0.05$), and the prevalence of musculoskeletal injuries were significantly greater in the High group than the Low group ($P<0.05$). Interestingly, the Low group had a greater prevalence of acute infections than the Med group ($P<0.05$). Further examination of stress fractures by estradiol status and sport impact were inconclusive, yet suggests that normal estradiol status in high impact sports may not be protective of stress fractures.

Predictive Model of Stress Fractures. Stepwise multiple logistic regression of variables related to bone health (i.e. estradiol, BMI, BMD, iron status, age of menarche, sport impact, and Bone F:B) identified two independent predictors of stress fractures, Bone F:B ($P<0.05$) and sport impact category ($P<0.01$). The following model was developed to predict the probability of developing a stress fracture in a sport impact category where Bone F:B is known.

$$\text{Probability (p)} = \exp(1.694 - 0.378X_1 - 0.915X_2 - 1.971X_3)/ (1 + \exp(1.694 - 0.378X_1 - 0.915X_2 - 1.971X_3)$$

Where:

- 1.694 is a constant
- X_1 is bone F:B
- X_2 is absence (0) or presence (1) of Low, X_3 is absence (0) or presence (1) of Med. The absence of both Low (0) and Med (0) represent the High group.

The Nagelkerke's R^2 of the equation was 0.311 (sport impact, $R^2 = 0.169$; Bone F:B, $R^2 = 0.142$). Prevalence of stress fractures was highest in the High group (n = 6) compared to the Med (n = 3) and the Low (n = 1) groups, and factors contributing to impaired bone health (delayed age of menarche, high bone breakdown) were characteristic of the High group.

Thus, Figure 5 highlights the High group and graphically depicts the estimated probability of stress fracture at predicted values of Bone F:B. The proportion of observed Bone F:B values for subjects with stress fracture (n = 6) and with no stress fracture (n = 13) (observed values for subject with stress fractures / total number of observed values) were plotted at the midpoint of Bone F:B ranges of 2.5 ($2.5 \geq 5$; $5 > 7.5$; $7.5 \geq 10$). The observed proportions suggest that the model may be useful to predict risk of stress fracture for athletes in high impact sports. A Bone F:B < 8 appears to increase the probability of stress fractures in high impact sports.

DISCUSSION

The purpose of this study was: 1. to examine the relationship between low, medium, and high sport impact (GRF) and anthropometric/biochemical bone status indices; and 2. to develop a model to predict stress fracture risk in female collegiate athletes. This study found that female athletes participating in high impact sports had the lowest Bone F:B prior to their collegiate careers, which increases their risk for developing stress fractures according to the logistic regression model.

No difference between impact groups was found in bone formation; however, bone resorption was greater ($P<0.05$) in the High group. Thus, the lower Bone F:B ratio observed in the High is due to the higher bone resorption rather than depressed bone formation. The high bone resorption observed in athletes participating in high impact sports confirms that reported by Matsumoto et al. (1997). Interestingly, the markers for bone resorption used by Matsumoto et al. (1997) (urinary pyridinoline and deoxypyridinoline) were not specific to bone collagen, in contrast to those used in the present study (serum CICP and NTx). In contrast, others have reported lower bone formation in non-weight-bearing sports, and no difference in bone resorption between the impact groups (using osteocalcin and NTx) (Creighton et al., 2001). The reason for these conflicting results may be due to the variation of bone markers used, thus warrants further research to better understand bone turnover and markers in young female athletes.

This study confirms previous studies, reporting that female athletes participating in weight-bearing exercise (i.e. Med and High impact sports) have higher BMD and BMC than

their non-weight bearing counterparts (Creighton et al., 2001; Dook et al., 1997; Fehling et al., 1995). The BMD among all impact groups was 1.20 ± 0.09 g/cm^2 (mean ±SD) and BMC was 2476 ± 265 g (mean ± SD). The BMD and BMC of a non-athletic peer population were 1.04 ± 0.08 g/ cm^2 and 2173 ± 360 g (mean ± SD), respectively (Faulkner et al., 1996). BMC ($t = 4.92$, $P<0.01$) and BMD ($t = 8.36$, $P<0.01$) were greater in this study's athletic population compared to the peer non-athlete population (Faulkner et al., 1996). Thus, the present study also confirms female athletes have significantly higher BMC and BMD than non-athletes (Alfredson et al., 1997).

Previous reports indicate that a low BMD increases the risk for stress fractures in women with menstrual irregularity and decreased estrogen levels (Bennell et al., 1999; Pettersson et al., 1999; Warren et al., 1986). However, in the present study the prevalence of stress fractures was greatest in the High group where the greatest BMD was observed. The high BMD in these athletes is likely related to the high mechanical stress of exercise, which stimulates osteogenic activity. Yet, the greater BMD in these athletes is apparently not sufficient to protect against the risk of stress fractures related to mechanical stress of the activity, or other underlying factors.

Numerous authors have reported that stress fractures are more prevalent in athletes with past or present menstrual disturbances (Bennell et al., 1996; Nelson et al., 1987; Warren et al. 1986; Myrburg et al. 1990; Marcus et al., 1985). Delayed menarche and amenorrhea reflect hypoestrogenism, which may predispose female athletes to stress fractures (Warren et al., 1986). The relationship between age of menarche and stress fractures is unclear; some studies have reported a relationship between late onset of menarche and increased risk for stress fractures (Warren et al., 1991; Warren et al., 1986) while others have not (Myburgh 1990; Frusztajer et al., 1990). In the present study, athletes participating in high impact sports had leaner body composition, later onset of menarche, higher prevalence of low estradiol status, and higher incidence of stress fractures than the other groups. It is possible that lean body composition delays age of menarche, decreasing estrogen levels, and results in increased bone resorption and bone loss.

Currently, there are no validated tools for athletic programs to assess and predict the development of stress fractures. Prouteau et al. (2004) reported that low BMI at birth might

predict female athletes who will develop stress fractures; however, this report has not been validated by other studies and BMI at birth is commonly not available to collegiate athletic programs. The present study introduces a new model to identify athletes at increased risk for stress fractures. The Nagelkerke's R^2 is an attempt to measure strength of association, rather than a goodness-of-fit, thus may have been affected by the small sample size of stress fractures (n = 6) in the High group. However, the proportion of observed Bone F:B values between subjects with stress fractures and subjects without stress fractures suggests that the prediction model for stress fractures in High impact sports is valid. In addition, the model suggests that a Bone F:B less than 8 increases the probability of having a stress fracture. However, due to small sample size, this model estimating the probability of developing a stress fracture requires further evaluation. This is the first study to distinguish female athletes with stress fractures by sport impact and markers of bone turnover.

Further research is required to examine the reliability and validity of the bone turnover markers, the prediction model using a larger sample size, and to confirm the relationship between sport impact and bone health in female athletes suggested in this study. Because of its relationship with body composition, age of menarche, BMD, BMC, and bone resorption, it is recommended that sport impact be considered when assessing bone status and stress fractures in female athletes. Currently, a number of bone markers are used in research and some lack standardized reference values. Bone marker analysis has limitations; young growing individuals have elevated bone turnover, the acute effect of exercise may increase bone turnover by 15% to 40%, and circadian variability occurs with peak values in the morning (Bernardi et al., 2004). Each of these can contribute to potential errors in bone turnover assessment. Thus, future research needs to identify valid and reliable bone markers with standardized reference values to facilitate the comparison of bone status among studies.

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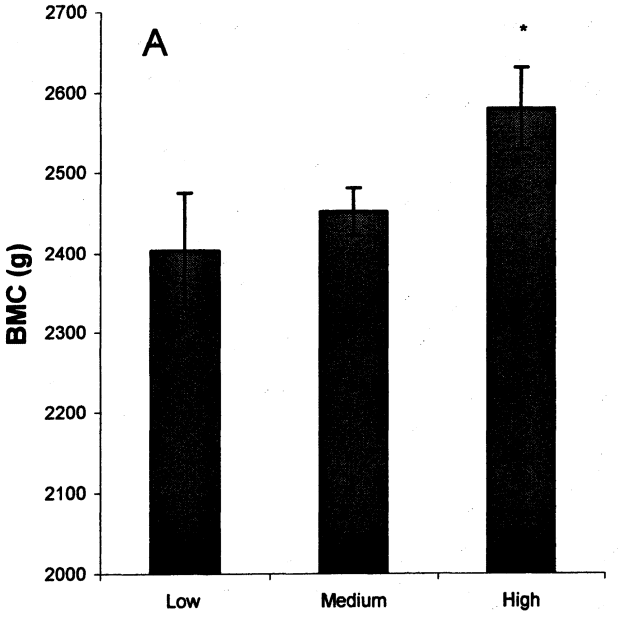
Table 1. Subject characteristics by sport impact

	Impact Group		
	Low (n = 15)	Medium (n = 74)	High (n = 28)
Age, yr	18 ± 1	18 ± 1	18 ± 1
Height, cm	170.3 ± 5.4	167.5 ± 5.5	171.4 ± 10.6
Weight, kg	67.5 ± 8.9	63.8 ± 8.8	64.9 ± 9.4
BMI (kg/m^2)	23.2 ± 2.3	22.7 ± 2.8	22.0 ± 1.6 [b]
Body fat, %	23.7 ± 4.5	23.0 ± 5.2	19.9 ± 4.3 [a, c]
Lean Body Mass, kg	49.7 ± 4.7	47.9 ± 4.8 [d]	50.8 ± 6.6
Menarche age, yr	13 ± 1	13 ± 1 [d]	14 ± 1
BMD, g/cm^3	1.14 ± 0.09 [e, f]	1.20 ± 0.08	1.22 ± 0.08
BMC, g	2404 ± 278 [d, f]	2453 ± 255 [d]	2580 ± 267
Estradiol			
%n <32 pg/ml	21	17	32
%n >32 pg/ml	79	83	68

Values are mean ± SD; n, number of subjects.
[a]Greater than Low ($P < 0.01$); [b]Less than Low ($P < 0.05$); [c] Less than Medium ($P < 0.05$); [d] Less than High ($P < 0.05$); [e] Less than Medium ($P < 0.01$); [f] Less than High ($P < 0.01$).



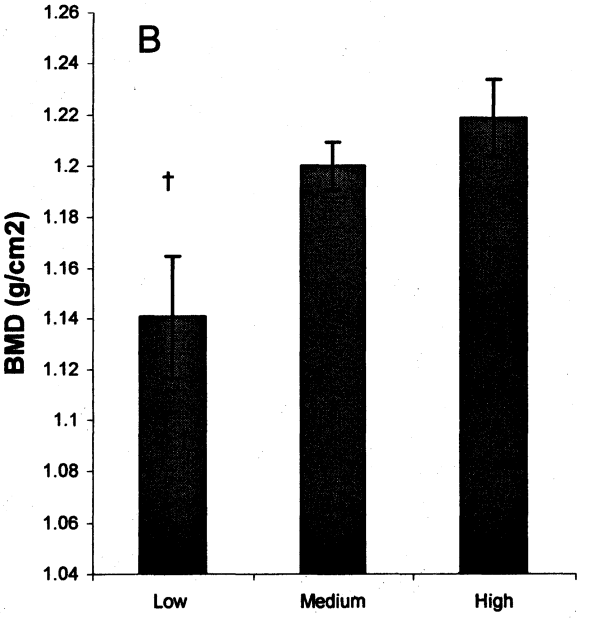


Figure 1. Mean ± SE of BMC (A) and BMD (B) by Sport Impact Category.
*Significantly greater than Low and Medium ($P<0.05$)
†Significantly lower than Medium and High ($P<0.01$)

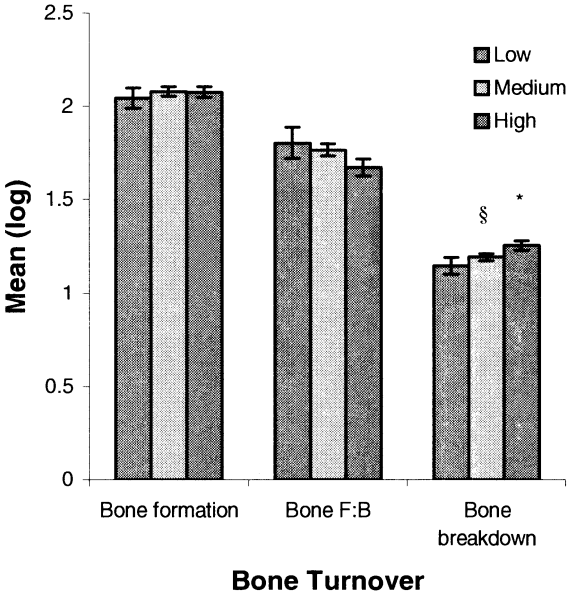


Figure 2. Mean ± SE of bone turnover markers by sport impact category. *Significantly greater than the Low group (P<0.05). § Marginally lower than the High group (P=0.09). The mean was log transformed due to unit differences and to allow for better visualization of the differences between the means.

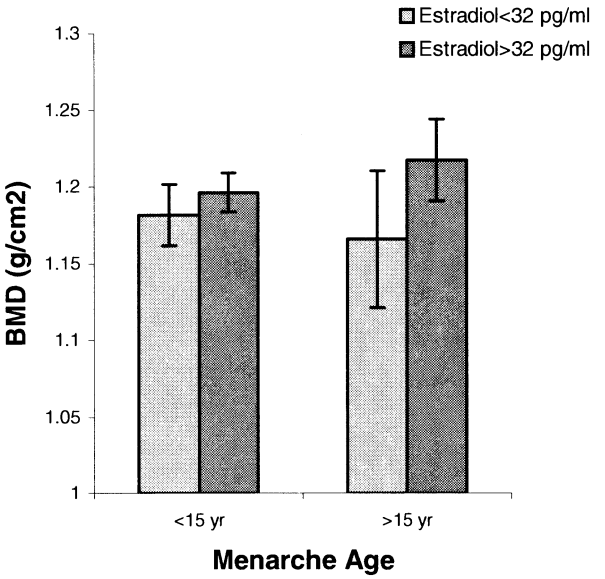


Figure 3. Mean ± SE of BMD by estradiol category and age of menarche. Athletes with delayed age of menarche (>15 yr) and low estradiol levels <32 pg/ml appear to have lower BMD.

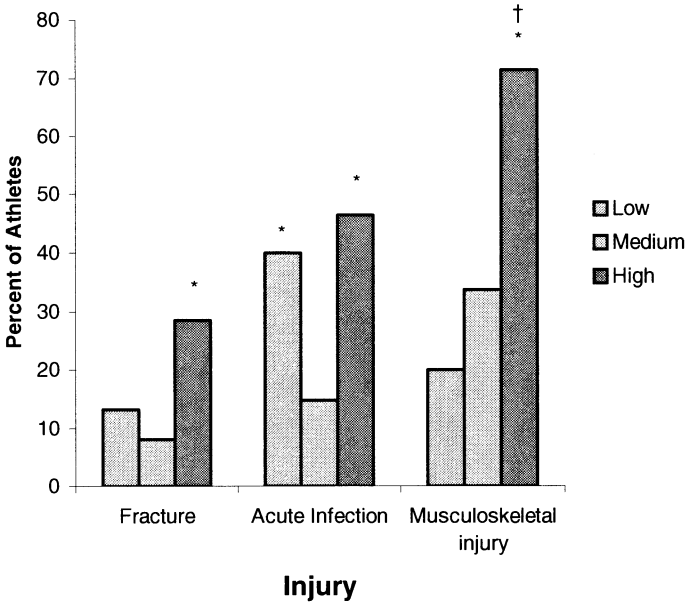


Figure 4. Prevalence (%) of injuries by sport impact category.
* Significantly higher prevalence than Medium P<0.05.
† Significantly higher prevalence than Low P<0.05.

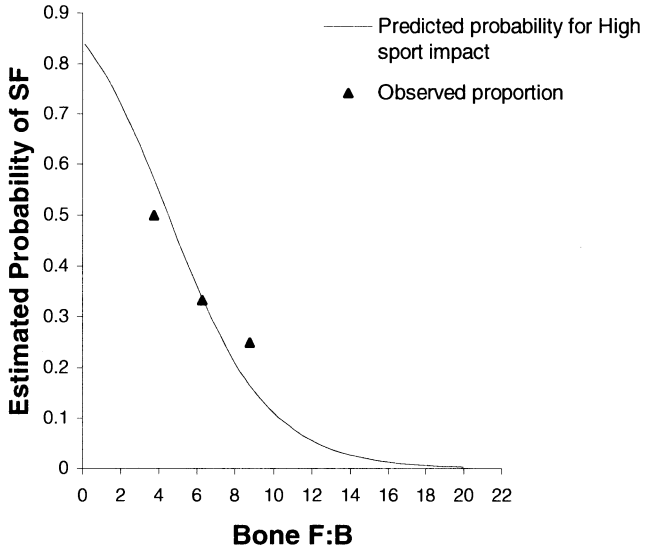


Figure 5. Estimated probability of stress fracture (SF) by predicted Bone F:B and High sport impact. ▲= proportion of observed values of subjects with stress fracture (n = 6) and subjects with no stress fracture (n = 13) within Bone F:B ranges of 2.5.

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CHAPTER 5. RELATIONSHIP BETWEEN IRON STATUS AND BONE HEALTH IN FEMALE ATHLETES

To be submitted to Calcified Tissue International

Abstract

The relationship between iron status and bone health was examined by collecting bone mineral density (BMD), bone mineral content (BMC), bone formation [C-terminal propeptide of type I collagen (CICP)], bone resorption [cross-linked N-telopeptides of type I collagen (NTx)], serum ferritin, serum transferrin receptor (sTfr), total body iron (TBI), and hemoglobin (Hb) in 80 female athletes ages 18 $\pm$ 0.5 years. Mean bone formation, bone resorption, and bone formation to breakdown ratio (Bone F:B) were analyzed by TBI (iron deficiency and normal), and iron status [ideal, iron depletion, iron deficiency, and iron deficiency anemia (IDA)]. Iron deficient subjects, categorized by TBI, had higher Bone F:B ($P<0.01$) and lower bone breakdown ($P<0.01$) than those with normal TBI. When subjects' overall iron status was classified as ideal, iron depletion, iron deficiency, or IDA, bone breakdown was higher ($P<0.05$) in the iron depleted group compared to the iron deficiency and IDA groups. Iron depleted subjects had lower Bone F:B ($P<0.01$) and higher bone formation ($P<0.01$) than iron deficient subjects. There was no significant relationship between iron status and BMD or BMC. Iron status was negatively associated with bone formation, contradicting a previous postulate that adequate iron promotes bone formation. A negative bone turnover, reported in this study, and previously reported reduction in endurance capacity (Brownlie et al., 2004) with iron depletion, stresses the need for adequate iron assessment to identify and treat iron depletion of female athletes.

Key Words: Bone turnover – Iron status – Serum ferritin – Serum transferrin receptor – Total body iron.

Introduction

Iron deficiency and osteoporosis are two of the most prevalent health concerns of women in the United States (Centers for Disease Control and Prevention, 2002; National Osteoporosis Foundation, 2004). Many female athletes engage in undesirable health practices to optimize physical performance. These practices, including excessive exercise and poor nutrition, make them particularly susceptible to iron deficiency and impaired bone health (Peterson, 1998; Otis et al., 1997).

Bone formation and resorption increase with weight-bearing exercise (Bernardi et al., 2004) and ideally bone formation will exceed resorption for a positive bone turnover. Unfortunately, the high intensity and frequency of exercise in highly competitive athletes may not permit sufficient bone formation, resulting in negative bone turnover, thus bone loss. Negative bone turnover can impair physical performance by contributing to stress fractures (Murguia et al., 1988).

Iron is a cofactor for iron-dependent enzymes, which play an important role in bone formation (Tuderman et al., 1977). Bone primarily consists of type I collagen (Crofton et al., 1996), formed in osteoblast cells. Proliferation and maturation of collagen in osteoblasts involves a cascade of biochemical reactions, including hydroxylation of prolyl and lysyl. These hydroxylations are catalyzed by the iron-dependent enzymes, prolyl and lysyl hydroxylase, and are essential steps prior to cross-linking of collagen fibrils. An elevated iron requirement would be expected with a high bone turnover related to the function of these iron dependent enzymes.

Unfortunately, 26% to 60% of female athletes suffer from iron deficiency (Dubov and Constantini, 2004; Malczewska et al., 2000; Peterson, 1998), and most athletes have suboptimal iron status (Brutsaert et al., 2003; Peterson, 1998; Weaver & Rajaram, 1992; Zhu & Haas, 1997). Iron deficiency can be classified as iron depletion, iron deficiency erythropoiesis, and iron deficiency anemia. Iron depletion, or reduced iron stores, has been shown to be a critical stage at which physical performance in athletes is reduced (Brownlie et al., 2004). Ultimately, iron deficiency in combination with high bone turnover may place female athletes at risk for low bone mass and stress fractures (Myburgh et al, 1990). Yet, the role of iron in bone is controversial. Some studies report that iron promotes bone formation

(Propckop, 1971; Tuderman et al., 1977), while others report a negative correlation between iron and bone formation (Diamond et al., 1991; Van de Vyver et al., 1988).

Numerous studies report a possible relationship between dietary iron, bone health, and the bone iron content of rats (Kipp et al., 2002; Kipp et al., 1998; Malecki et al., 2000; Massie et al., 1990; Medeiros et al., 2002); however, the relationship of iron status in humans has received limited attention (Diamond et al., 1991; Harris et al., 2003; Maillet et al., 1998). The purpose of this study was to examine the relationship between iron status, assessed by serum ferritin, serum transferrin receptor, and total body iron (TBI), and bone health, including bone formation, bone resorption, bone mineral content (BMC), and bone mineral density (BMD), in collegiate female athletes. TBI (mg/kg), a relatively new method to assess iron status, was used to identify individuals susceptible to iron deficiency. TBI is based on an equation using serum transferrin receptor and serum ferritin (Cook et al., 2003).

Methods

Subjects

Subjects for this study were 80 female freshmen collegiate athletes 18 $\pm$ 0.5 years of age recruited from the Iowa State University Athletic Program. The athletes were recruited based on their expertise in a particular sport, which they participated in prior to college. The subjects included 8 basketball players, 3 golfers, 6 gymnasts, 17 soccer players, 8 swimmers, 16 softball players, 4 tennis players, 13 track and field athletes, and 5 volleyball players. All protocols were approved by the Institutional Review Board at Iowa State University and all subjects/guardians signed an informed consent form.

Study protocol

Subjects were contacted and recruited during the summer or early fall prior to their collegiate athletic career. After receiving informed consent, subjects completed a radiological assessment of bone density (DXA scan). A physical exam required of all female athletes entering the athletic program was performed at the Student Health Center on campus. During the physical exam 7 ml of blood were drawn from the antecubital vein, stored on ice, and

centrifuged at 10°C at 2500 rpm for 15 minutes. Serum was stored at -80°C within 1 hour of collection in separate aliquots for the analysis of bone markers and iron status.

Anthropometrics and Bone Mineral Density Measurements

Each subject's height was measured, using a set square to the nearest 0.1 cm, and weight was measured using a triple beam balance scale to the nearest 0.1 kg. Total body density, total BMD and BMC were estimated (N=80) by a trained certified professional with dual-energy x-ray absorptiometry (DXA; Hologic Delphi W bone densitometer, software version 11.2; Hologic, Inc.; Bedford, MA).

Bone metabolic markers

Bone formation was assessed by Enzyme-Linked Immunosorbent Assay (ELISA) for the estimate of the C-terminal propeptide of type I collagen (CICP) (N=80) in serum [normal range for 4-18 years of age, 110-966 ng/ml (Quidel Corporations, San Diego)]. Bone resorption was assessed by ELISA for the estimate of cross-linked N-telopeptides of type I collagen (NTx) (N=80) in serum [normal range for 25-49 years of age, 6.2-19.0 nM BCE (Ostex International Inc., Seattle)]. The intra- and inter-assay %CV for CICP were 7.0% and 3.2%, and 5.0% and 1.6% for NTx, respectively. A bone formation to bone breakdown (Bone F:B) ratio was calculated to provide an estimate of bone turnover.

Iron status

Hemoglobin (Hb), Hematocrit (Hct), serum ferritin, and serum transferrin receptor (sTfr) status of the athletes were used to examine iron status. Serum ferritin (N= 80) was assayed by Immunoradiometric (RIA) procedure (Ramco Laboratories Inc., Stafford) with an intra-assay %CV range of 3.6%-10.8%. Serum transferrin receptor (N=74) was assayed by ELISA with an intra-assay %CV range of 6.1%-6.9% [normal range 2.9-8.3 mg/L (Ramco Laboratories Inc., Houston)]. Hb (N=73) and Hct (N=69) values were obtained from the physical exam. Total body iron (TBI) was calculated using the serum transferrin receptor to ferritin ratio {(body iron (mg/kg) = -[log(sTfr/serum ferritin) – 2.8229]/0.1207)} (N = 74), (Cook et al., 2003).

To examine the relationship between iron status and bone turnover, TBI was divided into iron deficiency (negative TBI) and normal (positive TBI) (Cook et al., 2003). The relationship between iron deficiency and bone status, (bone formation, bone resorption, BMD and BMC), was examined by categorizing the stages of iron deficiency into ideal (serum ferritin >40 μg/L) (n = 7), iron depletion (serum ferritin = 20-40 μg/L) (n = 35), iron deficiency (serum ferritin 12-20 μg/L and Hb>12 g/dL) (n = 35), and iron deficiency anemia (IDA) (serum ferritin <12 μg/L and Hb<12 g/dL) (n = 3) using criteria from Cook and Finch (1979) and Herbert (1986).

Statistical Analyses

Descriptive statistics (frequencies, percentiles, minimum, maximum, means, and standard deviations) were performed on the data. Data were examined to verify normality of distribution. The bone marker analysis data and iron status markers showed a skewed distribution, thus statistical analysis was performed on logarithmically transformed data. Descriptive statistics are reported as means ± SD, all other statistical analyses are reported as means ± SE. Group differences between the iron status and bone markers were analyzed using analysis of variance (ANOVA) followed by Tukey's post hoc analysis. Comparison of normal and iron deficient TBI was examined by independent sample *t*-test. Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS, version 11.5) software (SPSS, Inc.; Chicago, IL).

Results

Subject characteristics appear in Table 1. Ideal levels of serum ferritin (>40 μg/L) were observed in 9% of the subjects. Indicators of tissue iron deficiency, sTfr (>8 mg/L, Cook et al., 2003) and a negative TBI, were observed in 8% and 14% of the subjects, respectively.

The relationship between TBI and bone markers appears in Figure 1. There was no significant difference in bone formation between the iron deficient and normal groups. Bone F:B was higher ($P<0.01$) in the iron deficient group than the normal group. Conversely, bone breakdown was lower ($P<0.01$) in the iron deficient compared to the normal group.

Figure 2 displays the relationship between iron status and bone markers. Bone breakdown was significantly higher ($P<0.05$) in iron-depleted subjects compared to those classified as iron deficient or IDA; in addition, they were marginally higher than those with ideal iron status ($P=0.07$). Bone F:B was significantly lower ($P<0.01$) in subjects with iron depletion than those with iron deficiency. Bone formation was significantly higher ($P<0.01$) in subjects with iron deficiency than those with iron depletion and ideal iron status.

No significant differences in BMD and BMC were found between the stages of iron status. BMC tended to decrease with increasing severity of iron deficiency and tended to be highest in those with ideal iron status.

Discussion

The purpose of this study was to examine the relationship between iron status and bone status/turnover in collegiate female athletes. The data suggest that iron stores are negatively associated with bone formation and positively associated with bone breakdown.

Female athletes are highly susceptible to iron deficiency (Peterson, 1998) and impaired bone health (Braam et al., 2003; Otis et al., 1997). The mechanical stress of exercise induces bone turnover (Bernardi et al., 2004) and iron loss (Cowell et al., 2003). Iron losses (menstrual, fecal, urinary, and sweat iron losses), in combination with low dietary iron intake, increase the risk for iron deficiency (Brune et al., 1986; Hallberg & Magnusson, 1984; Siegel et al., 1979; Stewart et al., 1984). Iron is an essential component of bone cells and serves as a cofactor for prolyl and lysyl hydroxylase, which are important enzymes for collagen synthesis, thus may promote bone formation (Tuderman et al., 1977). However, numerous reports indicate that excessive iron levels may have an inhibitory effect on osteoblast function (Diamond et al., 1991; Van de Vyver et al. 1988 and Lynch et al., 1970) and increase the probability of decreased bone mass in humans (Diamond et al., 1989; Schnitzler et al, 1994; and Sherman et al., 1970; Sinigaglia et al. 1997).

In the present study, subjects were categorized by iron status (ideal and three stages of iron deficiency). The first stage of iron deficiency, iron depletion, is characterized by a decrease in tissue iron stores. Subjects with iron depletion in this study had significantly higher bone breakdown than those with iron deficiency and IDA, and lower Bone F:B than

those with iron deficiency. This suggests that an initial decrease in tissue iron stores may negatively impact bone turnover in female athletes. In addition, iron depletion has also been reported to reduce the ability to sustain endurance capacity (Brownlie et al., 2004). Thus, iron depletion appears to be a critical stage of iron deficiency where an athlete may experience compromised performance in both endurance and bone health and needs to be acknowledged. This results is noteworthy considering the majority of National Collegiate Athletic Association Division I-A schools inadequately assess iron status of female athletes, thus fail to identify athletes with iron depletion (Cowell et al., 2003). In addition, at this stage of iron deficiency individuals more readily respond to iron supplementation, thus compromised performance is more likely to be reversed. Conversely, in the present study, subjects with iron deficiency had significantly higher bone formation than the iron depleted and ideal groups, which contradicts the theory that adequate iron promotes bone formation (Tuderman et al., 1977).

The relationship between TBI and bone turnover showed that the iron deficient group had significantly higher Bone F:B than the normal group. In addition, bone breakdown was significantly lower in the iron deficient group than the normal group. However, there was no significant difference in bone formation between the groups. These results suggest that iron deficiency was associated with higher bone formation and lower bone breakdown, whereas elevated iron levels were associated with lower bone formation and higher bone breakdown. This observation was unexpected, given the biological function of iron in bone formation and previously postulated theories that iron would have a positive impact on bone status. A possible explanation is that the osteogenic effect of exercise induced bone turnover (Bernardi et al., 2004) superseded the negative impact of iron deficiency on bone formation. The acute effect of exercise may increase bone turnover by 15% to 40% (Bernardi et al., 2004). In addition, young growing individuals have greater bone turnover, and the circadian variability, with peak bone turnover in the morning, contributes to the limitations of bone marker assessment. These confounding factors may elevate bone turnover independent of iron status.

The effect of iron status on BMD and BMC remains uncertain. In humans, dietary intervention studies have reported that increased iron intake is associated with increased BMD (Michaelsson et al., 1995; Angus et al., 1988; Harris et al., 2003). A similar

relationship was reported in rats, where dietary iron deficiency impaired mineralization and collagen maturation of the rat femur, and resulted in decreased BMD and increased bone fragility (Smoliar et al 1984; Kipp et al., 2002). Conversely, Medeiros et al. (2002) reported that dietary iron in combination with calcium had a negative impact on bone mineral content, thus one would suspect a similar relationship with iron status.

There are limited data on iron status and bone health. Maillet et al. (1998) reported a positive correlation between serum ferritin concentrations and skull BMD in young females; however, this finding was not verified in the present study. In addition, bone iron content has been negatively correlated with calcium and collagen (Massie et al., 1990). This study did not find significant relationships between total body BMD, total body BMC, and iron status.

To our knowledge this is the first study to present the relationship between iron status and bone turnover in young female athletes. The results in the present study suggest that a negative iron status in female athletes is associated with a positive bone turnover. The negative relationship between iron status, TBI and bone turnover (CICP and NTx) was unexpected and warrants further research.

Table 1. Subject characteristics[a]

	Female Athletes (N = 80)	Range
Age, yr	18 ± 1	17 - 20
Height, cm	168.9 ± 7.4	155 - 189
Weight, kg	64.6 ± 9.0	47 - 99
BMD, g/cm[b]	1.2 ± 0.8	1.03 - 1.40
BMC, g	2465 ± 276.	1930 - 3424
CICP, ng/ml	128.7 ± 54.7	55 - 335
NTx, nM BCE[b]	16.8 ± 5.2	8 - 32
Bone F:B	8.2 ± 3.8	3 - 20
Ferritin, µg/L	23.0 ± 13.8	4 - 88
sTfr, mg/L	5.0 ± 2.1	2 - 15
TBI, mg/kg	3.4 ± 3.1	-4 - 10
Hb, g/dL	13.7 ± 0.9	11 - 17
Hct, %	39.8 ± 2.5	33 - 47

[a]Values are mean ± SD

[b] BCE = bone collagen equivalents

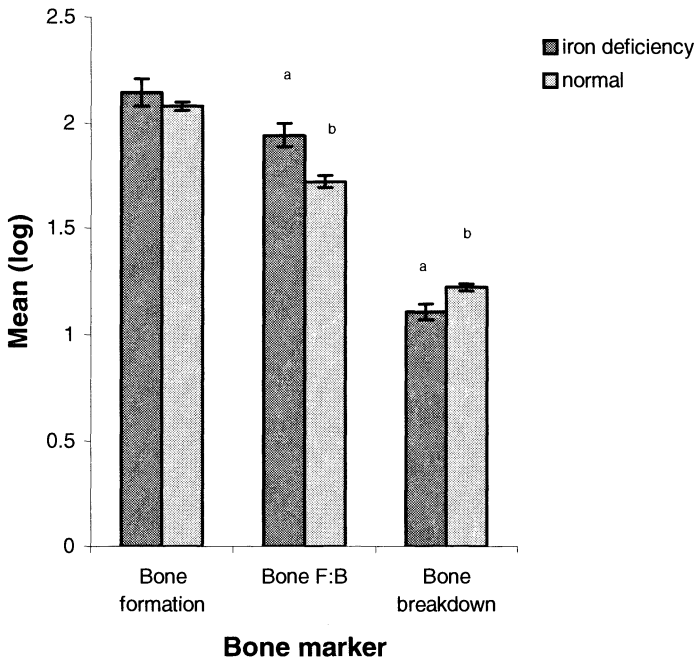

Figure 1. Values are means ± SE of bone marker status by TBI
Iron deficiency = negative TBI, normal = positive TBI
Different letters indicate significant difference between
iron deficiency and normal at a p-value of <0.01

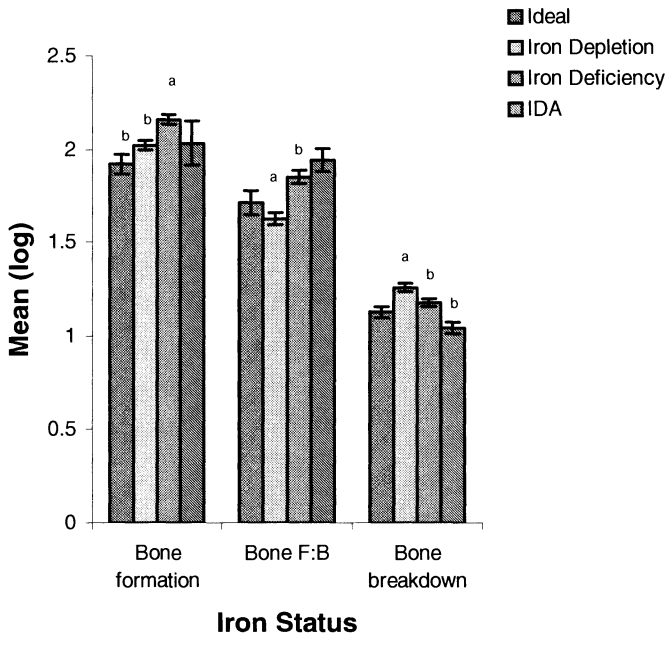

Figure 2. Values are means ± SE of bone marker status by iron status
Variables with different letters indicate significant difference at a p-value of <0.05.

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CHAPTER 6. GENERAL CONCLUSION

The physical stress of exercise and/or engagement in poor nutrition habits places female athletes at increased risk for weight loss, menstrual disturbances, and impaired bone health (Otis et al., 1997). Exercise increases bone turnover, which in a healthy state of hormonal balance and nutritional adequacy, has an osteogenic effect on bone. Consequently, female athletes tend to have greater bone mass density (BMD) and bone mineral content (BMC) than non-athletes (Alfredson et al., 1997). However, greater BMD and BMC may not be protective against stress fractures.

Bone turnover is affected by the physical impact of sports. High impact sports (i.e. Gymnastics, Volleyball, Basketball) have higher bone resorption rates than medium (i.e. Track, Soccer, Softball, Tennis) and low (i.e. Swimming) impact sports. Female athletes in this study participating in high impact sports had leaner body composition, later age of menarche, and higher prevalence of low estradiol levels than low and medium impact sports. These characteristics place the athletes at increased risk for amenorrhea, bone loss, stress fractures, and osteoporosis. The present study introduces a new model to identify athletes at increased risk for stress fractures. This model suggests that High impact sports and low Bone F:B are predictive of increased probability of stress fractures.

Bone turnover may be influenced by iron status. Iron is essential for iron-dependent enzymes involved in formation type I collagen of bone, playing a role in bone health (Tuderman et al., 1977). Many female athletes suffer from iron deficiency (Peterson, 1998), thus one would expect a negative bone turnover in these athletes. Numerous rat and human studies suggest a relationship between dietary iron, bone iron content, and bone (Diamond et al., 1989; Kipp et al., 2002; Kipp et al., 1998; Malecki et al., 2000; Massie et al., 1990; Medeiros et al., 2002; Schnitzler et al, 1994; Sherman et al., 1970). However, no previous human studies have examined the relationship between tissue iron (serum ferritin, sTfr, TBI) and bone status. This study reports that iron deficiency is negatively associated with bone formation and positively associated with bone breakdown. A theory that iron deficiency results in a negative bone turnover was not supported by the results of this study.

Future Considerations

- Sport impact may be related to body composition, age of menarche, bone turnover, and bone mineral density, which are key factors for optimal bone health, thus it is recommended that sport impact be included when assessing bone status and stress fractures in female athletes.
- Currently, a number of bone markers are used in research and some lack standardized reference values. Future research needs to identify valid and reliable bone markers with standardized reference values to facilitate the comparison of bone status among studies.
- The negative relationship between tissue iron status indicators (serum ferritin, sTfr, and TBI) and bone turnover (CICP and NTx) was unexpected and warrants further research.

APPENDIX A.
CONSENT FORM

Health and Nutrition Status of Female College Athletes
Consent Form

You are invited to be in a research study examining the health and nutrition status of female college athletes. You were selected as a possible participant because you are a new female athlete entering the Iowa State University Athletic program.

We ask that you read this document and ask any questions that you may have before agreeing to be in the study. This study is being conducted by faculty/staff in the Department of Food Science and Human Nutrition at Iowa State University.

Background Information:

The purpose of this research study is to examine the nutrition and health status among college female athletes. It is designed to examine eating attitudes and habits, blood tests, body composition, and bone density among the subjects. If you agree to be in this study, you will be asked to complete a 49-question survey, a blood draw, and body composition assessment. The surveys will take approximately 15 minutes to complete. The blood draw will take place as part of the physical you are required to complete for participation in the Athletics programs. The body composition assessment will take approximately 20 minutes to complete. Throughout your collegiate career your health status will be monitored for events such as stress fractures, anemia, and any acute health illness.

Procedures:

Surveys: The 49-question survey consists of basic health/nutrition habits and beliefs. Completion of the surveys will take approximately 15 minutes.

Blood Draw: blood draw as part of the health physical required for participation in ISU Athletics programs. The venous puncture required for the blood draw may cause emotional and/or physical discomfort for some subjects. Five ml of blood are drawn into an EDTA tube for a Complete Blood Count (CBC) and sickle cell anemia assay. An additional 6 ml of blood is drawn into a serum tube for rubella and cholesterol assays.

For this study, an additional 7 ml (a little more than a teaspoon) of blood will be drawn into another serum tube for ferritin and transferrin receptor assays. The additional amount of blood will not require an additional venous puncture. Only the results of the ferritin and transferring receptor assays will be used for this study.

Dual X-Ray Absorptiometry (DXA): body composition analysis during the scheduled ISU orientation visit. Height, weight, and age is required for the completion of this test. This test is non-invasive and conducted in full clothing in the supine position. There is minimal physical discomfort associated with this test. Some subjects may experience emotional discomfort with being weighed and having body composition assessed. This test will take approximately 20 minutes to complete.

Risks of Being in the Study:

- Bruising and infection (rare) with the blood draw.
- Exposure to .5 mRem of radiation with the DXA, which is less than that experienced in a cross-country airplane flight. There are no known risks associated with radiation exposure levels this low.

Benefits of participating in this study include:

- analysis and interpretation of body composition data.
- analysis and interpretation of bone density.

The information listed above will be provided to you in written form during an individual appointment to be scheduled during the fall semester 2003. At that time you may also request to receive a copy of the group results including survey results, blood work results, body composition results, and bone density results.

Injury:

Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance.

Confidentiality:

All data collected on each subject will be stored in a locked file cabinet and assigned a code number for analytical purposes. Complete confidentiality will be maintained, as participation identification will not be recorded in any way that it could be recalled and/or matched to a particular subject. Presentations or manuscripts pertaining to this study will only report group data. Absolute confidentiality cannot be guaranteed, since research documents are not protected from subpoena.

Voluntary Nature of the Study:

Your decision whether or not to participate will not affect your current or future relations with Iowa State University. If you decide to participate, you are free to withdraw at any time without affecting your relationship with Iowa State University or the ISU Athletics program.

New Information

You will be told of any new information that comes out while you are in this study that might make you change your mind about staying in the study. At the end of the study, you will be told when study results may be available and how you can find out about them.

Contacts and Questions:

Ruth Litchfield, PhD, RD, LD
Extension Specialist/Assistant Professor
133 MacKay
Iowa State University
Ames, IA 50011-1120
515-294-9484

Paul Flakoll, PhD
Director, Center for Designing Food to Improve Nutrition
1127 HNSB
Iowa State University
Ames, IA 50011-1120
515-294-8935

Manju Reddy, PhD
Associate Professor
1107 HNSB
Iowa State University
Ames, IA 50011-1120

Karin Westberg
Student/Athlete
133 MacKay
Iowa State University
Ames, IA 50011-1120
515-231-4577

If you would like to talk to someone other than the researchers:

Denise Harklau
Head Women's Athletic Trainer
Jacobson Building
Iowa State University
Ames, IA 50011-1140
515-294-8389

You will be given a copy of this form to keep for your records.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

Signature of Participant:

_____ Date _____

Signature of Parent (if participant < 18):

_____ Date _____

Signature of Investigator or Person Obtaining Consent:

_____ Date _____

APPENDIX B.
HIPAA FORM

Notice of Privacy Practices

This notice describes how medical information about you may be used and disclosed and how you can get access to this information.

Please review this information carefully.

This Notice of Privacy Practices describes how researchers of the "Nutritional Status of Female Athletes" project will use and disclose your protected health information to carry out research conducted by the Department of Food Science and Human Nutrition at Iowa State University (ISU). This Notice describes your rights to access and control of your protected health information. "Protected Health Information" is information about you, including demographic information, that may identify you and that relates to your past, present or future physical or mental health condition and related health care services. For purposes of this notice, we will refer to "Protected Health Information" as "PHI".

Description of information to be used

Participation in the project, "Nutritional Status of Female Athletes" will include monitoring your health status through your medical record kept in the Athletic Department at ISU. The researchers of this project will be collecting PHI from your this medical record, including treatment for injuries such as stress fractures or musculoskeletal injuries, and treatment for acute illnesses including respiratory infections, colds, and flu. This PHI will be used to examine the relationship between nutritional status and physical health status.

Who will use the information

Researchers involved with the project "Nutrition Status of Female Athletes" include:

- Ruth Litchfield, PhD, RD, LD, Assistant Professor in the Department of Food Science and Human Nutrition
- Paul Flakoll, PhD, Professor in the Department of Food Science and Human Nutrition and Director of the Center for Designing Foods to Improve Nutrition
- Manju Reddy, PhD, Associate Professor in the Department of Food Science and Human Nutrition
- Karin Westberg, Graduate Student in the Department of Food Science and Human Nutrition

Each of the researchers has completed Human Subjects Training and is familiar with the Health Insurance Portability and Accountability Act (HIPAA) regulations.

Who will receive the information

The researchers listed above are the only individuals that will have access to the PHI collected from your medical record. Any reports of the results of this research will be reported in group or aggregate data, where individual subjects will not be identifiable.

Purpose of the requested use or disclosure
Use of your PHI is for research purposes only. The researchers will be examining the relationship of nutritional status and physical health status in female athletes. The goal of the research is to optimize the overall health status of female athletes at ISU.

Expiration of the project
PHI will be collected from your medical record throughout your collegiate career until you graduate, leave the ISU Athletics Programs, transfer from ISU, or December 31, 2007, whichever event occurs first.

Your decision whether or not to authorize use of your PHI and participate in the research project "Nutritional Status of Female Athletes" is completely voluntary and will not affect your current or future relations with ISU or the ISU Athletics Program. If you decide to participate, you are free to withdraw from the project and revoke this authorization to your PHI at any time by informing any of the previously listed researchers in writing. Withdrawal from the project will not affect your relationship with ISU or the ISU Athletics program.

You will be given a copy of this form to keep for your records.

Statement of Authorization:

I have read and understand the above information. I authorize the researchers of the project, "Nutritional Status of Female Athletes" to access PHI from my medical records in the Athletic Department at ISU.

Signature of Participant:

_____ Date _____

Signature of Parent or Legal Guardian (if participant < 18):

_____ Date _____

Signature of Investigator or Person Obtaining Consent:

_____ Date _____

APPENDIX C.
EATING SURVEY

Please complete the following questionnaire as completely and honestly as possible. Do not worry about whether your answers are 'right' from a nutrition or health point of view. You may skip any questions that you are uncomfortable answering. Your responses to this questionnaire are strictly confidential and will not impact your student-athlete eligibility or participation.

KEY: Exercise = physical activity ≥ 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 ≥ 20 minutes in length on days that I have practice or competition.
   a. Frequently b. Sometimes c. Rarely d. Never

2. If I cannot exercise, I find myself worrying that I will gain weight.
   a. Frequently b. Sometimes c. Rarely d. Never

3. I believe that most female athletes have some form of disordered eating habits.
   a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

4. During training, I control my fat and calorie intake carefully.
   a. Frequently b. Sometimes c. Rarely d. Never

5. I do not eat foods that have more than 3 grams of fat.
   a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

6. My performance would improve if I lost weight.
   a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

7. If I get on the scale tomorrow and gained 2 pounds, I would practice or exercise harder or longer than usual.
   a. Frequently b. Sometimes c. Rarely d. Never

8. I weight myself _____.
   a. Daily b. 2 or more times a week c. Weekly d. Monthly or less

9. If I chose to exercise on the day of competition (Game/Meet), I exercise for
   a. 2 or more hours b. 45 minutes to 1 hour c. 30 to 45 minutes d. 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.
    a. Frequently b. Sometimes c. Rarely d. Never

11. I feel guilty if I choose fried foods for a meal.
    a. Frequently b. Sometimes c. Rarely d. Never

12. If I were to be injured, I would still exercise even if I was instructed no to do so by my athletic trainer or physician.
    a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

13. I take dietary or herbal supplements in order to increase my metabolism and/or to assist in burning fat.
    a. Frequently b. Sometimes c. Rarely d. Never

14. I am concerned about my percent body fat.
    a. Frequently b. Sometimes c. Rarely d. Never

15. Being an athlete, I am very conscious about consuming adequate calories and nutrients on a daily basis.
a. Frequently b. Sometimes c. Rarely d. Never

16. I am worried that if I were to gain weight, my performance would decrease.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

17. I think that being thin is associated with winning.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

18. I train intensely for my sport so I will not gain weight.
a. Frequently b. Sometimes c. Rarely d. Never

19. During season, I choose to exercise on my one day off from practice or competition.
a. Frequently b. Sometimes c. Rarely d. Never

20. My friends tell me that I am thin but I feel fat.
a. Frequently b. Sometimes c. Rarely d. Never

21. I fell uncomfortable eating around others
a. Frequently b. Sometimes c. Rarely d. Never

22. I limit the amount of carbohydrates that I eat.
a. Frequently b. Sometimes c. Rarely d. Never

23. I try to lose weight to please others.
a. Frequently b. Sometimes c. Rarely d. Never

24. If I were unable to compete in my sport, I would not feel good about myself.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

25. If I were injured and unable to exercise, I would restrict my calorie intake.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

26. In the past 2 years I have been unable to compete due to an injury.
a. 7 or more times b. 4 to 6 times c. 1 to 3 times d. No significant injuries

27. During practice I have trouble concentrating due to feelings of guilt about what I have eaten that day.
a. Frequently b. Sometimes c. Rarely d. Never

28. I feel that I have a lot of good qualities.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

29. At times I feel that I am no good at all.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

30. I strive for perfection in all aspects of my life.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

31. I avoid eat meat in order to stay thin.
a. Strongly Agree b. Agree c. Disagree d. Strongly Disagree

32. I am happy with my present weight
a. Yes b. No

33. I have done things to keep my weight down that I believe are unhealthy.
a. Frequently b. Sometimes c. Rarely d. Never

34. How many pounds over your desired weight were you at your maximum weight? _____

35. How often are you dieting?
    a. Rarely b. Sometimes
    c. Usually d. Always

36. Which best describes your behavior after you have eaten a "no allowed" food while on your diet?
    a. Return to diet
    b. Stop eating for an extended period of time in order to compensate
    c. Continue on a splurge, eating other "not allowed" foods

37. What is the maximum amount of weight that you have ever lost within 1 MONTH? _____

38. What is your maximum weight gain within a WEEK? _____

39. In a typical week, how much does your weight fluctuate (maximum – minimum)? _____

40. Would a weight fluctuation of 5 pounds affect the way you live your life?
    a. Not at all b. Slightly
    c. Moderately d. Extremely

41. Do you eat sensibly before others and make up for it alone?
    a. Never b. Rarely
    c. Often d. Always

42. Do you give too much time and thought to food?
    a. Never b. Rarely
    c. Often d. Always

43. Do you have feelings of guilt after overeating?
    a. Never b. Rarely
    c. Often d. Always

44. How conscious are you of what you're eating?
    a. Not at all b. Slightly
    c. Moderately d. Extremely

45. How would you describe your weight during childhood (ages 5-11)?
    a. Definitely underweight b. Somewhat underweight c. About right
    d. Somewhat overweight e. Definitely overweight

46. How would you describe your weight during adolescence (ages 12-18)?
    a. Definitely underweight b. Somewhat underweight c. About right
    d. Somewhat overweight e. Definitely overweight

47. How would you describe your weight NOW?
    a. Definitely underweight b. Somewhat underweight c. About right
    d. Somewhat overweight e. Definitely overweight

48. How would you describe your biological mother's weight?
    a. Definitely underweight b. Somewhat underweight c. About right
    d. Somewhat overweight e. Definitely overweight f. N/A

49. How would you describe your biological father's weight?
    a. Definitely underweight b. Somewhat underweight c. About right
    d. Somewhat overweight e. Definitely overweight f. N/A

APPENDIX D
NUTRITIONAL QUESTIONNAIRE

Intercollegiate Athletics – Iowa State University
Feldman Athletic Health Care Center – Jacobson Athletic Complex Ames, IA 50011
(515) 294-4441 (Phone) 515-294-6554 (Fax)

Nutritional Questionnaire

Name: _____ Age: _____

Sport: _____ Years participated in this sport: _____

Height: _____ Weight: _____

1. What is your desired body weight? _____
2. When were you last at that weight?

   ☐ Never ☐ 1 –2 years ☐ 4-5 years
   ☐ Within the last 6 months ☐ 2-3 years ☐ Other
   ☐ 6 months – 1 year ☐ 3-4 years

3. Assuming the following statements are true, what do you think you would weigh _____ if you:
   - Did not restrict your food selection
   - Ate meals both rich in nutrients and are tasty
   - Ate when hungry and stopped when full
4. How do you currently feel about your weight?
5. Are you currently taking any action towards that feeling?
   ☐ trying to lose weight ☐ stay the same weight
   ☐ trying to gain weight ☐ I am not doing anything about my weight.
6. At what approximate age did your menstrual period begin? _____
7. How long do your periods last? _____
8. How many periods have you had in the last year? _____
9. Have you ever gone for 3 or more months without having a period? ☐ Yes ☐ No
10. When was your last period? _____
11. Are you currently taking Birth Control Pills or other hormone replacement? ☐ Yes ☐ No
    a. If yes, how long have you been taking them? _____
    b. If yes, did you start on these for the reason of regulating your menstrual cycle? ☐ Yes ☐ No
12. In the past, have you had any type of sports injury? ☐ Yes ☐ No
    If yes, did you feel bad about your body or weight in response to how the injury affected you? (Explain)
13. Have you ever had a stress fracture? ☐ Yes ☐ No (If yes, please explain)
14. Have you ever been treated for anemia? ☐ Yes ☐ No
15. How many meals do you eat per day? _____
16. How many snacks do you eat during the day? _____
17. Do you frequently skip meals? ☐ Yes ☐ No
    If yes, explain: _____
18. How do you describe your eating habits? ☐ good ☐ fair ☐ poor

19. What percent of your day do you think about food?

☐ 0-15 ☐ 15-25 ☐ 25-50 ☐ 50-75 ☐ 75-100

20. Do you avoid any of the following foods? (Check all that apply)

☐ Red meat
☐ Poultry (chicken, turkey)
☐ Fish
☐ Dairy (milk, cheese)
☐ Vegetables
☐ Fruits
☐ Fried foods
☐ Breads
☐ Grains (pasta, rice)
☐ Fast foods
☐ Sweets (candy, dessert)
☐ Alcohol
☐ Fats/oils (mayo,butter, etc)

☐ Other (please specify) _____

Please briefly explain why you avoid these foods:

21. Do you eat or crave certain foods then feel guilty eating them? ☐ Yes ☐ No
If yes, please list those foods:

22. Do you currently take any supplements? ☐ Yes ☐ No (If yes, which ones?)

☐ Iron ☐ Vitamin C ☐ EnergyBoosters (e.g. Ephedra, Ma Huang)
☐ Calcium ☐ Multi-vitamin ☐ Herbs ☐ Creatine
☐ Other Please List: _____

23. **Please indicate which foods you eat.**

	Less than once a week	Not daily but at least once a week	Daily	Never or Rarely
Milk, yogurt				
Cheese				
Red meat (beef and pork)				
Poultry				
Fish				
Eggs				
Mixed dishes (casseroles, lasagna, etc.)				
Dried beans, legumes				
Peanut butter				
Nuts				
Breads, cereal				
Potatoes, pasta, rice				
Fruits				
Fruit Juice				
Vegetables				
Vegetable Juice (such as V8)				
Margarine, butter				
Cooking oil				
Sour cream, salad dressing				
Ice cream				

	Less than once a week	Not daily but at least once a week	Daily	Never or Rarely
Cookies, cake, pie				
Candy				
Soft drinks				
Coffee				
Tea, iced tea				
Alcohol				

24. Typically, about how many cups of water, juice, sports drink, or non-caffeinated beverages do you drink **during the 2 hours before exercise? (Check one)**
☐ None ☐ 3-5 cups
☐ 1-2 cups ☐ More then 5 cups

25. Typically, about how many cups of water, juice, sports drink, or non-caffeinated beverages do you drink **during each hour** of exercise? **(Check one)**
☐ None ☐ 3-5 cups
☐ 1-2 cups ☐ More then 5 cups

26. 30. Typically, about how many cups of water, juice, sports drink, or non-caffeinated beverages do you drink **during the first two hours after exercise? (Check one)**
☐ None ☐ 3-5 cups
☐ 1-2 cups ☐ More then 5 cups

27. How many hours per week, on the average, do you engage in planned exercise (including sport participation/practice)?
☐ 5-10 ☐ 15-20 ☐ More then 25
☐ 10-15 ☐ 20-25

28. How many hours per week, on average, do you engage in exercise for pleasure or weight loss?
☐ 0 ☐ 1 – 1.5 ☐ more than 2
☐ less than 1 ☐ 2

29. How many times a year do you intentionally lose weight for your sport? _____

30. Have any of your coaches asked you to lose weight for your sport? ☐ Yes ☐ No
If **yes**, please explain:

31. Do you think your performance would improve if you lost or gained weight? ☐ Yes ☐ No

32. Name some things that you like about your body?

33. Name some things you don't like about your body?

34. Overall, how satisfied are you with the physical appearance of your body? **(Check one)**
☐ very satisfied ☐ somewhat dissatisfied
☐ somewhat satisfied ☐ very dissatisfied

35. Overall, how satisfied are you with the physical performance of your body? **(Check one)**
- ☐ very satisfied
- ☐ somewhat satisfied
- ☐ somewhat dissatisfied
- ☐ very dissatisfied

36. How easy or difficult is it for you to maintain your in-season weight? **(Check one)**
- ☐ very easy
- ☐ somewhat easy
- ☐ somewhat difficult
- ☐ very difficult

37. Do you have any personal goals for body composition? ☐ Yes ☐ No
If yes, which ones? **(Check all that apply)**
- ☐ Gain lean mass/weight gain
- ☐ Decrease body fat
- ☐ Lose weight
- ☐ Maintain current body composition
- ☐ None

38. Do you know which dietary supplements are banned or restricted by the NCAA? ☐ Yes ☐ No

39. **Please indicate whether you agree or disagree with the following statements by placing a check (√) in the appropriate column.**

	Agree	Disagree	Don't Know
The lack of a menstrual cycle is acceptable for an athlete's overall health			
Carbohydrates and fats are the main source of energy for muscles.			
Protein is the primary source of energy for muscles.			
Sweets should not be eaten prior to an athletic event.			
Fluids should be replaced before, during and after athletic events.			
Sports drinks are better than water for replacing fluid losses.			
Protein supplements are needed in addition to dietary protein for muscle growth and development.			
Eating carbohydrates makes you fat.			
Meals high in fat should be consumed 2-3 Hours before training or competition.			
Athletes can rely on thirst to ensure fluid replacement during and after competition.			
Dehydration decreases athletic performance.			
Vitamin and mineral supplements increase energy level.			

40. If you would like to learn more about any of these topics place a check in the box.
- ☐ Nutrition programs for peak performance
- ☐ Weight gain
- ☐ Exercise and fitness programs
- ☐ Cooking demonstrations/meal preparation
- ☐ Fluids
- ☐ Carbo loading
- ☐ Off-season vs. in season nutrition
- ☐ Weight control
- ☐ Eating disorder counseling
- ☐ Grocery store tour
- ☐ Tips on eating out
- ☐ Supplements
- ☐ Eating on the road

Please list if there are any other topics you would like more information about that are not listed above:

41. When you eat out where are the 3 most common places you go:

_____

_____

_____

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