DOES RELATIVE ENERGY DEFICIENCY IN SPORT UNDERMINE BONE HEALTH?

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Relative Energy Deficiency in Sport (RED-S) is a term expanded from the female athlete triad the is inclusive to males and females and the negative physiological symptoms impacting athlete health and performance from low energy availability. Bone health is one of the ten health consequences of RED-S. Therefore, the purpose of this study is to investigate RED-S in female and male collegiate athletes and determine if there is an association with bone health. Thirteen participants completed an electronic survey containing the LEAF-Q and EAT-26, a three-day food diary and exercise log, and a DXA scan. Energy intake and exercise expenditure was analyzed using an ESHA food analysis processor. Participants at risk for RED-S had higher occurrences of injuries ($p<0.022$) and lower Z-scores ($p<0.063$) than those not at risk for RED-S. In conclusion, athletes at risk for RED-S may have higher occurrences of injuries and lower bone mineral density.

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DEDICATION

To my mother, who has always encouraged me.

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LIST OF ABBREVIATIONS

BEDA-Q ..... Brief Eating Disorder in Athletes Questionnaire

EEE ..... Exercise Energy Expenditure

EA ..... Energy Availability

Triad ..... The Female Athlete Triad

LEA ..... Low Energy Availability

RED-S ..... Relative Energy Deficiency in Sport

LIST OF DEFINITIONS

Amenorrhea	Absence of menstrual cycles for more than 90 days (Nattiv et al., 2007).
Disordered Eating	Various abnormal eating behaviors, including restrictive eating, fasting, frequently skipped meals, diet pills, laxative, diuretics, enemas, overeating, binge-eating and then purging or vomiting (Nattiv et al., 2007).
Eating Disorder	A clinical mental disorder defined by DSM-IV and characterized by abnormal eating behaviors, an irrational fear of gaining weight, and false beliefs about eating, weight, and shape (Nattiv et al., 2007).
Eating Disorder Screen for Primary care	Questions used to screen for eating disorders in primary care patients and university students and determination of more detailed assessment of potential eating disorder (Cotton, Ball, & Robinson, 2003).
Eating Disorders Not Otherwise Specified	A diagnosis applied when an individual's symptoms narrowly fall short of meeting the full criteria for anorexia, bulimia, binge eating disorder, or other categories (Le Grange, Swanson, Crow, & Merikangas, 2012).
Energy Availability	Dietary energy intake minus exercise energy expenditure normalized to fat-free mass (Nattiv et al., 2007).
Eumenorrhea	Menstrual cycles at intervals near the median interval or young adult women. In young adult women, menstrual cycles recur at a medial interval of 28 days that varies with a standard deviation of seven days (Nattiv et al., 2007).
Exercise Energy Expenditure	The energy expended during exercise training in excess of the energy that would have been expended in non-exercise activity during the same time interval. Neglecting the adjustment for non-exercise activity causes EA to be underestimated by a few kcal/kg/FFM/day, which is a negligible error for most purposes (Nattiv et al., 2007).

Female Athlete Triad	Relationships among energy availability, menstrual function, and BMD that may have clinical manifestation including eating disorders, functional hypothalamic amenorrhea, and osteoporosis (Nattiv et al., 2007).
Low Energy Availability	Occurs when energy intake is reduced or exercise load is increased, causing a change in body systems, hormones, and metabolic and functional characteristics to reduce energy expenditure (Mountjoy et al., 2014).
Luteal Phase	The second phase of the menstrual cycle wherein progesterone is produced, therefore inhibiting endometrial proliferation and determining endometrial receptivity. This phase normally runs for 11 to 17 days (Mesen & Young, 2015).
Oligomenorrhea	Menstrual cycles at intervals longer than 35 days (Nattiv et al., 2007).
Relative Energy Deficiency in Sport	Syndrome that impairs physiological functioning caused by relative energy deficiency, and includes but is not limited to impairments of metabolic rate, menstrual function, bone health, immunity, muscle protein synthesis, and cardiovascular health (Mountjoy et al., 2014).

INTRODUCTION

Relative energy deficiency in sport (RED-S) is a condition in which low energy availability (LEA) occurs in athletes and results in negative effects on health and performance in both females and males (Mountjoy et al., 2014). Relative energy deficiency in sport (RED-S), defined as a syndrome that impairs physiological functioning caused by RED-S, and includes but is not limited to impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis, and cardiovascular health, is commonly found in athletes and those training for recreational activities in which the individual is expending more energy than being consumed (Mountjoy et al., 2014). A lack of knowledge regarding RED-S exists among coaches, athletic trainers and athletes (Mountjoy, Costa, et al., 2018).

There are organizations for both recreational and professional sports that exist to protect athletes from being harmed. The International Sports Federation and the International Olympic Committee (IOC) are two programs tasked to manage the risks related to sports, thereby protecting athlete health. The IOC promotes mental and physical health for all athletes ("Who we are," n.d.). The International Sports Federations work in unison with the IOC, assuring that athletes in every sport are practicing within the guidelines and rules of the Olympic charter ("Who we are," n.d.). In 2012, Mountjoy and Junge created a publication detailing the activities and areas of priority for promoting health among International Sport Federations athletes and in the general population. In 2016, the 2012 study was repeated by Mountjoy and colleagues (2018) and included 28 International Sport Federations. Each federation was surveyed to determine their priorities and programming for athletes and for global health by ranking 11 health-related topics. The most significant identified topics, in order of importance, were 'fighting against doping', 'your sport is perceived as safe', and 'health of your elite athletes' (Mountjoy, Costa, et al.,

2018). Programs and guidelines for health-related activities were also reported by International Sport Federations. The most prevalent health related activities were ‘injury prevention by regulation for equipment/venues’ and ‘first aid/minimum safety standards at events’. The least common activities were ‘technology-based health risks’, ‘prevention of chronic disease in the general population’, and lastly, ‘relative energy deficiency in sports’. Furthermore, when comparing the 2016 survey to the 2012 survey, there was a decrease in identified health-related programs, importance for elite athletes such as, ‘elite athlete mental health’, ‘nutrition and hydration’, ‘injury surveillance’, ‘environmental conditions’, and ‘injury prevention programs’ (Mountjoy, Costa, et al., 2018; Mountjoy & Junge, 2013). Overall, the studies determined priority is given to promote health-related topics like preventing doping, injuries, and safety for the athlete and their health.

In an editorial by Mountjoy et al. (2018), Relative Energy Deficiency in Sport: The Tip of an Iceberg, it is suggested that the impact of RED-S is highly underestimated (Mountjoy, Burke, Stellingwerff, & Sundgot-Borgen, 2018). The authors compare the magnitude of concussion research to the importance of awareness for RED-S and the impact of future research. The information from the comparison of the International Sport Federation surveys from 2012 to 2016 states that nutrition and RED-S are given a low priority by programs, but to improve athlete health and performance and decrease injury, Mountjoy et al. (2018) suggests including an increase in RED-S knowledge education in a more comprehensive approach to achieve an all-encompassing approach to athlete health, with education as a vital tool, not only for injury prevention, but treatment as well. In the conclusion of the editorial the authors stated that RED-S should be better known as cause for injury in sport and that knowledge and awareness is important to maintaining overall athlete health (Mountjoy, Costa, et al., 2018). In combination

with this coaches, athletic trainers, and athletes should have a higher awareness of the negative effects RED-S has on health and performance and the long-term health consequences. For example, 21.8% of American athletes were found to have low bone mineral density and 20% of injuries treated in sports medicine clinics are stress fractures (Fredericson, Jennings, Beaulieu, & Matheson, 2006; Lambrinoudaki & Papadimitriou, 2010). Therefore, the purpose of this study was to investigate RED-S in female and male collegiate athletes and determine if there is an association with bone health. A study will be designed to answer the following questions: Do female and male long-distance runners who screen positive for RED-S have bone mineral density below recommended levels? Are female and male runners who screen positive for RED-S more susceptible to bone injuries? Do female and male runners who screen positive for RED-S have low energy availability? Do female and male runners who have low energy availability become more susceptible to bone injuries? Do female and male distance runners who are at risk for disordered eating also at risk for RED-S and low energy availability? Do female and male distance runners who are at risk for disordered eating become more susceptible to bone injury?

LITERATURE REVIEW

What is Relative Energy Deficiency in Sport

The etiology of relative energy deficiency in sport was identified and defined by the International Olympic Committee (IOC) in 2014 to serve as a more comprehensive explanation for all individuals who were experiencing symptoms similar to that of the Female Athlete Triad (Triad)(Mountjoy et al., 2014). The Triad is known as a condition influencing three components of female athlete health including energy availability, menstrual function, and bone health (Nattiv et al., 2007). Energy deficiency is the etiological factor at the base of the Triad; therefore, causing the identified physiological symptoms (Mountjoy et al., 2014). However, the Triad is not inclusive of males experiencing physiological symptoms of energy deficiency similar to this syndrome (Mountjoy et al., 2014). Therefore, the term RED-S was developed to include the physiological dysfunction that both males and females experience in a state of energy deficiency. To further understand RED-S and its role in overall athlete well-being, it is critical to describe the basic terms that are used to identify RED-S and past scientific literature to build on its background. Low energy availability is the etiological factor of RED-S that causes physiological impairment. To understand this, the term energy availability (EA) needs to be explained. Energy availability is the energy consumed through dietary intake minus the energy expended in exercise (Loucks et al., 2011). Exercise energy expenditure (EEE) is the expenditure of energy above the normal daily needs of living used during exercise (Anne B Loucks et al., 2011). The calculation is depicted in equation 1.

$$\text{Energy Availability (EA)} = [\text{Energy intake (EI)(kcal)} - \text{Exercise Energy Expenditure (EEE) (kcal)}] \div \text{Fat Free Mass (FFM)(kg)} \quad (1)$$

On average, optimal EA is achieved at 45 kilocalories per kilogram of fat free mass (FFM) per day (kcal/kg FFM/day) (Anne B Loucks et al., 2011). When EA reaches less than 30 kcal per kg FFM, negative physiological changes begin to occur. For example, energy use is diverted to essential functions, optimal metabolic function can become disturbed and physiological functions disabled (Dufour & Sauther, 2002; Jasienska, 2003). Such processes can impact different areas of health and performance such as menstrual function, endocrine systems, bone, psychological, cardiovascular, gastrointestinal, immunological health, metabolic function, hematologic function, growth and development, and have been correlated with depression, irritability, impaired judgement, increased risk for injury, decreased glycogen stores, decreased muscle strength, decreased endurance performance, impaired training response, decreased coordination, and decreased concentration (Mountjoy, Sundgot-Borgen, et al., 2018).

Low energy availability among female athletes

Recent studies have been conducted to estimate the occurrence of low energy availability (LEA) among athletes. A study investigated 13 female, elite national-level track athlete sprinters of mixed ethnicity (Sygo et al., 2018). The athletes were tested for indicators of LEA and the changes in these symptoms over the course a five-month season which ecompassed training and competitive indoor events. Data collected included blood tests, blood pressure, resting metabolic rate (RMR) measured using indirect calorimetry (Quark-Omnia, Cosmed, Italy), dual energy x-ray absorptiometry (DXA), anthropometric measures, and physiological symptoms using the Low Energy Availability in Female's Questionnaire (LEAF-Q) and medical history (see Appendix D). The LEAF-Q is a brief 25 item questionnaire assessing physiological symptoms related to energy deficiency such as menstrual dysfunction, history of injury, and gastrointestinal function. It is a tool used often in combination with eating disorder screens and can be used to

assess risk of the Triad in female athletes. Questions involve categories such as occupation, type of sport, age, height, weight, training, dizziness, gastrointestinal function, menstrual function, physical activity, contraceptive use and illness and injury in the past year. Answers are self-reported on dichotomous, nominal and Likert-type ordinal scales (Melin et al., 2014). Data were collected pre-training season and again post-training season. Primary indicators of LEA included amenorrhea (absence of menstrual cycle for at least three consecutive months), RMR less than 29 kcal/kg FFM, LEAF-Q score of greater than eight, low bone mineral density, low estradiol, follicle stimulating hormone (FSH), and/or luteinizing hormone (LH). Secondary indicators were fasting blood glucose less than 4 mmol/L, ferritin less than 25 μg/L, fasting insulin less than 20 pmol/L, free triiodothyronine less than 3.5 pmol/L, LDL cholesterol greater than 3 mmol/L, low insulin-like growth factor-1 (IGF-1), systolic blood pressure less than 90 mm Hg, and diastolic blood pressure less than 60 mm Hg. Researchers searched for connections between indicators of LEA and the data collected to determine if the athletes in season training was correlated with LEA (Sygo et al., 2018). At the end of the study, seven out of thirteen athletes had one primary and one secondary indicator of LEA. Body composition did not change after training, however, athletes with indicators of LEA had higher fat mass proportions in comparison to athletes without indicators of LEA. These results are similar to findings of other studies measuring the body composition of LEA and non-LEA athletes (Deutz, Benardot, Martin, & Cody, 2000). In the pre-season physiological symptom questionnaire, five of thirteen athletes reported a past history of stress fractures. Post-season, no athletes reported stress fractures during training; there were no significant relationships found between LEA indicators and stress fractures. As for blood measures, IGF-1 was increased pre- to post-season. In cases of LEA, IGF-1 is normally suppressed (Anne B Loucks & Thuma, 2003; Sygo et al., 2018). However, in this study levels

increased from pre- to post-season. Total cholesterol and blood pressure were also increased; three athletes reported low serum ferritin without iron deficiency anemia, and seven athletes had one or more occurrences of low sex hormones such as luteinizing hormone, follicle stimulating hormone, or estradiol during the study. LEA is known to be associated with and can serve as an indicator of menstrual dysfunction. Ten of the thirteen athletes did not use oral contraceptives; one reported menstrual abnormality; six recorded low sex hormones post-season. Compared to overall endurance athletes, sprinters historically present with a lower prevalence of injuries, higher bone density and fewer cases of menstrual abnormalities (Bennell & Crossley, 1996; Bennell et al., 1997; Ikedo et al., 2016). This study observed symptoms of LEA and RED-S in elite female athlete sprinters. In pre-season data, four of the thirteen athletes showed at least one primary and secondary LEA indicator, and in post-season data, seven out of thirteen athletes (Sygo et al., 2018). This data suggests that female elite athlete sprinters may be at risk of LEA and RED-S after training periods and also enter the season with LEA after a period of off-season rest.

In another study, both male and female athletes were tested using a cross-sectional design (Heikura et al., 2018). Athletes were recruited from the Finnish Athletics Federation and the Athletics Canada, Australia, and U.S. runners/race walkers in Flagstaff, Arizona. The following were measured in the athletes during seven days of high intensity pre-competition training: EA (seven-day food diaries), EEE (self-recorded training diaries), metabolic and reproductive hormone function (fasting blood samples), bone mineral density (BMD) and body composition measured (DXA) and self-reported illness and injury rates (informational questionnaire). Also measured in this study were the strength of the Triad and RED-S diagnostic tools (Joy et al., 2014; Melin et al., 2014). Fifty-nine elite long-distance female and male runners and walkers

between the age of 18 and 40 were recruited. Men and women were categorized into groups of suboptimal and normal EA status. This was determined by measuring EA in one of two ways: by an objective measure of reproductive status with assessments using the Triad cumulative risk assessment tool and LEAF-Q or, by using 7-day food intake and training diaries. The principal investigator analyzed dietary records using Nutri-Flow software (Flow Team, Oulu, Finland) and Food Processor software (ESHA, Salem, OR). Participants kept training diaries listing exercise mode, duration, and intensity. A metabolic equivalent (METs) (Ainsworth et al., 2000) value was used in combination with a resting energy expenditure, estimated using the Cunningham equation (Cunningham, 1991), to sum total energy expenditure. Blood samples were taken to measure insulin, testosterone in males, estradiol in females, triiodothyronine, and IGF-1. Body composition and BMD were measured by DXA. Females took the LEAF-Q and the Triad cumulative risk assessment tool to assess LEA and amenorrhea. Males used the Triad cumulative risk assessment tool as well. To modify this tool for males, aspects concerning amenorrhea were replaced with a screen for low testosterone, recognized as a score of one point in the diagnostic tool (Heikura et al., 2018). For both the Triad and RED-S diagnostic tools, the athletes were grouped into rankings of low, moderate, or high risk. Forty percent of the female and male athletes had amenorrhea or low testosterone, respectively. Compared to athletes with eumenorrhea or normal testosterone levels, sex hormones and triiodothyronine concentrations were lower in athletes with amenorrhea or low testosterone levels (Heikura et al., 2018). Females were more likely to be amenorrheic, have low testosterone levels and were 4.5 times more likely to have bone injuries than athletes with normal levels of sex hormones and triiodothyronine and who were eumenorrheic. The cumulative risk score for the Triad of amenorrheic females was higher than those of eumenorrheic females (5.2±1.9 vs. 1.7±1.4 points, $p<0.001$). The men with

low testosterone had higher Triad scores in comparison to men with normal TES (2.0±1.1 vs. 0.1±0.4 points, $p=0.009$). Using the RED-S scoring system for LEA, a significant difference was found in scores of the amenorrheic and eumenorrheic females ($p<0.001$), as well as the low and moderate EA males ($p<0.001$). Compared to seven-day food and training diary estimated results, the Triad and RED-S screening tools were more accurate and reliable in measuring low EA; and were able to identify endocrine-metabolic function and bone health symptoms experienced in both sexes (Heikura et al., 2018).

Both Sygo et al. (2018) and Heikura et al. (2018) assessed similar indicators of health and also used many of the same methods (e.g LEAF-Q, biometrics, and anthropometrics). Likewise, consistent results were also found. Both studies resulted in considerable rates of LEA among the group of athletes tested and found similar negative physiological effects, such as a disruption in reproductive function. An occurrence of LEA was discovered in both samples of athletes.

Athletes, among different sports, consume a variety of different diets with the intention of following the one that will provide the most benefit for health and performance. More specifically, among track and field athletes, some athletes may follow "fad" diets or prescribed diets in order to bring performance to a higher level: gluten-free, low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP), vegetarian, and fasting diets are becoming more common (Costa, Snipe, Kitic, & Gibson, 2017). Besides benefit to performance, these diets may be consumed for reasons such as a food allergy, food intolerance, toxin exposure or excess histamine, a metabolic error, or malabsorptive problem with certain foods (Schäfer et al., 2001; Turnbull, Adams, & Gorard, 2015). Some of the most common food allergies are shellfish, peanut, tree nuts, and fish (de Silva et al., 2014; Schäfer et al., 2001). The most reported food intolerances are lactose and fructose from malabsorption (Lis,

Kings, & Larson-Meyer, 2019). Allergies and intolerances of food should be tested by a licensed medical specialist such as an allergist or immunologist. Many food intolerance tests have not been validated and are not recommended. Some athletes may become mislead by unreliable tests and unnecessarily avoid or restrict foods (Turnbull et al., 2015). Additionally, many of the symptoms associated with food intolerances may be misinterpreted as they overlap with symptoms of irritable bowel syndrome, functional gastrointestinal disorders, or exercise-induced gastrointestinal syndrome (Costa et al., 2017; Turnbull et al., 2015). Gluten-free diets may be followed because of the belief that gluten causes gastrointestinal injury and symptoms, triggering inflammation. Another reason to follow a gluten free diet is because it is generally "healthier", so as many as 41% of athletes without celiac disease follow a gluten free diet (Lis, Ahuja, Stellingwerff, Kitic, & Fell, 2016). A low FODMAP diet has become increasingly popular, with lactose-containing foods being the most eliminated food (Gaskell & Costa, 2019). Without proper diagnosis, elimination of food that is unnecessarily restricted can cause disturbances in healthy gut microbiota, short-chain fatty acid production, increased arsenic intake, increased psychosocial anxiety, orthorexia nervosa and disordered eating, and decreased energy and nutrient intake (Hill, Muir, & Gibson, 2017; Staudacher et al., 2017). Nutrients impacted by a gluten free diet are iron, B vitamins, and protein; plus, a higher intake of sugar and fat. Vegetarian and vegan diets, though associated with some benefits like reduced chronic disease risk, can compromise nutrient intake of omega-3 fatty acids, zinc, calcium, iron, vitamin D, vitamin B12, iodine, and overall needed energy intake, and may also mask restrictive eating or eating disorders (Cialdella-Kam, Kulpins, & Manore, 2016). Lastly, fasting practices can impact nutrient and energy intake. Though some fasting is based on religious belief, others are trends believed to benefit performance such as intermittent fasting, with fasting and refeeding

throughout 24 hours, and time restricted feeding, which requires 16-20 hours of fasting and 4-8 hours allowed for feeding (Heilbronn, Smith, Martin, Anton, & Ravussin, 2005; Tinsley et al., 2017). Regardless, all can negatively impact caloric intake, recovery strategies, body composition physical performance, and psychological performance (Chamari et al., 2016; Kirkendall, Chaouachi, Aziz, & Chamari, 2012; Meckel, Ismaeel, & Eliakim, 2008; Norouzy et al., 2013).

Food restriction and low energy availability

As discussed, low energy availability (LEA) can have negative impacts on health and performance for female athletes and physically active women. Papageorgiou et al. (2017) found that five days of LEA, at 15 kcal/kg/lean body mass/day, decreased bone formation and increased bone resorption among women. In another study, by Schaal et al., 2017, elite female swimmers with intense training had lowered energy availability, which were significantly associated with fatigue, endocrine and metabolic signs of energy conservation like decreases in glucose and leptin and increases in cortisol and ghrelin, and self-reported decreased performance (Schaal et al., 2017). Methods and strategies for dietary intervention to reverse negative effects of LEA, such as resumption of menses or improved BMD, have been investigated to determine what treatments can used for nonpharmacological treatment.

Two females, one with three-month amenorrhea (age 24), and the other with eleven-month amenorrhea (11 months) (age 19) were provided a dietary intervention with increased energy intake to determine its impact on the resumption of menses and recovery of bone health (Mallinson et al., 2013). Both women engaged in greater than seven hours of physical activity per week. Inclusion criteria for the study was as follows: 18-35 years, BMI 16-25 $kg \cdot m^{-2}$, weight stable (±2 kg) for six months, no serious medical conditions or current clinical diagnosis of an

eating disorder, non-smoker, no medication that would alter metabolic or reproductive hormone concentrations, greater than three hours of aerobic exercise per week, no menses for three months, no clinical diagnosis of polycystic ovarian syndrome, or a free androgen index greater than six. Participants height and weight were measured and a questionnaire was provided assessing medical, exercise, and menstrual history, eating behaviors, and psychological health. DXA scan was taken for BMD and body composition, as well as a physical exam and blood sample for general health. To be sure participants were not experiencing any major psychiatric disorders, they underwent a psychological interview and eating patterns and food preferences were assessed by a registered dietitian. To begin the study, four weeks of baseline information were collected. The presence of amenorrhea was confirmed by analyzing urinary excretion for 28 days of estrone-1-glucuronide and pregnanediol glucuronide metabolites. Body weight was measured weekly by a digital scale. During week three, baseline measurements were recorded for leptin, ghrelin, and total triiodothyronine, markers of bone formation and resorption, body composition, resting energy expenditure (REE), and dietary intake. Also, a baseline test for aerobic fitness with a progressive treadmill test using an on-line MedGraphics Modular VO2 system (St. Paul, MN) or SensoryMedics Vmax metabolic cart (Yorba Linda, Calif., USA) was completed. For dietary intervention, participants were provided energy bars containing about 250-300 kilocalories. Participants had a goal to continue their normal exercise training, but increase caloric intake 20-30% above their normal baseline total energy expenditure (Mallinson et al., 2013). A registered dietitian and psychologist met with participants regularly to help reach target caloric goals and monitor psychological health. Caloric intake was increased slowly at the beginning of the study to aid compliance. Monitoring of menstrual status was continued daily by urinary analysis of estrone-1-glucuronide, pregnanediol glucuronide, and luteinizing hormone

metabolites. Menses was self-reported on monthly calendars. Recovery of menses was classified into three categories: first occurrence of menstrual bleeding during intervention; resumption of menses followed by ovulation; and resumption of menses with at least two menstrual cycles (<36 days). During intervention body weight was measured every other week. Eating behavior was assessed during screening and months two, three, six, nine, and thirteen using the Three Factor Eating Questionnaire and Eating Disorder Inventory-2 (De Souza et al., 2007; Stunkard & Messick, 1985). Bone mineral density was assessed at screening, baseline, month six and month thirteen. Body composition was measured at screening, baseline, and months one, two, three, six, nine, and thirteen. To assess energy intake participants recorded a three-day diet log consisting of two weekdays and one weekend day, at week three of baseline and once a month throughout the duration of the 12-month intervention. In addition to meeting with a registered dietitian to provide guidance on accurate recording of energy intake, participants also received written guidelines for proper measurement of food intake. Indirect calorimetry was used to determine resting energy expenditure during week three of baseline and months two, three, six, nine, and thirteen (Sensormedics Vmax metabolic cart, Yorba Linda, CA). Participants also recorded seven-day exercise logs for any exercise lasting longer than 10 minutes and also logged type of exercise. Exercise energy expenditure from recorded exercise activities was calculated using the OwnCal feature of the Polar S610 or RS400 heart rate monitor at baseline and each month of the intervention (Polar Electro OY, Kempele, Finland). Blood samples were collected at baseline and every month except one, seven, eight, and twelve. The 19-year-old, participant one with long-term amenorrhea (11 months), averaged 12 hours of physical activity each week at baseline and 9 hours of physical activity each week of the intervention (Mallinson et al., 2013). At screening the participant had a BMI of 20.4 $kg \cdot m^{-2}$, body fat percentage of 20.6% and had no

history of or current eating disorders with a caloric intake of 2,143 kcal/day. Body mass index is classified as the following: underweight (<18.5), normal (18.5-24.9), overweight (25.0-29.9), obesity class 1 (30.0-34.9), obesity class 2 (35.0-39.9), extreme obesity class 3 (≥40.0) ("About adult BMI," (n.d.). Recommended body fat percentages at are minimum of 2% to 5% for men and 10% to 13% for women. When body fat percentage decreases below the suggested minimum range, bone development and production of sex-specific hormones, such as estrogen, becomes impaired (Karpinski & Rosenbloom, 2017, pg. 178). For obesity, body fat percentage >22% to >31% for men and >35% to >38% for women (Gibson et al., 2019, p. 290). For dietary intervention, a gradual increase of 500 $kcal \cdot day^{-1}$ was prescribed to reach 2,600 $kcal \cdot day^{-1}$. Participant one's intake reached 2,419 $kcal \cdot day^{-1}$ during the intervention and averaged 685 $kcal \cdot day^{-1}$ for EEE, ranging from 319-1,013 kcal/day. During the intervention participant one continually gained weight; 1.8 kg at month three, 2.1 kg at month 6, 4.2 kg after month twelve. BMI increased to 22.0 kg/m2, percent body fat increased to 21.1%, fat mass increased 11.7%, and lean mass increased 8.3%. Resting energy expenditure increased from 27.20 to 32.61 $kcal \cdot day^{-1} \cdot kg^{-1}$ lean body mass and the REE/predicted REE ratio increased from 0.8 to 1.01. Menses resumed two and a half months (74 days) into the intervention. Menses continued for two months following, though without ovulation. Six months into the intervention another stint of amenorrhea occurred for 92 days before resuming menses again. The first cycle after resumption was 68 days, in which time caloric intake decreased 400 $kcal \cdot day^{-1}$. The participant was made aware of this decrease and was encouraged to increase energy levels again. The participant was able to increase energy intake to 2,350 $kcal \cdot day^{-1}$. During this second resumption of menses ovulation occurred, through the luteal phase was inadequate. At baseline the participant presented with low BMD at the lumbar spine. There was no increase in BMD at the

end of the intervention, however, pro-collagen type 1 amino-terminal propeptide, a marker of bone formation, increased 49.6%.

Participant two was a 24-year-old with short-term amenorrhea of three months, though she had past occurrences of amenorrhea (Mallinson et al., 2013). At baseline she was physically active about seven hours per week at baseline with a BMI of 19.7 $kg \cdot m^{-2}$ and body fat percentage of 22.7%. The psychological interview reported a history of clinical diagnosis of anorexia nervosa, though she no longer met the criteria for a clinical eating disorder she did have some associated characteristics. For dietary intervention, the participant was told to increase dietary intake 400 $kcal \cdot day^{-1}$ (27%) above baseline intake with a target intake of 1,900 $kcal \cdot day^{-1}$. The participant was able to increase to 1,917 $kcal \cdot day^{-1}$ in the first six months of the study, and averaged 1,838 $kcal \cdot day^{-1}$ in the last six months. Hours of exercise ranged from 3-7 hours per week except for one month where 10 hours were averaged. An EEE of 237 $kcal \cdot day^{-1}$ was averaged with a range of 30 to 508 $kcal \cdot day^{-1}$. The participant put on most of the weight in the first six months, gaining 2.4 kg, and by the end of the intervention she had gained 2.8 kg total. Her BMI also increased to 20.7 $kg \cdot m^{-2}$, fat mass increased 17.5% and lean mass decreased 3.3%. Resting energy expenditure increased from 28.1 to 32.8 $kcal \cdot day^{-1} \cdot kg^{-1}$ lean body mass, with an increase in the REE/pREE ratio from 0.87 to 0.94. Resumption of menses occurred by day 23 followed by ovulation, followed by another bout of amenorrhea for the lasting four months during which time caloric intake and body weight decreased. When caloric intake and body weight increased again menses resumed for the last seven months of the intervention. During the study the participant had 10 menstrual cycles, but only six were ovulatory, and each consisted of a luteal phase defect. At baseline the participant presented with low BMD in the hip and lumbar spine. There was no significant increase in BMD during the intervention but there was an

increase in bone marker pro-collagen type 1 amino-terminal propeptide (51.6%), and a decrease in collagen type 1 cross-linked C-telopeptide.

To conclude, both participants increased caloric intake and gained weight following personalized intervention plans without decreasing exercise. Coinciding with weight gain was the resumption of menses. Energy status improved for both, defined in this study as a ratio of REE/pREE to indicate energy deficiency at <0.90 and both ended with ratios higher than cut-point (Mallinson et al., 2013). As REE and body weight increased so did total triiodothyronine, ghrelin, and leptin concentration. When caloric intake and weight decreased, a loss of menstruation also occurred. Though there was an increase in bone markers for both women, there was no significant increase in BMD. Also, ovulation was not consistent and the menstrual cycles that occurred had luteal defects. For both BMD and ovulation, longer interventions may be needed to see significant improvement. Based on the difference between the two participants it is suggested that resumption of menses and ovulation may require a longer recovery time for women who have experienced amenorrhea for longer amounts of time (Mallinson et al., 2013).

A dietary intervention was trialed among eight endurance trained women with amenorrhea (age 22.6±3.3y) and a control group of ten women with eumenorrhea (age 23.1±4.3y) lasting six months (Cialdella-Kam, Guebels, Maddalozzo, & Manore, 2014). A collection of baseline information was taken; questionnaires for general health, exercise training, menstrual, and dietary history, subscales for Drive for Thinness and Body Dissatisfaction on the EDI-2 questionnaire, and a mood state assessment (Profile of Mood State, 1992). A maximum of 60 could be given for a total mood disturbance score. For inclusion criteria participants were required to have exercise equal to or greater than seven hours per week for the last two years, have a VO^2max of greater than 38 $mL \cdot kg^{-1} \cdot min^{-1}$, not be taking oral contraceptives or hormonal

replacement therapy within the last six months, have a EDI-2 subscale score of less than fourteen, and no self-reported primary amenorrhea or non-exercise related amenorrhea. Amenorrhea and ovulation status were measured daily for one month (Clearblue ® Easy Fertility Monitor, Waltham, MA). A standardized treadmill test using indirect calorimetry was used to test VO^2max (ParvoMedics Metabolic Cart, Sandy, UT). Fasting blood samples were collected for iron, vitamin B12, folate, 25-OH vitamin D, triiodothyronine, estradiol, luteinizing hormone, follicle stimulating hormone and progesterone. Blood draws for bone formation (osteocalcin, procollagen type I intact N-terminal propeptide) and bone resorption (carboxyterminal telopeptide of type I collagen) were also collected (Cialdella-Kam et al., 2014).

To measure energy intake, a seven consecutive day weighed food diary was recorded by each participant (Cialdella-Kam et al., 2014). Seven-day physical activity logs were recorded and participants also wore accelerometers (ActiGraph LLC, Pensacola, FL). Both were analyzed using nutrient and activity analysis programs (Food Processor QL, ESHA Research, Salem, OR). Any under reporters were identified using the Goldberg et al. (1991) criteria and were eliminated from the study. Indirect calorimetry was used to measure total energy expenditure (TEE), running energy expenditure (RunEE), and RMR. Total energy expenditure was calculated by adding RMR, EEE and any activities of daily living, and the thermic effect of food. Energy balance (EB= EI-TEE) and EA (EA= EI-EEE) were also calculated. To remove activities of daily living from the EEE calculation, exercise was defined as physical activity with a metabolic equivalent greater than four metabolic equivalents. Total energy expenditure and energy intake were measured at baseline and at three months. Logs for performance, injury, and menstrual cycle were collected every month. Bone mineral density and body composition was assessed using DXA. A dynamometer measured isokinetic strength and torque of the knee extension and

flexion, and plantar and dorsiflexion (Biodex Medical Systems, Inc. Shirly, NY). A muscle biopsy was also collected using a percutaneous needle from the vastus lateralis. This was completed after participants had fasted eight hours and completed a 45-minute run at 75% of VO2max. A CHO-PRO nutrition shake (Gatorade® Nutrition Drink; 325mL; 360 kcal) was consumed after VO2 max was measured. A 60-minute rest was given before the biopsy.

For a dietary intervention, participants consumed a CHO-PRO supplement using a Gatorade® Nutrition Drink every day for the six-month intervention (325 mL, 360 kcal, 54 g carbohydrate, 20 g protein, 8 g fat, 300 mg of calcium, 100 IU of vitamin D, 0.4 mg of vitamin B6, 1.2 μg of vitamin B12). Supplements were consumed 30-60 minutes after exercise. On days without exercise the supplement could be consumed when it was convenient. Participants met with the researchers once a week. During this time, they discussed any issues, provided a 24-hour recall, returned empty supplement cans and were asked if they drank them, and also received more cans.

As a result of the dietary intervention 75% gained weight, with a mean weight gain of 1.6 kg. Energy balance improved in five out of eight women (62%) and EA improved in six out of the eight women (75%). Energy availability was <30 kcal·kg·FFM·day^{-1} at baseline for three women. At the end of the intervention, only one woman had EA <30 kcal·kg·FFM·day^{-1}. All eight women with amenorrhea resumed menses, and seven of the eight (88%) ovulated. Mean time to resumption of menses was 2.63 months, however the range was 1-7 months (longer for two women had experienced amenorrhea for longer than one year). Two women with amenorrhea presented with low BMD in their spine at baseline; one of the two had improved status at the end of the intervention. One woman with amenorrhea presented with spinal osteoporosis and also had improved status by the end of the intervention. There were no

significant changes in mood, muscle power, and torque in either group at the end of the intervention. Overall, EA (+382) and energy balance (+417) improved almost reaching zero, though not significant. This is potentially due to the fact that some participants moved in and out of season during the study (Cialdella-Kam et al., 2014). At baseline, there were no difference in energy, macronutrient, or micronutrients intake between groups, suggesting that some women may be more sensitive to LEA than others.

A study by McKinnon et. al (2019) examined the knowledge of US adults on typical daily caloric needs with data gathered from the National Health and Nutrition Examination Survey. Results from the 2007-2008 and 2009-2010 surveys were collected from a total of 6,267 respondents (3,681 women and 2,586 men) 21 years or older who responded to the following question, "About how many calories do you think a [man/women] of your age and physical activity needs to consume a day to maintain [your] current weights?" Suggested responses were provided to answer this question: <500 calories, 500 to 1,000 calories, 1,001 to 1500 calories, 1,501 to 2,000 calories, 2,001 to 2,500 calorie, 2,501 to 3,000 calories, > 3,000 calories, or don't know. Demographic data was collected from the respondents such as sex, age, race/ethnicity, annual household income, education, and BMI (McKinnon et al., 2019). However, the number of participants varied for each demographic variable as not all surveys were fully completed, and some answers were not provided.

As a result, more men (37.1%) gave responses showing a lack of knowledge about typical calorie needs in comparison to women (16.5%). Men were 3.8 (95% CI 2.8 to 5.1) times more likely than women to show a lack of caloric knowledge. Compared to other racial/ethnic groups, black and Hispanic participants were more likely to incorrectly estimate typical daily caloric needs (McKinnon et al., 2019). Both black and Hispanic groups were 3.1(95% CI 2.2 to 4.5)

more likely to show a lack of knowledge on typical caloric intake in comparison to whites. Lower education and lower income groups also had an increased lack of knowledge regarding typical daily caloric intake. Compared to participants who had graduated high school, had some college education, or were college graduates, participants who had less than high school education were 1.7 (95% CI 1.1 to 2.5) times, 2.3 (95% CI 1.5 to 3.5) times, and 3.3 (95% CI 2.5 to 5.0) times more likely to indicate a lack of typical daily caloric intake. Very low-income (<$20,000) and low-income ($20,000 to $44,999) were 1.8 (95% CI 1.1 to 3.0) and 1.5 (95% CI 1.0 to 2.3) times more likely to lack knowledge of typical daily caloric intake than those with and income of $45,000 to $74,999. Additionally, they were 1.9 (95% CI 1.2 to 3.0) and 1.6 (95% 1.1 to 2.4) times more likely than those with a $75,000 to $99,999 income and 2.0 (95% CI 1.3 to 3.1) and 1.7 (95% CI 1.1 to 2.5) times more likely than those with and income >$100,000 (McKinnon et al., 2019). Males were more likely to lack knowledge of typical daily caloric intake at every income level compared to women of the same income. As a whole, participants of all demographics tended to underestimate versus overestimate caloric needs (McKinnon et al., 2019). This study highlighted the lack of knowledge for typical daily caloric intake in demographic groups, specifically in attainment of education no higher than high school, black and Hispanic groups, low-income populations, and men.

Physiological Impact of RED-S

The IOC Consensus Statement (Mountjoy et al., 2014) highlights consideration of RED-S and its impact on athletes. Studies described in this article are used to provide evidence that RED-S causes a multi-faceted, deleterious impact on physiological functions and can increase risk of illness, infection, and chronic fatigue. The 2018 updated IOC consensus statement further describes the medical and physical complications of RED-S and its potential impact on the body

e.g. low BMD and associated stress fractures, amenorrhea, iron deficiency, depression or impaired judgement, atherosclerosis, alterations of hunger and reproductive hormones, lowered RMR, increased illness, and slower digestion (Mountjoy, Ackerman, et al., 2018). These health consequences have been investigated in several studies on athlete health and performance (see figure 1).

One of these studies, a cross-sectional examination of the plausibility of these physiological effects occurring in states of LEA in athletes was conducted using an online questionnaire. A questionnaire was developed to assess a sample of 1000 female athletes aged 15 to 30 who participated in greater than four hours of physical activity per week for the last 6 months. Participants were patients at the Boston Children's Hospital in the Division of Sports Medicine who presented for any medical conditional related to sport participation. The survey had a response rate of 84.5%. Questions in the survey asked participants about general health, illness, injury, sports performance and Triad and RED-S risk factors. More specifically, the questions address the ten RED-S health consequences suggested by the IOC; menstrual function, endocrine function, immunological function, bone health, metabolic abnormality, hematologic health, growth and development, psychological functioning, cardiovascular risk and gastrointestinal health (Mountjoy et al., 2014) (figure 1).

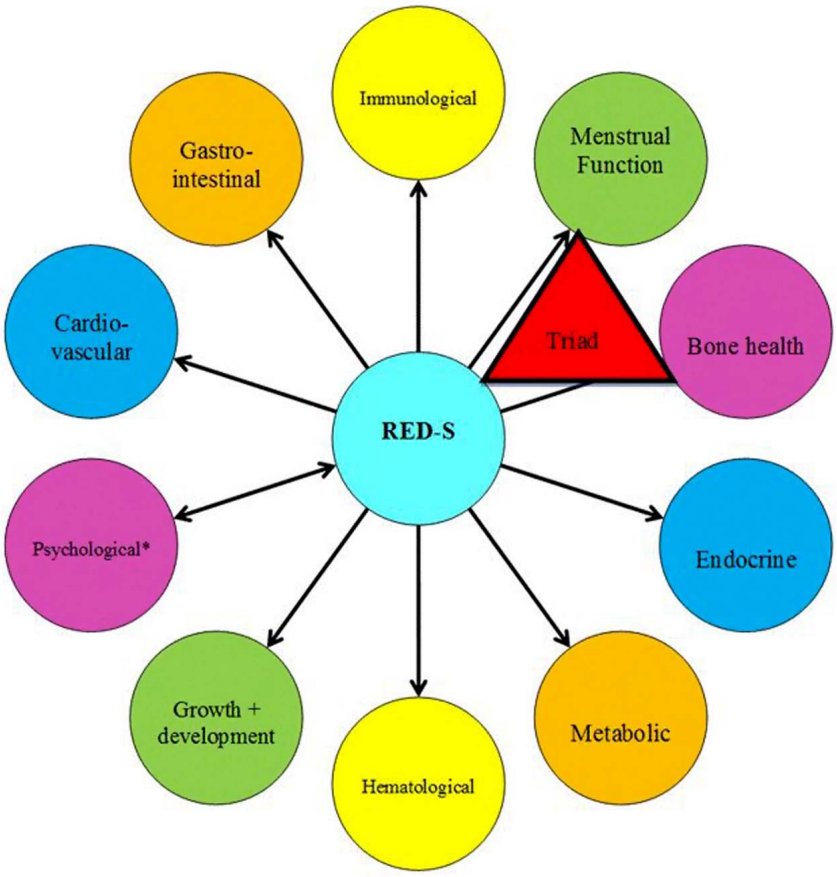

Figure 1. Ten health consequences of RED-S (Mountjoy et al., 2014)

To classify participants as low energy availability (LEA) or adequate energy availability (EA) they took a series of eating disorder (ED) and disordered eating (DE) screens; these were Brief Eating Disorder in Athletes Questionnaire (BEDA-Q), Eating Disorder Screen for Primary Care (ESP), and self-reported current or past history of ED or DE. These short screeners can help determine LEA for large sample sizes (Ackerman et al., 2018). Using a conglomerate result of these surveys, athletes were put into two groups, LEA (n=473, 47.3%) and adequate EA (n=527, 52.7%). Researchers found that the LEA group had a higher prevalence of all IOC suggested health consequences ($p<0.05$) except growth and development and immunologic functions. The athletes with LEA were more likely to experience the following problems: metabolic impairment (3x), cardiovascular symptoms (2.5x), psychological disorders (2.4x), hematological

abnormalities (1.6x), and gastrointestinal symptoms (1.5x). Overall, athletes in the LEA group were much more likely to have these health consequences than the adequate EA group (Ackerman et al., 2018). They also had impaired training response (2.1x), decreased coordination (1.6x), decreased concentration (2x), impaired judgment (4.3x), increased irritability (1.6x), increased depression (2.3x), and impaired endurance performance (1.5x). This study highlighted the connection between RED-S and its suggested health consequences. The following sections will further detail the physiological impact of RED-S.

Bone health

The impact of low energy availability (LEA) on bone health is a well-studied topic largely based on the similar syndrome: the Triad (Nattiv et al., 2007). The impact of LEA on bone health is an important consideration for athletes in preventing injuries that are particularly difficult to heal such as stress fractures of the foot (Papageorgiou et al., 2018). Henceforth, bone loss is an area of high concern for athletes with LEA and RED-S and is seen as an independent risk factor for poor health. Increased energy expenditure with less energy intake results in a negative energy balance causing a landslide of hormonal disruptions such as lowered growth hormone, IGF-1, triiodothyronine and increased cortisol all together disrupting bone synthesis (Papageorgiou et al., 2018). For females, a normal menstrual cycle is important to maintain bone health but is commonly disrupted in female athletes as a consequence of LEA (Papageorgiou et al., 2018). Relative energy deficiency in sport can cause a decrease in endogenous estrogen and can lead to low BMD through the disruption of calcium deposition into the bone (Elliot-Sale, Tenforde, Parziale, Holtzman, & Ackerman, 2018). Low testosterone in male athletes can have the same negative influence on BMD (Hind, Truscott, & Evans, 2006). Maintaining adequate EA

can help prevent occurrences of energy deficit that can lead to hormonal disruptions ending in detriment of bone health.

A cross-sectional analysis on male and female athletes aimed to find relationships among EA, metabolic and reproductive function (menstrual status and testosterone levels), BMD and illness and injury rates (Heikura et al., 2018). Participants in the study were divided into two groups, athletes with normal EA or low EA (cut point of 30 kcal·kg·FFM·day^{-1}). Based on the assessment of reproductive function and EA, 37% (n=13) of females were amenorrheic and 40% (n=10) of males had low testosterone. Results of LEA were found to be poorly correlated to factors such as bone health, metabolic and reproductive function however, this is in line with findings from other studies (Koehler, Achtzehn, Braun, Mester, & Schaenzer, 2013; Melin et al., 2014) and could be contingent on timing of EA assessment. Nevertheless, calculations of low reproductive hormones on injury revealed that amenorrheic and low testosterone athletes had 4.5 times higher rate of absences in training due to bone injury. Overall, these results showed that higher risk scores on RED-S and Triad were associated with lower testosterone concentrations and higher occurrence of all-time fractures for both males and females (Heikura et al., 2018). Another study suggested that amenorrheic athletes lack the potential benefits of weight bearing exercise to BMD due to their menstrual status and are more susceptible to stress fractures (Ackerman et al., 2015).

Metabolic rate

The effects of LEA and RED-S on metabolic rate have been studied among female athletes. In an observational study, the effects of EA and reproductive function on energy metabolism in female endurance athletes were investigated (Melin et al., 2015). Elite female endurance athletes (N=40) between the ages of 18 and 38 (26.3±5.7) were recruited for the two-

visit study. The first day, bone health was measured using DXA and a questionnaire was used to screen for reproductive function, and blood pressure and heart rate were measured with an electronic sphygmomanometer. The second visit involved a collection of measurements including energy assessment, aerobic capacity, and assessment and screening for ED. To assess EA, subjects kept a seven-day food and exercise diary, which was calculated to time of data collection and analyzed by a dietitian using two nutrient databases, Dankost 2000 (Copenhagen, Denmark) and Dietist XP (Kost och Näringsdata AB, Bromma, Sweden) (Melin et al., 2015). To assess EEE, subjects wore heart rate monitors and kept records of exercise in training logs to record exercise activities. An Eating Disorder Examination (EDE)-certified member of the research team interviewed subjects and assessed eating behavior using the Eating Disorder Inventory (EDI-3) (Garner, 2004). This EDI-3 is a questionnaire to assess behavior and attitudes of DE and overt eating disorders (Cooper, Cooper, & Fairburn, 1989). An EDI-3 subscale score for 'Drive for Thinness' of greater than or equal to 14 classified DE, and a 'Body Dissatisfaction' score of equal to or greater than 19 diagnosed ED. Resting metabolic rate was assessed in the morning from mean oxygen consumption (VO^2) and mean carbon dioxide production (VCO^2) using the Weir equation with a ventilated open hood system (Weir, n.d.). Resting metabolic rate ratio was calculated using the Cunningham equation (Cunningham, 1980); low RMR was defined as a RMR ratio of <0.90 (De Souza et al., 2008). To calculate EEE and exercise capacity a cycling test was used. Anthropometric measures, blood pressure, BMD and various blood samples were obtained. The LEAF-Q (Appendix D) was used to assess reproductive function, menstrual status, and to screen for other signs and symptoms of LEA. Researchers found a negative impact of LEA on RMR, menstrual function, and bone health. Overall, 63% of female subjects in this study presented with low or reduced EA (n=15 optimal

EA, n=17 reduced EA, n=8 low EA), 28% diagnosed with DE or ED (n=10 ED and n=1 DE), 60% (n=24) had menstrual dysfunction, and 45% (n=18) had impaired bone health. Low RMR was found in 53% (n=21) of subjects. Athletes with low or reduced EA and/or menstrual dysfunction had lower RMR than athletes with optimal EA. Athletes with optimal EA had a RMR 7% higher in than athletes with low or reduced EA. Similarly, eumenorrheic athletes had a RMR 6% higher than those with menstrual dysfunction. Active women who have amenorrhea were found to have lower resting energy expenditure (REE) than women who are eumenorrheic (De Souza, Lee, et al., 2007). The adaptation seen on metabolic rate in this study of female athletes shows that LEA can have a negative impact on the body's normal metabolic functions.

Endocrine system

Similar to the metabolic system, the endocrine system can also be negatively influenced by low energy availability (LEA). As discussed in previous paragraphs, when EA becomes severely low, the body begins to divert its resources to processes and functions that are most necessary to maintain survival. More specifically, this kind of stress can impact the reproductive system, growth and development, and hormonal pathways (Elliot-Sale et al., 2018). In addition, effects of LEA on the endocrine system can affect both sexes. However, most of the previous research has been conducted on females, with the following hormones found to be negatively impacted by LEA: hypothalamic-pituitary-gonadal-axis, thyroid, appetite regulating hormone (i.e. leptin), insulin, growth hormone, and cortisol (Hackney, Sinning, & Bruot, 1988; MacConnie, Barkan, Lampman, Schork, & Beitins, 1986; McColl, Wheeler, Gomes, Bhambhani, & Cumming, 1989). Though less is understood about LEA effects in men, some researchers have found men to have lower luteinizing hormone and reductions in testosterone with LEA (Hackney

et al., 1988; MacConnie et al., 1986; McColl et al., 1989). The following paragraphs will discuss the effect of LEA and the risk of RED-S on select hormones.

Leptin

In low energy availability (LEA), when food intake is manipulated or reduced, eating behaviors can alter appetite-regulating hormones in response. For example, leptin, an appetite regulating hormone, is impacted by the lack of energy intake and is measurably lower in states of LEA. In addition, adipokine leptin is an anorexigenic adipose hormone that is secreted by the adipose tissue and is involved in reproductive functions, such as menstruation (Elliot-Sale et al., 2018). It is thought that adipokines inform the hypothalamus about states of LEA, however, the exact mechanism to which this message is delivered to the hypothalamus it is not clearly described (O'Donnell & De Souza, 2011; Russell & Misra, 2010). Furthermore, low leptin levels have been found in both males and females with LEA and predict sex hormone concentrations such as estradiol, testosterone, and luteinizing hormone (Ackerman et al., 2012; Christo et al., 2008). Low leptin levels are also correlated with decreased fat mass in female athletes and especially females with anorexia (Ackerman et al., 2012; Grinspoon et al., 1996).

Ackerman et al., 2012 conducted a cross-sectional study comparing amenorrheic athletes, eumenorrheic athletes, and nonathletes to determine if luteinizing hormone and leptin were lower in amenorrheic athletes than eumenorrheic athletes and nonathletes and if ghrelin levels were higher. As a secondary purpose, the association of low luteinizing hormone secretion with high ghrelin and low leptin levels were also investigated. Participants were 14-21 years of age and divided into three groups, n = 21 amenorrheic athletes (20 ± 0.4y), n = 18 eumenorrheic athletes (18.7 ± 0.4y) and n = 20 non-athletes (19.1±0.4y). Data collection initiated at a hospital where participants were required to stay overnight for laboratory screenings: physical examination,

bone age assessment using the Greulich and Pyle methods (Southern Society for Clinical Investigation (U.S.), 1959a), and body composition (DXA). Also, overnight (8 hours) blood samples were taken for leptin every 10 minutes and ghrelin every 20 minutes through an intravenous catheter. A four-day self-reported energy intake was assessed using the Minnesota Nutrition Data System Software and energy expenditure using the Bouchard 3-day activity record (Ackerman et al., 2012; Hart, Ainsworth, & Tudor-Locke, 2011). Body fat was lower in amenorrheic athletes (21% ± 1.0%) than EA (23.5 %± 1.0%) and nonathletes (26.3% ± 1.2%)($p= 0.003$). In comparison to eumenorrheic athletes and nonathletes, amenorrheic athletes had lower luteinizing hormone total pulse secretion, higher ghrelin, and lower leptin. In conclusion, low leptin and high ghrelin levels were found in amenorrheic athletes with lower fat mass. Altered levels of these hormones related to a low fat mass can contribute to low luteinizing hormone pulsatility and amenorrhea (Ackerman et al., 2012). It can be inferred from these results that amenorrheic athletes have menstrual dysfunction in part from low energy intake, thus being in a state of LEA and having lower fat mass from this lack of energy intake.

A similar study by Christo et al. (2008) also compared amenorrheic athletes (n=21), eumenorrheic athletes (n=19), and nonathletes (n=18) levels of ghrelin and leptin, however replacing levels of luteinizing hormone with testosterone instead. Similar to the results in the previous study, amenorrheic athletes in this study also had lower leptin ($p<0.0001$) and higher ghrelin levels than eumenorrheic athletes and nonathletes. Testosterone levels were found to be lower in amenorrheic athletes than eumenorrheic athletes and nonathletes with association to increased ghrelin levels from LEA ($p<0.002$) (Christo et al., 2008).

Adiponectin

Adiponectin, another type of anorexigenic adipose hormone, has been reported to be higher in female athletes and general exercisers (Elliot-Sale et al., 2018). In a study of women with delayed menstruation, referred to as delayed menarche from LEA, it was hypothesized that increased adiponectin levels, as well as elevated ghrelin and reduced leptin, may also predict suppressed gonadal steroids, like estrogen (O'Donnell & De Souza, 2011). A relationship between adiponectin, body fat, and bone health were tested to determine if they were nutritionally mediated by factors such as energy deficiency. An observational study design was used to test women of three different groups; sedentary menstruating women (n=10, 27.8±1.8y), exercising menstruating women (n=15, 24.7±0.9y), and exercising amenorrheic women (n=9, 24.0±1.5y). The women were monitored for two to three consecutive menstrual cycles and for amenorrheic participants, two to three consecutive thirty-day periods. The following data were collected during the study: anthropometric measures, body composition and BMD through DXA, REE using the Weir equation, VO^2 peak by a progressive treadmill test, eight hour fasted serum levels of total adiponectin, leptin, total ghrelin, and total triiodothyronine, and daily urinary samples. Data collected on adiponectin, leptin and urinary progesterone were analyzed using a one-way ANOVA test. At the end of the study, adiponectin levels ($p = 0.056$) were higher in exercising amenorrheic females compared to sedentary or exercising menstruating women. Serum blood tests resulted in lower leptin ($p = 0.012$) and total triiodothyronine in exercising amenorrheic women than in sedentary ovulating women, serum ghrelin ($p = 0.011$) was higher in exercising amenorrheic women than sedentary ovulating women, and there were no differences in insulin levels ($p = 0.327$) among all groups (O'Donnell & De Souza, 2011). Data from urinary collections showed estrogen levels ($p = 0.002$) were also lower in exercising amenorrheic

woman than the other two groups. Overall, there was no relationship between adiponectin and gonadal status. In combination with low estrogen levels, exercising amenorrheic women with higher adiponectin levels had lower BMD, impacting bone health. From the results it was also demonstrated that total triiodothyronine predicted estrogen exposure, meaning gonadal status is altered by metabolic factors changed by energy deficiency (O'Donnell & De Souza, 2011) demonstrating that metabolic and hormonal factors are impacted by energy deficiency, that potentially result in harmful effects on bone health.

These results are similar to another study among ballet dancers which found increased adiponectin levels have a negative correlation with fat mass and also suggested a link to bone health through adiponectin (Donoso et al., 2010). In this study, 22 white, female ballet dancers in Tanner stage II (11.3 ± 0.8 years), performing 18 hours of exercise per week were studied. Along with a control group of 30 girls exercising three hours or less per week in different stages of puberty, Tanner stages II, III and V (10.5 ± 1.4 years, 11.9 ± 1.6 years, and 15.1 ± 1 years). The goal of this study was to determine the progression of puberty, weight, height, BMI, growth velocity, and bone maturity in adolescent ballet dancers. A secondary goal was analyzing adiponectin and leptin levels, and soluble receptors, while also determining the relationship of these listed parameters to body composition. The study lasted for 36 months, measuring the ballet dancers every 6 months. The following methods were used for data collection; anthropometric measures to calculate nutritional status using height, weight and BMI, growth velocity using cm/year, bone age determined by X-ray on the left hand and wrist, and food intake using a 5-day food recall survey, analyzed using the Nutritionist IV software package (San Bruno, CA). Serum leptin, leptin soluble receptors, and adiponectin were analyzed with serum blood draws. Bone density, body fat, and lean mass were measured using DXA of the trunk,

arms, legs and total body. As a result, by the end of 36 months, BMI for ballet dancers recovered and growth velocity was normal, while bone age was delayed (-0.5 ± 0.8 years). The mean age of menarche for ballet dancers was 13.3 ± 0.4 years and 12 ± 0.1 years for the control group (Donoso et al., 2010). Height for ballet dancers was within 0.6 ± 0.7 standard deviation in regards to their target height. As for caloric intake, it was based on the guidelines of the Food and Nutrition Board of the Institute of Medicine of the National Academy, and was found to be insufficient for the ballet dancers for the amount of physical exercise performed (Trumbo et al., 2002). At the beginning of the study, ballet dancers had lower leptin levels and ratio of leptin to soluble leptin receptors than the controls. As the ballet dancers progressed through puberty leptin and leptin to soluble leptin receptor levels increased. There was also a positive correlation between leptin levels and total body fat at the beginning of the study ($r = 0.777$, $p < 0.05$, $p < 0.01$) and BMI ($r = 0.521$, $p < 0.05$). Adiponectin levels were also increased compared to the controls ($p < 0.01$) and increased as the ballet dancers progress through the Tanner stages (Donoso et al., 2010). When BMD of the ballet dancers was compared to the controls, BMD was normal. Overall, the researchers found that in adolescent ballet dancers' caloric intake was insufficient and menarche or menstruation and skeletal maturation though delayed, were recovered as puberty progressed. It is suggested that the extensive exercise and insufficient caloric intake is likely what caused lower fat mass to be found in the ballet dancers, and could also be a reason for delayed menarche and bone maturation (Donoso et al., 2010). Furthermore, adiponectin levels were increased in ballet dancers and continued to increase with onset of puberty. Researchers also found a positive correlation with adiponectin and lean mass. While increased adiponectin is sometimes associated with decreased BMD, increased adiponectin in this study was correlated with normal BMD. This may be due to the increased exercise in ballet

dancers and that adiponectin promotes osteogenesis (Donoso et al., 2010; Lee et al., 2009). In conclusion, this study demonstrates that lowered fat mass, which is connected to excessive exercise and insufficient caloric intake, causes undesirable changes in leptin and adiponectin.

Interestingly, the same results have not been reported for men in relation to exercise, EA and adiponectin levels. A systematic review on the effects of exercise on adiponectin revealed studies evaluating adiponectin levels in men during exercise did not directly measure the relation of EA and no change in levels were found in association with exercise (Simpson & Singh, 2008). Though it is known that increased levels in these hormones occur during states of LEA, particularly in women, the exact consequences on regulatory functions has yet to be determined. More research is needed in men in states of LEA to determine if there is an impact on adiponectin levels.

Ghrelin and peptide YY

Ghrelin and peptide YY, produced mainly in the stomach, are also important hormones involved in appetite regulation. Ghrelin is a marker for energy status, so when levels of ghrelin are high, low energy availability (LEA) is present. Ghrelin acts on the hypothalamus and the pituitary gland to release growth hormone, ultimately to increase appetite (Ackerman et al., 2012; Christo et al., 2008; Misra et al., 2005; Tolle et al., 2003). Interestingly, female athletes with amenorrhea and anorexia have elevated levels of ghrelin indicating they are in a LEA state (De Souza, Lee, et al., 2007; De Souza, Leidy, O'Donnell, Lasley, & Williams, 2004). Exercise that creates a negative energy balance or low energy availability, increases ghrelin levels (Borer, Wuorinen, Ku, & Burant, 2009). Additionally, elevated ghrelin does not induce hunger, and it has been suggested that ghrelin disrupts reproductive function by suppressing luteinizing hormone, potentially contributing to amenorrhea in states of LEA (Scheid & De Souza, 2010). In

combination, it is hypothesized that elevated levels of ghrelin occur in amenorrheic athletes that do not have increased hunger, though they are energy deficient (Elliot-Sale et al., 2018). Women with amenorrhea can have a high drive for thinness, which may suppress appetite.

Peptide YY opposes ghrelin and "tells" the hypothalamus to suppress appetite after caloric intake. Peptide YY is also increased in states of LEA for females and athletes with amenorrhea. Due to its correlations with BMI and energy status, it is believed that the levels of Peptide YY has a role in long-term energy homeostasis (Scheid & De Souza, 2010). Furthermore, it is hypothesized that peptide YY causes ghrelin resistance and can play a part in eating disorders of those with anorexia or amenorrhea (Elliot-Sale et al., 2018). Similar to ghrelin, peptide YY may also play a role in suppressing reproductive function through luteinizing hormone. However, further research is needed for the assessment of peptide YY and ghrelin's interaction and contributions to metabolism during LEA.

In a study by Misra et al. (2006) of adolescent girls with anorexia it was hypothesized that peptide YY would be elevated in relation to low food intake and that it would be a predictor of reduced bone turnover. To assess peptide YY levels 23 adolescent girls (16.2 ± 1.6 years) with anorexia were compared to 21 healthy girls (15.4 ± 1.8 years). Several blood samples were taken for the following hormones; growth hormone, cortisol, leptin, ghrelin, and fasting blood samples of peptide YY, glucose, insulin, IGF-1, total triiodothyronine, and estradiol. X-ray was used to determine bone age of the girls and a DXA scan was used for bone density and body composition (Misra et al., 2006). Serum markers for bone formation were also taken such as carboxy-terminal propeptide of type 1 collagen, osteocalcin, and bone specific alkaline phosphatase along with N-telopeptide and deoxypyridinoline which are two markers of bone resorption. To calculate caloric intake four-day food diaries were recorded and analyzed with

NDS-R software (version 4.03; nutrient database 31; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

Peptide YY was higher in girls with anorexia (17.8 ± 10.2 pg/ml) compared to healthy girls (4.8 ± 4.3 pg/ml) ($p < 0.0001$) and in 10 girls with anorexia who had a weight recovery of 10% increase in BMI their levels peptide YY levels decreased. Log peptide YY was also found to be inversely correlated with BMI, which was one of the independent predictors of log peptide YY. As for predictors of bone markers, all but carboxy-terminal propeptide of type 1 collagen correlated inversely with log peptide YY (Misra et al., 2006). From these results researchers concluded that peptide YY may be associated with bone metabolism due to the high levels of peptide YY being found with low levels of bone markers, though more research is needed to confirm a relationship. Higher levels of peptide YY were also correlated with a decreased fat intake, suggesting that peptide YY may also contribute to a decreased food intake.

Oxytocin

Evidence from research has shown that levels of the hormone oxytocin are lower in female athletes. A study of nocturnal oxytocin levels was conducted on female athletes (Lawson et al., 2013). Participants were grouped into three categories, amenorrheic athletes (n=15), eumenorrheic athletes (n=15), and nonathlete controls (n=15) all aged 14-21. To meet the criteria of an athlete, women had to have at least four hours of aerobic weight-bearing activity or twenty miles of running weekly for the previous six months to meet the criteria. To be considered a nonathlete, the participant had to perform less than two hours of weight bearing activities per week. A physical examination and medical screening were performed to rule out any other medical causes for amenorrhea besides excessive exercise and insufficient caloric intake. Participants were required to stay overnight in a hospital and undergo the following tests: history

and physical examination, four-day food log, and blood sampling for oxytocin every sixty minutes between eleven pm and seven am. A bone age assessment using the Greulich & Pyle method was completed to assess bone microarchitecture (Southern Society for Clinical Investigation (U.S.), 1959b). At the end, nocturnal levels of oxytocin for amenorrheic athletes were lower (245±22 $pg \cdot ml^{-1}$) compared to eumenorrheic athletes (344±53 $pg \cdot ml^{-1}$) and nonathletes (446±40 $pg \cdot ml^{-1}$). The difference remained significant ($p=0.0001$) when comparing amenorrheic athletes and nonathletes after controlling for serum estradiol levels. Moreover, this study also found, when compared to nonathletes, lower levels of oxytocin in amenorrheic athletes were moderately associated with impaired bone microarchitecture (cortical density at the distal tibia $r=0.57$, $p=0.03$; trabecular thickness at the distal radius $r=0.52$, $p=0.049$).

Insulin, amylin, and incretins

In states of low energy availability (LEA), insulin, amylin and incretins are all suspected to be reduced or down regulated in response to the lack of energy intake. Insulin is a hormone well known for its role in the energy store of carbohydrates, protein and fat. In athletes with amenorrhea or LEA, insulin secretion is down regulated to support more substrate availability and insulin sensitivity increases (Laughlin & Yen, 1996; Martin et al., 2008; Rickenlund et al., 2004). Levels of insulin were also decreased in studies of exercising males with lowered energy intake, suggesting that insulin is decreased in states of LEA in both males and females (Chan et al., 2003; Elliot-Sale et al., 2018; Koehler et al., 2016; Mäestu et al., 2010). Amylin, secreted together with insulin, and incretins such as glucagon-like peptide 1 and gastric inhibitory peptide, which stimulate insulin release, are also reduced in females with anorexia (Elliot-Sale et al., 2018; Misra & Klibanski, 2014). Though there is limited research on the mechanisms that

cause a decrease in these hormones, the research that has been conducted confirms that there is some connection with LEA.

Additional Hormones

There are a few other hormones that are suspected to be impacted by low energy availability (LEA). In LEA, growth hormone is increased due to resistance caused by a decrease in IGF-1 (Misra & Klibanski, 2014). Of the thyroid hormones triiodothyronine and throxyine, triiodothyronine is commonly reduced in women with anorexia, amenorrhea, and LEA; however, the effect on throxyine is variable but in some studies has been found to be low during LEA (Berga et al., 1989; Counts et al., 1992; De Souza, Lee, et al., 2007; Estour et al., 2010; Gordon, 2010; Harber et al., 1998; A B Loucks & Heath, 1994; A B Loucks et al., 1992; Misra & Klibanski, 2014; Misra et al., 2004, 2003; Støving et al., 1999). Some researchers suggest that triiodothyronine levels could be used as an indicator of LEA (Loucks & Heath, 1994). Another hormone involved with LEA is cortisol. In women with anorexia or amenorrheic athletes, cortisol levels are increased during times of decreased caloric intake, prolonged exercise, or if an individual is underweight (Loucks et al., 1989; Martin et al., 2008; Nakamura et al. , 2016; Schaal et al., 2011; Schorr et al., 2015). Lastly, LEA is strongly connected to a decrease in menstrual function, therefore causing changes in normal reproductive hormone distribution. Secretions of these hormones are decreased as an energy conservation tool by the body to save energy for vital processes (De Souza, Lee, et al., 2007; De Souza, Nattiv, et al., 2014; Gordon et al., 2017; Jasienska, 2003). Hormones that are involved include estradiol, luteinizing hormone, progesterone, and testosterone (Loucks et al., 1989; Loucks & Thuma, 2003). Other hormones are reduced in most states of LEA from reduced energy intake and increased training, but more

specifically in athletes participating in sports that have an emphasis on leanness (Bennell, Brukner, & Malcolm, 1996; Hackney et al., 1998; Heikura et al., 2018).

Hematological

Iron deficiency, a familiar problem to female athletes, is another suspect involved in energy deficiency (Mountjoy, Sundgot-Borgen, et al., 2018). Iron deficiency is associated with an increase in anxiety, potentially contributing to eating disorders and disordered eating in women, especially in exercising women (Petkus et al., 2017a). Furthermore, a reduced appetite is associated with iron deficiency (Petkus et al., 2017a). Metabolic efficiency can also be reduced by iron deficiency, which ultimately increases exercise energy expenditure (EEE) and energy expenditure at rest, furthering energy deficiency. Iron can impact cortisol synthesis as a cofactor which could negatively affect metabolic fuel availability and can also impact glucose concentration and growth hormone, potentially causing muscle protein degradation. Further research is needed to better understand the relationships of iron deficiency to low energy availability (LEA). It is recommended that monitoring serum ferritin can help identify women at risk for iron deficiency and its impact (Petkus, Murray-Kolb, & De Souza, 2017b).

A study previously mentioned that surveyed female athletes ages 15 to 30 years at the Division of Sports Medicine, Boston Children's Hospital who exercised at least 4 hours of physical activity per week, looked at the health consequences of RED-S (Ackerman et al., 2019). One of those health consequences was hematological impact. The participants were assessed for a history of anemia, low hemoglobin, or ferritin, or any abnormal bruising. Of the 1,000 participants who answered the survey, those with LEA (47.3%), also were 1.6 times more likely to have a history of hematological issues.

Growth and development

Studies that research the impact of anorexia nervosa on adolescent male and female growth and development can be used to infer the effects of RED-S on growth and development. This connection is made due to anorexia nervosa being a state of LEA, similar to that of RED-S. A study of 211 anorexic female patients at a pediatric children's hospital were used to investigate the effect of calorie restriction or undernutrition on growth and final height (Modan-Moses et al., 2012). The aim was to assess the prevalence of growth retardation, impact of weight restoration on growth and final height, and what other physiological factors were involved (Modan-Moses et al., 2012). The mean age of patients was 16.6±4.2 years and the mean BMI was 15.7±1.02 $kg \cdot m^{-2}$. While hospitalized, patients followed an intervention for weight gain setting a target weight, gain of 0.5-1.0 $kg \cdot week^{-1}$. After final target weight was achieved patients had to maintain it for two weeks before being discharged. Thereafter, a follow up weight check occurred biweekly for the first two months, once a month for the next four months, then, every three months until they reached the age of 18. Target weight was adjusted every three months for those who had not achieved full height. Height was measured using a wall mounted stadiometer at admission and each follow-up until final height was reached. Pre-morbid height was recorded for those where that data was available. Reaching the age of 18 or more and being at least three years after menarche was defined to be final height. The CDC 2000 sex-specific growth charts were used to determine height standard deviation. Paired t-tests were used to compare height and weight at admission and discharge. An ANOVA with repeated measures compared height standard deviation for patients with multiple measurements. A p-value of <0.05 was considered significant.

Patients in the study had a significant weight gain ($p<0.001$) of 7.98±6.43 kg with an average hospital stay of 5.3±8.2 months. It was determined that growth and final height of female adolescents were impacted by anorexia nervosa or undernutrition. At admission the patients had significantly lower height standard deviation than that of the normal population (-0.231±1.103 cm, $p<0.001$)(Modan-Moses et al., 2012). Additionally, those patients closer to menarche, or less than one-year menarche, were more affected than those more than one year past menarche. This could be due to the possibility that those more than one year past menarche have reached or are closer to their final height before the onset of anorexia nervosa. Though weight restoration did improve linear growth, full catch-up growth was not obtained and final height standard deviation was significantly lower than the normal population. Furthermore, the longer the duration of the disease, the less chance that maximal catch-up growth can be achieved. To conclude, more research is needed to fully determine whether RED-S slows or is a detriment to growth and development and if this impact can be recovered.

Cardiovascular impact of relative energy deficiency in sport

Decreased estrogen levels seen during amenorrhea or menopause are associated with increased risk for cardiovascular disease. It is also found that female athletes who become amenorrheic commonly due to low energy availability (LEA), develop endothelial dysfunction (Rickenlund et al., 2005). The generally accepted range for resting heart rate is 60 to 100 beats per minute. The “red flag” range for cardiac abnormality is the lower threshold of 50 beats per minute (Karpinski & Rosenbloom, 2017, p. 398). A retrospective follow-up study of amenorrheic athletes with reduced flow-mediated dilation included an intervention to regain menstrual function. Amenorrheic athletes who regained menstrual function had more significant increase in flow-mediated dilation than those who did not (Hoch et al., 2007). Additionally, it is

known that estrogen levels can have an impact on lipid profiles of women. When estrogen levels are lowered because of an energy deficiency leading to a condition such as anorexia or amenorrhea, a negative change in low-density lipoproteins (LDL) occurs with elevated LDL and total cholesterol (Rickenlund et al., 2005).

Changes in cardiovascular health can be seen in postmenopausal women as increased blood pressure and an accelerated progression of atherosclerosis. However, for premenopausal women with functional hypothalamic amenorrhea, blood pressure has shown to be lower (O'Donnell et al., 2015). A study was conducted to determine the effects of hypoestrogenemia on premenopausal women and its impact on blood pressure regulation. Participants were between the ages of 18 and 35 and had to meet the following inclusion criteria: good health (absence of chronic illness), normal menstrual status for three months prior, no medications, nonsmoker, not participating in dieting, stable weight for three months prior, no hormonal therapy for at least six months prior, no history or diagnosis of eating disorders, exercising for more than two hours per week, and no other factors that would implicate participation. There were two groups, physically active eumenorrheic ovulating women (n=17), and physically active women with amenorrhea (n=12). For eumenorrheic women measures were taken in the early follicular phase of the menstrual cycle while amenorrhea women were taken on a random day so that the comparison could be made between cyclically low estrogen versus chronically low estrogen as estrogen is lower in the beginning phase of the menstrual cycle (days 2-6). Anthropometric measures and body composition were also recorded. A physician's balance scale (Detecto, Webb City, MO) was used to measure total body mass and height. Body composition was determined using DXA scan to measure central fat mass (kg) from the trunk and peripheral fat mass (kg) in the arms and legs. In a supine position, systolic, diastolic, mean arterial blood

pressure, and heart rate were taken using an automated device (Dinamap Pro 100, Critikon) as well as a continuous recording using lead II of an ECG and a photophlethysmographic device placed on the index finger (O'Donnell et al., 2015). Also, in a supine position, postganglionic muscle sympathetic nerve activity was recorded using a unipolar tungsten electrode as lower body negative pressure with the lower body in a chamber to simulate orthostatic stress. Amenorrheic women had significant differences in lower systolic blood pressure ($p<0.01$) and heart rate ($p<0.01$) as a response to graded lower body negative pressure or orthostatic stress, than the eumenorrheic women. There was no difference in diastolic blood pressure between groups ($p=0.45$). From the results it was concluded that blood pressure and heart rate during orthostatic stress is lower and at rest is lower in amenorrheic women than eumenorrheic women. Also, normal counter-regulatory responses of plasma renin, angiotensin, and aldosterone were disrupted as they did not increase during the orthostatic stress. There was a significant difference ($p<0.05$) between eumenorrheic women and amenorrheic women. Renin and angiotensin did not increase in amenorrheic women, and aldosterone decreased, but both increased in eumenorrheic women (O'Donnell et al., 2015). Overall, menstrual dysfunction correlated with hypoestrogenemia can have a negative impact on cardiovascular function. In cases of energy deficiency that become severe, such as anorexia nervosa, cardiovascular health is increasingly impacted (Spaulding-Barclay, Stern, & Mehler, 2016). Some of these negative effects are a decrease in left ventricular mass, pericardial effusion, bradycardia, and valve abnormalities like mitral valve prolapse (Spaulding-Barclay et al., 2016).

Immunological

As discussed previously, female athletes with amenorrhea typically also have lower levels of estrogen. When estrogen is low the physiological impact can be seen in different areas,

for example, disruptions in reproductive function and the cardiovascular system. Athletes who endure intense training are susceptible to a weakened immune system and can undergo conditions like and upper respiratory tract infection. Symptoms of a comprised immune system can include a sore throat, runny nose, coughing, headache, emesis, and fatigue (Shimizu et al., 2012). Furthermore, intense exercise can reduce salivary secretory immunoglobin A, which helps to inhibit pathogens from attaching to mucosal surfaces to enter the body. Salivary secretory immunoglobin A is also correlated with serum estradiol (Shimizu et al., 2012). Due to this, it is believed that estrogen deficiency (e.g. amenorrhea) might play a role in suppression of mucosal immune function and ultimately increasing the likelihood of infection (Shimizu et al., 2012).

The possible connection between amenorrhea and low estrogen levels on mucosal immune function and likelihood of upper respiratory tract infections was investigated in a group of amenorrheic and eumenorrheic runners (Shimizu et al., 2012). For this study, amenorrheic (n=8) and eumenorrheic (n=13) elite, collegiate, Japanese female distance runners were used to compare salivary secretory immunogloblin A and the incidence of upper respiratory tract infections. In each participant body mass, fat percentage, fat-free mass, and body water were recorded using a bioeletrical impedance analysis digital scale. Saliva and blood samples were taken in the morning, before breakfast with only consumption of water allowed. Serum blood samples were collected to measure serum progesterone and serum estradiol. To determine occurrences of upper respiratory tract infection participants were given a questionnaire regarding symptoms within the last month (Shimizu et al., 2012). Female athletes with amenorrhea had significantly lower body mass, body mass index ($p<0.05$), menstrual frequency ($p<0.05$), and estradiol concentration ($p<0.05$). There was no significant difference in progesterone concentration between groups. Salivary secretory immunoglobin A concentration was lower

($p=0.09$), and the secretion rate was significantly lower ($p<0.05$) in amenorrheic females than eumenorrheic females (Shimizu et al., 2012). There were also more symptoms reported for upper respiratory tract infections in amenorrheic females than eumenorrheic females. Based on these results, the researchers advocate that athletic amenorrhea plays a part in the disruption of mucosal immune function through estrogen levels and salivary secretory immunoglobin A, therefore enhancing the likelihood of upper respiratory tract infections.

Additionally, two studies were conducted on Australian Olympic athletes prior to the 2016 Rio Olympics. The first study was nine months before, and the second study was just three months before the Olympics began. These studies provided questionnaires to Australian Olympic athletes investigating factors that contributed to illness in the athletes. The first study included questionnaires, encompassing the following topics: hygiene, psychology, sleep, travel, probiotic use or nutrition (Drew et al., 2017). Eighty-one male (n=26 age 25.4±6.2 years) and female (n=55, age 25.5±7.4 years) athletes participated in the questionnaires from the following sports: cycling, equestrian, hockey, swimming, gymnastics, rowing, rugby sevens, sailing, triathlon, and water polo. Interestingly, the risk factors for illness that were most significant were being a female and LEA. Fifty-three percent of the sample females reported LEA using the LEAF-Q. If LEA was viewed separately from the results, it would account for 76% of reported illnesses (Drew et al., 2017).

The second study was done with a larger sample, closer to the beginning of the 2016 Rio Olympics (Drew et al., 2018). Two hundred and six athletes (male, n=47, age 25.8±4.1 years; female, n=85, age 24.3±3.9 years) completed this survey from the following sports: equestrian, soccer, boxing, hockey, gymnastics, rowing, rugby sevens, triathlon, sailing, and water polo. Forty percent of females reported LEA on the LEAF-Q. Reporting LEA had higher odds of

upper respiratory tract infections, bodily aches, head symptoms, and gastrointestinal disturbances. Again, LEA was found to be a leading risk factor for illness in Olympic athletes.

Gastrointestinal

Gastrointestinal function becomes compromised when LEA is extreme and progresses to the point of anorexia nervosa (Mountjoy, Sundgot-Borgen, et al., 2018). Changes to normal gastrointestinal function include delayed gastric emptying, increased transit time, altered sphincter function, and constipation (Norris et al., 2016). Slowed colonic transit time causes many individuals with eating disorders to experience constipation. However, function can return to normal once a diet sufficient in caloric intake is reestablished (Chun, Sokol, Kaye, Hutson, & Wald, 1997). A study conducted by Melin et al. (2014) aimed to create a screening tool for RED-S that would help recognize female athletes at risk for the Triad, known as the LEAF-Q (Appendix D), and evaluate the questionnaires reliability, validity, consistency and discriminants. Participants included 84 female athletes from Sweden and Denmark, between ages 18 to 39 who trained five or more times per week. The study was broken up into two parts. In part one, endurance athletes and dancers (n=37) were asked to complete the LEAF-Q twice to test its reliability. The LEAF-Q consists of self-reported questions about gastrointestinal and reproductive function, and injuries. In the second part of the study, clinical assessments on another group of endurance athletes (N=45) were used to verify the self-reported answers on the LEAF-Q. Internal consistency and reliability for the LEAF-Q were highly significant (alpha 0.86). Questions regarding gastrointestinal function asked about occurrences of gastrointestinal symptoms such as pain, cramps, bloating, stool frequency and stool consistency. Gastrointestinal symptoms were negatively correlated with EA ($p=0.023$).

Ackerman et al. (2018) asked 133 questions addressing general health, sports performance, illness, injury, and Triad and RED-S risk factors. Participants were asked to take the LEAF-Q and the Faecal Incontinence Questionnaire (Reilly, Talley, Pemberton, & Zinsmeister, 2000) which provided questions to assess gastrointestinal disturbances. The results concluded that individuals with LEA compared to individuals with adequate EA were 1.5 times more like to experience gastrointestinal symptoms. Both of these studies highlight the connection between LEA and gastrointestinal symptoms.

Menstrual function

A connection between energy deficiency, excessive exercise, and menstrual dysfunction (i.e. amenorrhea) has been determined. Diagnosis of amenorrhea is determined if the menstrual cycle commonly exceeds 45 days or amenorrhea persists for three or more additional months (Gordon et al., 2017). Evaluation of amenorrhea focuses on diet, exercise/athletic training, presence of eating disorders, and presence of attitudes like perfectionism, and need for social approval. Additional evaluation involves weight fluctuations, stressors, substance abuse, fractures, sleep, menstrual patterns, family and personal history of eating and reproductive disorders, and more (Gordon et al., 2017). The degree to how severe nutritional or energy deficiency is can be very important in amenorrhea because of its potential of having a negative impact on skeletal health causing fragility. It is estimated that this disruption in menstrual status begins when energy intake sinks to 30 $kcal \cdot kg^{-1}$ or less (Anne B Loucks & Thuma, 2003). Correction of the negative energy balance by increased caloric intake and a decreased exercise expenditure often leading to weight gain is commonly part of the treatment for amenorrhea along with several other clinical interventions (Gordon et al., 2017).

In a randomized experiment, sedentary, regularly menstruating women (n=29) participated in a repeated measures design that controlled for dietary intake and energy expenditure (Anne B Loucks & Thuma, 2003). After being screened, any women with underlying health concerns or physical activity of more than 60 min/week were excluded from the study. Participants were also required to have a hematocrit above 35% and an energy intake regularly above 35 or below 55 kcal·kg·lean body mass·day^{-1}. The last three months of menstrual cycles had to be regular, meaning intervals of 26-32 days with an average luteal length of eleven days as to not skew the results of the study (Anne B Loucks & Thuma, 2003). Additionally, women were all between the ages of 18 and 30 years old. In the three pre-treatment days participants wore an accelerometric physical activity monitor to estimate energy expenditure. In the first round of the experiment, participants consumed a balanced intake (45 kcal·kg·lean body mass·day^{-1}) with an exercise protocol for five days that expended X = 15 kcal·kg^{-1} lean body mass of 70% maximum oxygen uptake (VO^2max). Exercise treatment consisted of walking on a treadmill up a grade for 30-40-minutes with 10-minute rest periods (Anne B Loucks & Thuma, 2003). In the second round, participants were placed in one of three different energy restricted diets for five days (10, 20, or 30 kcal·kg·lean body mass·day^{-1}). These diets consisted of 28% fat, 15% protein, and 58% carbohydrates. Standardized meals, three times a day, provided all foods and fluids along with a daily multivitamin and mineral tablet. After the fifth day of the treatment participants stayed for 24 hours in a hospital for continuous blood sampling though venous catheter at 10-minute intervals (Anne B Loucks & Thuma, 2003). Blood samples taken included luteinizing hormone, estrogen (E2), glucose, follicle stimulating hormone, triiodothyronine, insulin, IGF-1, IGF-1 binding protein, and IGFBP-3, growth hormone, leptin, cortisol, and B-hydroxybutyrate. For statistical analysis, the various energy

availabilities (10, 20, or 30 kcal·kg·lean body mass·day^{-1}) were analyzed through one-sided, single sample tests as well as repeated measures ANOVA along with post-hoc, single-sided, two sample least significance difference to determine if the restricted EA treatments had any effects.

As a result of these treatments, body weight was reduced at each of the energy restricted levels, 3.4%, 1.8%, and 2.1% of body weight respectively. There were no effects seen on luteinizing hormone pulse frequency or pulse amplitude with the balanced diet or the 30 kcal·kg·lean body mass·day^{-1} treatment, but with the 10 and 20 kcal·kg·lean body mass·day^{-1} restricted EA treatments luteinizing hormone pulse frequency was suppressed and luteinizing hormone pulse amplitude was increased (all $p<0.04$). Additionally, mean follicle stimulating hormone was not altered by any of the restricted EA treatments. However, the 24-hour mean estrogen was only suppressed by the 10 kcal·kg·lean body mass·day^{-1}restricted EA treatment, by 15% ($p<0.01$). Effects seen by the restricted EA treatments on luteinizing hormone pulsatility were bimodal, meaning they had a larger effect and were more significant in women with shorter luteal phases. Lastly, the effects on luteinizing hormone pulsatility were similar to that on glucose, B-hydroxybutyrate, growth hormone, and cortisol (Anne B Loucks & Thuma, 2003). Therefore, women with a shorter luteal phase can potentially be identified as more susceptible to the disruption of reproduction function by a lack of EA.

The female athlete triad

The Triad, originally defined by the American College of Sports Medicine, is a condition that is closely related to RED-S. In fact, RED-S was developed as an "expansion" on the Triad to provide a broader term for the symptoms experienced by low energy availability (LEA) or relative energy deficiency (Mountjoy et al., 2014). The Triad affects women who are physically active and is present when one or more of the three components occur: LEA with or without

disordered eating, menstrual dysfunction, and low BMD (Nattiv et al., 2007). Much of the physiological impact that occurs when women experience the Triad are similar to those seen while experiencing RED-S. These health consequences include menstrual disturbances, disruption of estrogen and other reproductive hormones, musculoskeletal effects such as bone stress injuries, and cardiovascular, renal, gastrointestinal, endocrine, and neuropsychiatric comprised systems (Nattiv et al., 2007). Understanding the components, health consequences, identification, and treatment of the Triad is essential and also beneficial to completely understand the syndrome of RED-S, prevent, and treat it.

Identification of the Triad can be difficult due to the fact that there are three components, and each of these components runs along a spectrum. An athlete can fall on these spectrums anywhere from good health to disease. Good health involving these three components is comprised of optimal EA, optimal bone health, and eumenorrhea. Moving along the spectrum towards disease the components are reduced EA with or without disordered eating, low BMD, and subclinical menstrual disorders. Finally, reaching the opposite end of the spectrum where the athlete experiences LEA, osteoporosis, and functional hypothalamic amenorrhea occur (Nattiv et al., 2007).

The thick arrows in the female athlete triad figure show both an indirect and direct impact of LEA on bone health and development through amenorrhea and estrogen disruption and by suppression of hormones that are involved in bone formation (Nattiv et al., 2007). Thin arrows in the female athlete triad figure show intermediate levels of the three components (Nattiv et al., 2007). An athlete can move back and forth on the spectrum for each component at different rates. This is affected by current EA and EEE of the athlete.

Energy availability can be compromised in different ways depending on the athlete. Athletes can increase exercise, expending more than they are putting in, or the opposite can occur where there is less energy intake. This lack of energy intake can support eating disorders or psychiatric illnesses such as anorexia nervosa or bulimia (Nattiv et al., 2007). Menstrual function moves form eumenorrhea to oligomenorrhea, which is a menstrual cycle longer than 35 days, to the point of amenorrhea, which is an absence of a menstrual cycle for more than three months (Practice Committee of the American Society for Reproductive Medicine, 2004; Vollman, 1977). Lastly, BMD ranges from optimal bone health to osteoporosis. Screening and diagnosis of osteoporosis is determined by BMD and the level that the risk of fracture is unacceptable (Bonnick, 2002). The World Health Organization diagnosis osteoporosis in women of *T*-scores based on average peak BMD. However, this is a problem for adolescents and premenopausal women due to a lack of research for these populations making it hard to determine standards for these ages (Leib et al., 2004; Writing Group for the ISCD Position Development Conference, 2004). The International Society for Clinical Densitometry (ISCD) instead recommended *Z*-scores be used to determine BMD in the adolescent and premenopausal populations (Leib, 2005; Leslie et al., 2006; Writing Group for the ISCD Position Development Conference, 2004). *Z*-scores below -2.0 are considered low BMD. The ISCD recommends that osteoporosis be diagnosed when in the presence of secondary clinical risk factors including chronic malnutrition, eating disorders, hypogonadism, glucocorticoid exposure, and previous fractures as these can signal an increased risk of low BMD and fractures (Nattiv et al., 2007; Writing Group for the ISCD Position Development Conference, 2004). A BMD score of <-1.0 calls for further assessment. The American College of Sports Medicine (ACSM) says low BMD is defined as history of hypoestrogenism, nutritional deficiencies, stress fractures, along with

other secondary risk factures for fracture and a BMD Z-score of -1.0 to – 2.0 (Khan et al., 2004; Nattiv et al., 2007; Writing Group for the ISCD Position Development Conference, 2004).

An ideal time to screen for the Triad is during annual health visits and preparticipation physical exams as well as when athletes are being assessed for related problems, such as stress fractures, amenorrhea, and reoccurring injury and illness (Nattiv et al., 2007). Furthermore, if the athlete has one component of the Triad, they should be assessed for the other components as well (Otis, Drinkwater, Johnson, Loucks, & Wilmore, 1997). Early and comprehensive screening and intervention for eating disorders or disordered eating should be conducted to prevent or catch any behaviors causing energy deficiency. An extensive health history should be recorded, with information such as energy, supplement, or medication intake, normal dietary practices, planned and unplanned weight changes, eating behaviors, body image, exercise expenditure, menstrual status, stress fractures and other injuries. A complete physical exam should occur for athletes who have or have had one or more component of the Triad. Physical symptoms may be found for athletes with eating disorders such as bradycardia, chronic cold hands and feet, hypercarotenemia, lanugo hair, parotid gland enlargement, and prolong (irregular heart rate) QT interval (Nattiv et al., 2007). Additionally for those with functional hypothalamic amenorrhea there could be hypoestrogenism or vaginal atrophy (Becker, Grinspoon, Klibanski, & Herzog, 1999). Laboratory tests should include electrolytes, chemistry profile, complete blood count, erythrocyte sedimentation rate, thyroid function tests, urinalysis, as well as tests to exclude other causes of amenorrhea (Nattiv et al., 2007). If the athlete has experienced a stress or low impact fracture or has had 6 months of amenorrhea, oligomenorrhea, eating disorders or disordered eating, DXA is recommended (Nattiv et al., 2007).

To help determine if female athletes are at risk or are currently experiencing aspects of the Triad, screening questions and tools have been developed to be used as part of a preparticipation physical evaluation (Joy et al., 2014). Examples of self-reported screening questions are, “have you ever had a menstrual period?”, “How many periods have you had in the last 12 months?”, “Have you ever had an eating disorder?”, “Have you ever had a stress fracture?”. Appendix K illustrates the cumulative risk assessment tool (Joy et al., 2014). It is an objective measure used to determine an athlete’s risk for the Triad by risk stratification and evidence-based risk factors. The cumulative risk assessment tool can be used to determine clearance for sport participation.

Appendix K is the Triad clearance and return to play guidelines by medical risk stratification. The sums of each risk factor from the cumulative risk assessment is used to determine the cumulative risk score. The outcomes are low, moderate, or high risk (Joy et al., 2014).

When treating the Triad, the goal is to reverse the effects, such as regaining a normal menstrual cycle, increasing BMD, and having sufficient to normal energy intake (Nattiv et al., 2007). Health care professionals that should be involved in treatment include, a physician, registered dietitian, and a mental health practitioner for those athletes with eating disorders (Nattiv et al., 2007). Other professionals that may be involved are an athletic trainer, exercise physiologist, and the athlete’s coach (Nattiv et al., 2007). Dependent on the reason for LEA, whether it be unintentional or intentional interdisciplinary health care providers are able to provide comprehensive care for treatment. The first step in nonpharmacological treatment is to increase energy intake and reduce energy expenditure to increase EA. An increase in EA will help restore normal menstruation. A normalization of body weight is important in regaining

menstruation and bone health, and the amount of weight gain needed is different for each individual (Joy et al., 2014). As body weight increases, BMD can increase as well for athletes with anorexia or amenorrhea (Nattiv et al., 2007). An intake of 30 kcal·kg·lean body mass·day^{-1} or more is thought to restore normal menstruation, but an intake of up to 45 kcal·kg·lean body mass·day^{-1} may be more effective in improving BMD with relation to weight gain. Additionally, energy intake should increase gradually, starting at 20% increments (Joy et al., 2014). Furthermore, supplementation of calcium and vitamin D are suggested as they support bone building (Nattiv et al., 2007). An increased protein intake of 1.2-1.6 g·kg·day^{-1} may also be beneficial since protein needs for athletes with intense exercise is normally recommended for their higher needs (Thomas, Erdman, & Burke, 2016). For those athletes who have an eating disorder or disordered eating, it is hopeful that they can trust their care provider and use support from family to help resolve any emotional issues or unhealthy thought processes to regain normal eating behavior.

Pharmacological treatment is necessary if nonpharmacological treatments have been unsuccessful after one year and there is occurrence of more fractures (Joy et al., 2014). Pharmaceutical agents such as antidepressants that may be helpful for treating eating disorders such as anorexia nervosa or bulimia nervosa are not proven to be helpful in restoring BMD (Nattiv et al., 2007). Other treatments such as hormone replacement therapy and oral contraceptive pills may be helpful in regaining BMD, but have mixed results for effectiveness that warrant a need for more research. Bisphosphonates have also been implicated in the treatment of osteoporosis; for postmenopausal women and should not be used on young women (Nattiv et al., 2007).

Psychological

There is an association between negative psychological factors and low energy availability (LEA) (Melin et al., 2014). The LEAF-Q is an assessment tool that is meant to compliment disordered eating screeners. Amenorrhea, a menstrual disturbance commonly seen among female athletes, is linked to LEA. The occurrence of amenorrhea is also predictive of females with eating disorders (Melin et al., 2014). In a study to validate the LEAF-Q, 84 female athletes (18-39 years old), training five or more days per week completed the LEAF-Q (Melin et al., 2014). Questions on the LEAF-Q regarding disordered eating and eating disorders were verified against clinical assessment tools. These tools consisted of the Eating Disorder Inventory (EDI-3) questionnaire and the Eating Disorder Examination (EDE-16.0), with results classified according to the Diagnostic and Statistical Manual Mental Disorders, Fourth Edition (DSM-IV) (Fairburn et al., 2008; Garner, 2004). Additionally, interviews were conducted with EDE-certified professionals. In the study there were more participants with LEA associated with disordered eating and eating disorders. A total of 12 participants (27%) presented with disordered eating (n=11) or an eating disorder (n=1). Eight of the twelve participants classified as disordered eating or an eating disorder also had LEA. Though the LEAF-Q was not created to identify eating disorders, it can be used to identify characteristics associated with the Triad and screens for further assessment need, suggesting that LEA may be predictive of undesirable eating behaviors and vice versa.

A study by De Souza et al., (2007) investigated the hormonal adaptations to energy deficiency and its potential relationship to drive for thinness as an indicator. Over a time period of three years, 52 participants were recruited for the study. Inclusion criteria for the study encompassed; women 18-35 years old, medical exam determining good health, no chronic

illness, stable menstrual status for three months, non-smoker, weight stable for the three months, not dieting, no forms of hormonal therapy in the last 12 months, and no history of a clinical eating disorder. Women with normal menstruation were monitored for two to three menstrual cycles. Women with oligomenorrhea were followed for 100 days or until their first menses, and for women with amenorrhea, they were followed for two to three 30-day periods. Height was measured once at the beginning of the study, total body mass was collected once a week on a physician's scale (Detecto, Webb City, MO), and BMI was calculated using the average weekly weight. A DXA scan was used to determine body composition. Resting energy expenditure was measured at the beginning of each menstrual cycle (day 2-6) using indirect calorimetry (SensorMedics Vmax Series, Yorba Linda, CA). The Harris-Benedict equation was used to estimate REE and used as a ratio of actual to predicted REE. At the same time, three-day food diaries were collected. The food diaries contained food intake from two week days and one weekend day. Energy and nutrient intake from the diaries were analyzed using Nutritionist Pro software (Version 1.3.36, First Databank Inc., Indianapolis, IN). Participants were asked to record daily physical activity logs, along with measure of heart rate within three minutes of beginning exercise, taken manually through palpation of an artery. A progressive treadmill test was completed for measurement of VO^2 peak. The Eating Disorder Inventory was used to assess drive for thinness once during the study. This consisted of a 91-item self-reported measure of attitudes and behaviors concerning weight, eating, and shape. Additionally, it also assesses psychological traits relevant to eating disorders such as ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation, and social insecurity. The three-factor eating questionnaire was also provided (De Souza, Hontscharuk, et al., 2007; Stunkard & Messick, 1985). These questions are comprised of dietary

cognitive restraint, disinhibition, and hunger. Fasting blood samples were also collected at the beginning of each menstrual cycle for the following hormones: total triiodothyronine, insulin, serum leptin and ghrelin. According to exercise status, participants were grouped into three groups, exercising 'high drive for thinness' (n=9), exercising 'normal drive for thinness'(n=34), and sedentary 'normal drive for thinness'(n=9). Normal drive for thinness scores were defined as less than or equal to six, and a high drive for thinness score was classified as seven or greater on the Eating Disorder Inventory. Exercising women with 'high drive for thinness' scored significantly higher for bulimia ($p=0.012$) and infectiveness ($p<0.001$) compared to the exercising women with 'normal drive for thinness' group (De Souza, Hontscharuk, et al., 2007). Exercising women with 'high drive for thinness' also scored significantly higher for dietary cognitive restraint ($p<0.001$), moreover, 'drive for thinness' had a significant positive correlation to dietary cognitive restraint ($r=0.602$, $p<0.001$). Resting energy expenditure for exercising women with 'high drive for thinness' was significantly lower ($p=0.023$) and 66% could be classified as energy deficient compared to 27% of the other group. Exercising women with 'high drive for thinness' had lower total triiodothyronine levels ($p=0.007$) and higher ghrelin levels ($p=0.004$), and combined total triiodothyronine concentration, adjusted REE, and the ratio of actual REE to predicted REE had a significant negative correlation ($p=0.018$). A positive correlation ($p=0.004$) between 'drive for thinness' scores and ghrelin concentration was found. To conclude, this study demonstrated the relationships between psychological factors dealing with eating behaviors, particularly 'drive for thinness', to hormonal markers of energy deficiency such as total triiodothyronine, REE, ghrelin, and abnormal menstrual cycles (De Souza, Hontscharuk, et al., 2007). Therefore, the Eating Disorder Inventory and 'drive for thinness' subscale could be used as helpful tools for screening and identification of energy deficiency.

The psychological diagnosis of anorexia nervosa and occurrence of amenorrhea were compared in a study (Bomba et al., 2014). Three groups of girls participated in this study, one group with amenorrhea only, one with anorexia nervosa only, and one control group. Each group had 21 girls all of similar age (16±1y). For screening, all groups underwent a clinical examination, BMI calculation, evaluation of physical activity by recall, blood tests, fasting hormone assays and a psychiatric evaluation with a neuropsychiatrist. Each of the participants also took the following self-administered tests: Children Depression Inventory, Eating Disorder Inventory-2 (EDI-2), and the Toronto Alexithymia Scale-20 (Bagby, Parker, 1994; Kovac, 1987; Garner, 2004). On the EDI-2, girls with anorexia had scores two to three times higher than the girls with amenorrhea in the following subscales: drive for thinness, ineffectiveness, interpersonal distrust, and interoceptive awareness. Girls with amenorrhea had scores five times higher in drive for thinness, two times higher in maturity fears and social insecurity subscales than the control group. Similar to the EDI-2, scores on the Children Depression Inventory had higher scores for the girls with anorexia than for girls with amenorrhea, and both groups were had higher scores than the control group. Thirteen (69.0%) of girls with anorexia and two (9.5%) girls with amenorrhea had depressive traits on the Children Depression Inventory, shown as a score of 19 or greater. Both girls with anorexia (+46%) and girls with amenorrhea (+32%) had higher scores on the alexithymia scale, on the overall Toronto Alexithymia Scale-20 score. Also, both groups had higher scores in "difficulties in describing feelings" subscale (+70% and +62%). Lastly, 15 (71.4% of girls with anorexia, 12 (57.1%) of girls with amenorrhea, and 4 (19%) of controls had alexithymic traits on the Toronto Alexithymia Scale-20 (Bagby, Parker, 1994).

Overall, girls with anorexia had more severe scores for the Children Depression Inventory, EDI-2, and Toronto Alexithymia Scale-20 than both the girls with amenorrhea and the

controls. However, the girls with amenorrhea had similar psychological patterns and also had scores significantly higher than the control group. Girls with anorexia and girls with amenorrhea had subthreshold or higher eating disorders. This was evidenced by their drive for thinness scores on the EDI-2. This score included concerns about body image, dieting, and fear of gaining weight (Bomba et al., 2014). Based on their score, girls with amenorrhea are classified as a "restrictive type" (Bomba et al., 2014). In conclusion, the researchers suggested amenorrhea can be considered as a model of psychosomatic disease and females experiencing amenorrhea should undergo assessment and treatment for both physiological and psychological aspects of the condition (Bomba et al., 2014).

Eating disorders

It is suggested that athletes are potentially at higher risk for eating disorders than non-athletes. This is likely due to the pressure and focus put on the body for performance, or particularly for sports that have more focus on aesthetic characteristics (Martinsen & Sundgot-Borgen, 2013). Body dissatisfaction and concerns about eating are common in the athletic population and are referred to as normative dissatisfaction, reportedly a predictor of the beginning of an eating disorder (Karpinski & Rosenbloom, 2017, p. 405). Twenty-one small studies were used to examine the prevalence of eating disorders in athletes. For disordered eating the estimate ranges from 6.0% to 45.0% in females and 0% to 19.0% in males. Specific eating disorders in females, anorexia ranges from 0% to 6.7%, bulimia is 0% to 12.1%, and eating disorders not otherwise specified are 2.0% to 13.4%. In males, the prevalence of eating disorders is anorexia 0%, bulimia 0% to 7.5%, and eating disorders not otherwise specified 0% to 9.7% (Karpinski & Rosenbloom, 2017, p. 392).

The prevalence of eating disorders among a sample of adolescent female and male athletes was examined against a control group using a two-tiered approach of both questionnaires and clinical interviews for risk factors of eating disorders (Martinsen & Sundgot-Borgen, 2013). Among participants in 16 different elite sport high schools in Norway, born in the year 1992 and first-year student. 611 males and females were surveyed with a response rate of 90%. Nonathlete controls, with a response rate of 85% of 355, participated in the study. Athletes were classified as weight-sensitive and less weight-sensitive sports participants. 'Weight-sensitive' included gymnastics, dancing, martial arts, track and field runners and jumpers only, swimming, cycling, skiing, and others. Less weight-sensitive sports entailed tennis, basketball, soccer, golf, hockey, track and field throwers only, volleyball, and others. The first part of the study required participants to answer a series of questionnaires. Categories within the questionnaires were training history, physical activity, menstrual history, nutritional patterns, oral contraceptive use, weight fluctuation history, dieting, injuries, pathogenic weight control methods, and previous or current eating disorders. Participants also complete the Eating Disorder Inventory-2 and the Hopkins Symptom Checklist (Strand, Dalgard, Tambs, & Rognerud, 2003). At least one of the following criteria had to be met be classified as "at risk" for an eating disorder: Drive for thinness score ≥15 for girls and ≥10 for boys, body dissatisfaction ≥14 for girls ≥10 for boys, underweight body mass index, currently trying to lose weight, tried to lose weight in the past three or more times, self-reported amenorrhea or secondary amenorrhea, use of pathogenic weight loss methods: diet pills, laxatives, diuretics, vomiting. Physical activity included physical education lessons, recreational sport, and activities of daily living. In the second part of the study, participants underwent a clinical interview for which the questions were based on the Eating Disorder Examination 16:0 with sport specific questions and the Eating Disorder

Examination Questionnaire 6.0 (Fairburn & Beglin, 2008). One psychiatrist and three people trained for the Eating Disorder Examination, conducted the interviews. Participants found to be "at risk" in the first part of the study were chosen to complete the second part. All of the 153 athletes that were "at risk" were invited to the clinical interview and 91 (50%, 57 girls, 34 boys) of the control group were invited. Also, 102 girls and 51 boys in the athlete group that were not "at risk", and 22 girls and 66 boy controls not "at risk", were invited to the clinical interview.

In part one, there were more controls (50%) found to be "at risk" for eating disorders than athletes (25%), as well as more females compared to males (athletes: 46.2% vs. 13.1%; controls 72.4% vs. 33.7%). For weight-sensitive (26.4%) and less weight-sensitive (24.6%) sports there was no significant difference in participants classified as "at risk". However, in part two, the same results were not found. For the clinical interview, 96 female athletes and 48 male athletes participated. Of these, 24 of the females and 5 of the males, were classified as "at risk" based on criteria for eating disorders. Thirty-four athletes met the criteria for the DSM-IV, and 73.5% (25) of them were diagnosed with eating disorders not otherwise specified. Of the controls, 4 of the 57 females met the criteria. Overall, 7.0% of athletes and 2.3% controls met the criteria for eating disorders. Female athletes (14.0%) had a higher prevalence than female controls (5.1%). Female athletes had a higher prevalence than male athletes (3.2%). Again, no difference was found between weight-sensitive sports and less-weight sensitive sports. Only 5 of 136 (3.7%) athletes classified as healthy. The subscale of shape had the highest score for concern for athletes and controls with eating disorders. Other subscales reported for those with eating disorders were dieting (n=34), pathogenic weight control methods (n=25), vomiting (n=22), binging and vomiting (n=8).

In conclusion, the prevalence of eating disorders was higher among athletes, moreover, it was higher among female athletes versus male athletes. In the first part of the study, controls presented with a higher prevalence, however, researchers suggest based on the information found in the second part of the study that athletes may be less willing to report symptoms, due in part to feeling these behaviors are rational to achieve optimal performance. Clinical interviews were judged to better identify more athletes at risk (Martinsen & Sundgot-Borgen, 2013).

There is an array of studies that investigate the risk factors and vulnerabilities that may cause athletes to develop an eating disorder or have a higher prevalence of eating disorders compared to the "healthy" non-athletic population. Relationships between coaches and teammates can influence athletes in combination with performance pressure (Shanmugam, Jowett, & Meyer, 2014). Additionally, athletes may feel justified or encouraged to participate in behaviors of weight regulation if they feel it will help them enhance their performance (Krentz & Warschburger, 2013). Moreover, athletes experience more sport specific triggers in addition to pressure for performance such as injuries or weigh-ins, whereas more common triggers for eating disorders in the general population are bullying or family dysfunction (Arthur-Cameselle, Sossin, & Quatromoni, 2017). Reasons for body dissatisfaction and pressure may vary based on sport or other situational factors.

The purpose of a study by Kong & Harris (2015) was to determine if dieting behaviors, disordered eating, and level of body dissatisfaction for female athletes was impacted by the level of sport (elite, recreational, or noncompetitive) and whether it was a leanness or non-leanness sport they participated in. A total of 320 participants were gathered by use of recruitment emails to Australian elite and recreational sporting organizations. Inclusion criteria consisted of the following: female, age 17-30 years, regular participation in a sport at elite, recreational, or

noncompetitive level, and participated in a leanness or non-leanness focused sport (leanness: gymnastics, cheerleading, cycling, long distance running, lightweight boxing or rowing; non-leanness: football, netball, soccer, hockey, cricket, baseball, racquet sports, waterpolo, heavyweight rowing) (Kong & Harris, 2015). A total of 174 athlete participants were in leanness sports and 146 in non-leanness sports. For sport level, 128 were elite athletes, 112 recreational, and 80 were noncompetitive. Participants were asked to provide the following demographic information: age, sex, years of education, height, weight, highest weight, ideal weight, lowest weight, if they are breastfeeding, type of sport, hours training per week, years of participation, level of participation, and if their coach monitored their body weight. Through an online survey, they also answered a series of questions from two different tests. The first, the Eating Attitudes Test (EAT-26)(Garner, Olmsted, Bohr, & Garfinkel, 1982). Participant scores of 20 or greater indicated disordered eating or an eating disorder. The other test to measure body shape perception was the Figure Rating Scale (Stunkard, Sørensen, & Schulsinger, 1983).

Leanness athletes trained for significantly more hours that non-leanness athletes per week ($p=0.014$), and elite athletes trained over twice as long as recreational and noncompetitive athletes ($p<0.001$). When answering whether they felt pressure from their coach to keep a low body weight, there was no significant difference between elite leanness and elite non-leanness sports ($p=0.71$). Results for the EAT-26 survey questions indicated that leanness athletes scored higher than non-leanness athletes ($p<0.001$). Additionally, elite athletes had greater disordered eating symptomatology in comparison to recreational athletes and noncompetitive athletes ($p<0.001$). A score of 20 or higher was reached by 74 female athletes (23.0%), 39 of these elite and leanness athletes. More leanness athletes (n=61, 35.1%) scored higher on the EAT-26 than non-leanness athletes (n=13, 8.9%). Over twice the number of elite athletes (n=43, 33.6%)

scored more than 20 on the EAT-26 than the recreational athletes (n=19, 17%) and noncompetitive athletes (n=12, 15%). Significantly more leanness athletes reported self-induced vomiting ($p=0.01$) and laxative use ($p=0.22$) compared to non-leanness athletes. For ideal weight, leanness athletes had a significantly higher current and ideal weight in comparison to non-athletes ($p=0.045$). For the Figure Rating Scale ideal figure, leanness athletes wanted significantly leaner figures than non-leanness athletes ($p<0.001$), and elite athletes picked significantly leaner figures than both the recreational and noncompetitive athletes ($p<0.001$). Together, elite leanness athletes had significantly leaner ideal figures than all other groups ($p<0.001$). Similar results were found for Figure Rating Scale sport figures, where a significantly leaner ideal figure was reported by the leanness focused athletes ($p<0.001$). Leanness athletes reported greater body dissatisfaction than non-leanness athletes ($p=0.044$). Lastly, leanness athletes also had a greater sporting dissatisfaction report than the non-leanness athletes ($p<0.001$).

Overall, more disordered eating patterns were seen in both leanness athletes and elite athletes. Of the 74 women who reported a score of 20 or more on the EAT-26, 61 (82.4%) were leanness athletes and two thirds of those 61 were elite athletes. The average score for elite athletes on the EAT-26 was 23.3, (comparison to the cut point of 20 for identifying risk of eating disorders). Similarly, elite athletes from leanness sports also had higher numbers of vomiting and laxative use for intentional weight loss and a leaner ideal figure. Elite athletes also had greater levels of eating disorder symptomatology compared to recreational and noncompetitive athletes and 60% from leanness sports reported experiencing pressure from a coach to maintain a lean figure (Kong & Harris, 2015). Elite athletes may feel pressured to have lean physiques in order to perform well, leading to higher levels of body dissatisfaction. Although, it was noted that non-

leanness athletes and athletes from different sport levels also experienced symptoms of disordered eating, elite athletes, particularly from leanness sports, may have a particularly high risk for development of disordered eating problems (Kong & Harris, 2015).

Performance Effects

Low energy availability is associated with decreased performance as indicated by the RED-S model (figure 2) for health and performance consequences (Mountjoy et al., 2014). The ten performance effects include depression, irritability, impaired judgement, and decreased endurance performance, muscle strength, glycogen stores, concentration, coordination, training response, and increased injury risk. For female athletes with menstrual dysfunction, increased risk of injury may particularly be seen as bone injury because the benefit of weight bearing exercise is not received due to LEA (Ackerman et al., 2015). Additionally, LEA can be associated with increased likelihood of illness (e.g. colds or flu), and consequently, reducing sport performance (Drew et al., 2017; Drew et al., 2018). Furthermore, it is suggested that RED-S has an impact on sports performance through the categories displayed on the RED-S model (figure 2).

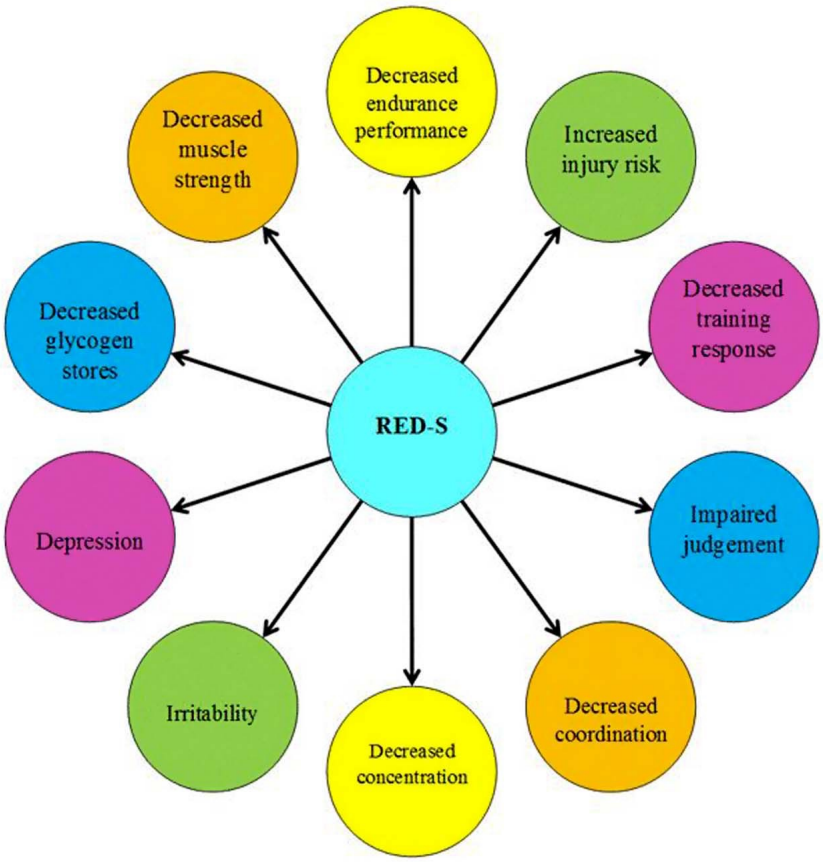

Figure 2. Possible negative performance effects of RED-S (Mountjoy et al., 2014)

A study by Woods et al. (2017) investigated the effects on RMR and exercise regulation of elite Australian rowers to an intensified training load, and also the impact on performance. Seventeen elite rowers completed a four-week training cycle, training six days per week. Participants consisted of 10 males and 7 females; ages 21-30 years old. Training load was measured as T2 minutes, used to calculate training duration, intensity, and mode in various formats for elite rowing. The equivalent to a T2 minute is one minute of on-water scull at T2 intensity (60-72.0% VO^2max). The different training formats included on-water, ergometer, strength and cross-training. The Douglas Bag method of indirect calorimetry was used to calculate RMR before and after the four-week training period (Woods, Garvican-Lewis, Rice, & Thompson, 2016). Participants were measured in a rested and fasted state, early in the morning

with no exercise eight hours prior. Before each RMR calculation, participants recorded energy intake by keeping a three-day food diary. A dietitian analyzed total energy and macronutrient intake using FoodWorks Professional v7.0.3016 software (Xyrics Software Pty Ltd, Australia). Body composition was measured using DXA before and after the four-week training period. A 5 km time trial one week before and after the four-week training period was used to evaluate on-water rowing performance and pacing strategy. Participants also took the Multicomponent Training Distress Scale to assess training-related mood disturbance every week of the training period and the week before and after training began and ended. Questions were related to indicators of training overload: depressed mood, vigor, physical signs and symptoms, sleep disturbance, perceived stress and fatigue. Heart rate for every day on-water training was tracked for each individual using a software program (Sportlyzer, Tartu, Estonia). Responses to training were monitored by heart rate, velocity, distance, and stroke rate during the sessions. Participants also had 30-minute sets in their individual T2 training zone that were monitored for power output, heart rate, rating of perceived exertion, and blood lactate concentration by an ergometer (Model D, concept2, Victoria, Australia).

Seven athletes were eliminated from the study due to injury, deliberate change in body composition, or hyperventilation during RMR measurement. Overall, training load increased by 21±7% from the beginning to the end of the study (Woods, Garvican-Lewis, Lundy, Rice, & Thompson, 2017). Absolute RMR decreased significantly ($p=0.01$) from baseline to after training increase. Body mass ($p=0.003$) and fat mass ($p=0.0001$) both decreased significantly. There was no change observed in energy or macronutrient intake. A significant difference was found in 5 km on-water trials for rowing performance ($p>0.05$). As the training cycle progressed, responses to the Multicomponent Training Distress Scale (Main & Grove, 2009) significantly

increased in fatigue (p=0.001) and total mood disturbance (p=0.02). Lastly, training monitoring of heart rate concluded with insufficient data.

In conclusion, an increased training load was associated with decreased RMR, rowing performance, body composition, increased fatigue, and mood disturbances. The decrease in RMR may be related to the lack of change in energy intake (Woods et al., 2017). Therefore, EEE increased without a coinciding increase in energy intake, causing a negative balance in EA, contributing to a decrease in RMR as a potential form of energy conservation. Researchers suggest the lack of energy intake along with changes in mood may have impacted their performance and indicate that athletes were training while fatigued. Also, changes in mood that coincided with an increase in training in addition to slower rowing race times, lowered blood lactate concentrations, and reduced power output during ergometer sessions also suggest experienced fatigue. Overall, failing to increase energy intake with increased intensity of training load may impair performance through psychophysiological factors such as changes in mood and a decreased in RMR.

A study by Tornberg et al. (2017) investigated if there was a relationship between reproductive function, metabolic and endocrine changes, and neuromuscular performance. The study involved 30 elite female endurance athletes between the ages of 18 and 39 years. Exclusion criteria included the following: pregnancy or chronic illness, smoking, use of hormonal contraceptives six weeks prior to the study, injuries that kept athletes from training for two weeks or more, and menstrual dysfunction caused by something other than secondary amenorrhea (absence of three or more consecutive menstrual cycles). Participants with eumenorrhea (n=16) who were currently menstruating completed testing in the early follicular phase (first 3-5 days). Testing was completed within two consecutive days. Participants were

required to limit exercise the day before and the first day of testing to 30 minutes at low to moderate level and to arrive both days in a rested and fasted state. The first day, bone health and reproductive function were examined. Participants were asked to take a pregnancy test and a menstrual function examination was completed by a gynecologist with an ultra-sound machine to assess ovarian volume (Ultrasound Scanner, Class 1 type B, B-K Medical REF TYPE 2202). A total of 12 out of the 30 participants, 5 with secondary amenorrhea and 7 with eumenorrhea, were taking hormonal contraceptives. These participants were asked to discontinue contraceptive use six weeks prior to the start of the study to allow a washout period. To further assess reproductive function, participants completed a LEAF-Q (Appendix D) to provide information such as onset of menarche, history if menstrual irregularity, number of cycles in the last year, and past use of hormonal contraceptives. Additionally, participants kept a menstrual bleeding calendar for three months in order for researchers to classify them as having eumenorrhea, secondary amenorrhea, oligomenorrhea, or polycystic ovarian syndrome. A DXA scan was used to assess body composition while participants were in a fasted state. The second day of testing energy metabolism, aerobic capacity, and neuromuscular performance was examined. In a fasted state between 7:00 and 8:30 AM, RMR was determined using a ventilated open hood system (Oxycon Pro 4, Jaeger, Hoechberg, Germany). Directly after, a blood sample was taken for hormonal concentrations to confirm participants were in a fasted state. Remaining in a fasted state, participants then completed a bicycle ergometer test to determine work efficiency. A capillary blood glucose test was taken in the rested state before the bicycle test, and taken after light activity. To determine EA, food intake and exercise were recorded on 7-day food and exercise logs. A heart rate monitor was worn during exercise and transportation such as bicycling (Polar RS400®, Polar, Oulu, Finland). Nonexercise activity thermogenesis was assessed using

actigraphy and data analysis software Actilife 5 (ActiGraph GT3X, Pensacola, FL). Participants wore an accelerometer at all times except while showering, swimming, bicycling, and training. For exercise capacity, a VO^2 peak test was conducted two hours after a standardized breakfast. Next, reaction time was tested using a portable computer connected to a linear encoder (MuscleLab 4010: Ergotest Innnovation, Langensun, Norway). Each participant performed three familiarization trials and five reaction trials. Knee muscular strength and knee muscular endurance were also tested. Isokinetic dynamometry was performed on a computerized dynamometer using the right leg (Biodex Multi-Joint System IV, Shirley, NY). Lastly, the following blood samples were collected: estradiol, androstenedione, total testosterone, progesterone, serum sex hormone-binding globulin, serum triiodothyronine, cortisol and thyroid-stimulating hormone, insulin, and capillary blood glucose. Athletes with secondary amenorrhea weighed less, had lower BMI, and lower fat mass and fat free mass compared to athletes with eumenorrhea (Tornberg et al., 2017). Energy intake, energy expenditure, or endurance training did not differ between athletes with secondary amenorrhea and athletes with eumenorrhea. However, athletes with secondary amenorrhea did perform more resistance training. Also, athletes with secondary amenorrhea had lower RMR, triiodothyronine, blood glucose, higher cortisol, and work efficiency. A positive association was found between fat mass and triiodothyronine ($p<0.05$), RMR ($p<0.05$), RMR ratio ($p<0.05$), and between triiodothyronine and RMR ($p<0.05$) and RMR ratio ($p<0.05$). In athletes with secondary amenorrhea, a negative association was found between triiodothyronine and fat free mass ($p<0.01$). Of the 14 athletes with secondary amenorrhea, 12 (86%) had hypoglycemia in a fasted state at rest and after exercise, compared to 2 (13%) of the 16 athletes with eumenorrhea. Cortisol-to-insulin ratio was higher (39%) and estrogen-to-cortisol ratio was lower (50%) in athletes with amenorrhea. A

faster reaction time was associated with higher blood glucose, triiodothyronine, estrogen, and lower cortisol levels. Reaction time in athletes with eumenorrhea was 7% shorter, and knee muscular strength (11%) and knee muscular endurance (20%) were greater. However, there was no significant difference between groups when knee muscle endurance was divided by fat free mass. A positive association was found between knee muscular strength and knee muscular endurance with fat free mass of the leg and triiodothyronine ($p<0.001$), and a negative association with cortisol ($p<0.05$, $p<0.001$).

In conclusion, there was a lower neuromuscular performance in athletes with secondary amenorrhea in comparison to athletes with eumenorrhea. This reduced performance was linked with lower blood glucose, triiodothyronine, estrogen, fat free mass of the leg, and higher cortisol. Researchers suggest that the threshold for LEA may be higher for athletes with secondary amenorrhea as they did not find a difference in EA between groups, but did see a difference in metabolic alterations (Tornberg et al., 2017).

Screening and Diagnosis

Early screening and detection can help to identify RED-S before health and performance consequences begin to develop. Screening procedures for RED-S are parallel to those of the Triad. If one of the three components of the Triad are identified, the individual should also be tested for rest of the components of the Triad (Nattiv et al., 2007). Overall screening should include assessment of energy intake, menstrual function (in females), bone health, and risk of disordered eating or eating disorders. Further assignment with physical exams and laboratory tests may also be needed. A pre-health examination should be given annually to athletes along with a pre-participation physical evaluation. Both examinations provide answers linked to the spectrum of symptoms experienced with the Triad and also with RED-S (De Souza, Nattiv, et al.,

2014). If an athlete presents with amenorrhea, stress fractures, recurrent injury or illness, disordered eating, eating disorders, weight loss, poor normal growth and development, decreased performance, or mood changes, this is an opportunity to further evaluate for RED-S and or the Triad (Mountjoy et al., 2014; Nattiv et al., 2007).

It is imperative to screen for disordered eating and eating disorders in order to recognize early onset and prevent intensification. If athletes do not meet full criteria for anorexia nervosa or bulimia nervosa this does not determine they are not at risk, and further more comprehensive screening may be needed. Identification of eating disorders not otherwise specified e.g. restricting or purging is important as EA and bone health can be affected (Nattiv et al., 2007). The Eating Disorder Examination interview (EDE) is the gold standard for eating disorder diagnosis (Martinsen et al., 2014). A validated screening tool that is recommended to help identify athletes with or without disordered eating or an eating disorder is the Brief Eating Disorder in Athletes Questionnaire (BEDA-Q) (Mountjoy et al., 2014). Especially for athletes who may be motivated to hide signs and symptoms of eating disorders, in-depth interviews and assessment are more useful in identification for disordered eating and eating disorders (Mountjoy, Sundgot-Borgen, et al., 2018). This is particularly important because athletes tend to have higher drive for thinness scores, specifically those with amenorrhea (Gibbs, Williams, Scheid, Toombs, & De Souza, 2011).

Menstrual function can be assessed by self-reported questions, but it may also call for more exhaustive physical examinations or laboratory tests. A menstrual history questionnaire is essential to ask questions about age of menarche, irregular menses and patterns, medication use such as oral contraceptives or hormonal therapy, and family history of menstrual issues (De Souza et al., 2014; Mountjoy et al., 2014). Physical examination, pelvic examination, laboratory

assessment of reproductive hormones, or even a pelvic ultra-sound and endometrial sampling may be necessary to determine menstrual status such as eumenorrhea, amenorrhea, oligomenorrhea, or other types of menstrual dysfunction (Mountjoy et al., 2014; Nattiv et al., 2007).

Evaluation of energy intake and EEE to determine EA is also an important element of screening. When diagnosing LEA, it is important not to use energy balance alone. This is because the body may adapt to a negative energy balance, changing physiological functions and stabilizing weight. Therefore, an athlete could be at a zero-energy balance, but still have LEA. Calculation of energy of food intake can be completed by having athletes complete 24-hour recalls, or a three, four, or seven-day food diaries. Further assessment with a registered dietitian may be necessary if a nutrient deficiency is suspected (De Souza, Nattiv, et al., 2014). Exercise logs along with heart rate monitors and accelerometers can be utilized to calculate energy expenditure. The 2011 Compendium of Physical Activities (Ainsworth et al., 2011) is recommended to calculate EEE (De Souza, Nattiv, et al., 2014). A DXA scan, anthropometric skinfolds measurement, bioelectrical impedance, or air displacement plethysmography can be used to determine fat free mass. Low energy availability (LEA) may be indicated by a BMI of <17.5 kg/m^2 or $<85\%$ of body weight for adolescents (De Souza, Nattiv, et al., 2014). There is an EA calculator on the Female Athlete Triad Coalition website ("Energy Availability Calculator - The Female and Male Athlete Triad Coalition," n.d.) that can be utilized to find EA.

During LEA bone health can become compromised and it is critical to detect this early on to prevent any long-term negative effects. A whole body DXA scan including lumbar spine should be completed for athletes that have had LEA, eating disorder, disordered eating, or amenorrhea for the last six months (Mountjoy et al., 2014). An adolescent or older athlete with a

Z score between -1.0 and -2.0 in combination with nutritional deficiency, hypoestrogenism, and/or a stress fracture is considered to have low BMD scores and require further investigation (Nattiv et al., 2007). For those at risk for bone loss, bone mineral density should be reevaluated every 12 months for adults and 6 months for adolescents (Mountjoy et al., 2014). For adolescents between the ages of 5 and 19, osteoporosis is diagnosed as a Z score of ≤-2.0 in combination with a clinically significant fracture such as a low bone fracture of the lower extremities, vertebral compression fracture, or two or more long bone fractures of the upper extremities. To diagnose osteoporosis in premenopausal women, a Z score of ≤-2.0 along with secondary causes of osteoporosis is recommended (Lewiecki et al., 2008).

In addition to the periodic health examination, the preparticipation physical evaluation, and the LEAF-Q, the RED-S clinical assessment tool (CAT) that is not yet validated, may be used to help screen for RED-S (Mountjoy et al., 2015b). This tool is meant to aid medical professionals in evaluating athletes with RED-S syndrome. The risk assessment chart uses a stop light metaphor with red, yellow, and green to categorize athlete risk. Red is high risk, yellow is moderate risk, and green is low risk. Additionally, the tool provides guidance with a RED-S treatment contract template and assessment for return to play (Mountjoy et al., 2015). The return to play model also uses the stop light metaphor to categorize and assess the appropriate level of participation based on signs and symptoms of RED-S. Red signifies high risk and no competition or training, and a written contract for treatment and recovery should be signed by the athlete. Symptoms in the red category include eating disorders, psychological and physiological conditions, bradycardia, or use of extreme weight loss techniques. The yellow category is moderate risk. If the athlete is following the treatment plan, they can train and compete after being medically cleared. Signs of moderate risk include 5% to 10% weight loss in a month, an

abnormally low body fat percentage for an extended period of time, interrupted growth or development, prolonged LEA, abnormal menstruation (>3 months), low BMD, or stress fractures which are associated with hormonal abnormality or LEA. Finally, the green category means the athlete can have full sport participation. Athletes in the green category manage an appropriate physique through healthy diet and exercise habits, proper EA, and are psychologically and physiologically healthy (Karpinski & Rosenbloom, 2017, p. 411). Registered dietitian nutritionists (RDN) are considered to be the nutrition expert as part of the professional team and are trained in counseling and coaching skills. Dietitians are positioned to support patients and athletes during return to play by provision of nutritional treatment and rehabilitation by using counseling and coaching skills during the nutrition care process (Karpinski & Rosenbloom, 2017, p. 407).

METHODS

The purpose of this study was to investigate the relationship(s) of screening for RED-S with bone health, and how nutrition may influence those relationships, among collegiate long-distance runners. The information gathered during this study may be beneficial to coaches, athletes, and health care professionals in understanding the impact nutrition has on performance through physical and psychological aspects. Potentially preventing bone injuries and eating disorders from occurring. The following methods detail how this data was collected and analyzed.

Participants

A cross-sectional study was implemented to recruit collegiate female and male competitive long-distance runners from North Dakota State University. Participants had to be eighteen to twenty-five years old, not injured, and currently participating as part of a collegiate competitive team. Additionally, they had to be training a minimum of five hours per week during recruitment and during testing week of the study. Cross-country coaches were contacted and meetings set up allowing the researchers to explain and discuss the study. Each male and female athlete was invited to participate in an electronic survey (Appendix H), DXA scan, three-day food diary, and exercise log. The electronic survey combined three surveys. A DXA screener survey, to screen for DXA safety, an EAT-26 survey to screen for eating disorders, and a LEAF-Q, to screen for RED-S. Study procedures were clearly presented in the written informed consent (both list of procedures and estimated time for each procedure).

Procedures and Materials

Applicable athletes were invited to an informational meeting to learn about the study; and (1) the benefits of logging both food and exercise and (2) the benefits of bone health for

performance and long-term health. The coach or coaching staff were not part of this meeting. During the meeting study details and instructions were presented on how to complete a three-day food diary and exercise log as an educational component to benefit knowledge of student athletes. A hand-out was provided regarding bone health.

Self-regulation theory is established around self-awareness and/or self-monitoring of behaviors. Assuming that balanced nutrition is important to all competing athletes, an educational session was led on food logging and the importance of logging foods at the *start* of any *change* in training program when energy needs may be higher or lower (this is known as periodized training). Self-monitoring is central to weight management (Burke, Wang, and Sevick, 2011), and management of weight is central to athletic performance. Therefore, a short 20-minute educational session was offered by the registered dietitian nutritionist (RDN) on the benefits of, and how to fill out a food diary. Examples were used to demonstrate the most accurate way to record food intake, what time, what food, and how much was eaten. At the same meeting, instruction was given on how to properly record exercise by including type of exercise, duration, and intensity on the exercise log. The food and exercise logging were tied together at the end of the presentation, showing participants the energy balance equation:

$$\text{Energy intake/expenditure=energy balance} \quad (2)$$

If expenditure increases, such as starting a new training program, intake must increase or weight loss will occur. If energy intake stays the same, but training decreases, such as during the "off-season", weight gain will occur; other examples will be given as well.

Each participant was given a food and exercise diary to complete on their own time. Next, a short presentation was given regarding bone health.

Participants were given a brief overview (5 to 10 minutes) about the research study and each athlete was given (1) informed consent form (2) HHQ (3) PAR-Q (4) SASE (self-addressed stamped envelope) to return the carefully, neatly, completed forms and food and exercise diary/log within one week, if interested in study participation. Willing participants were encouraged to call or email with questions before signing the consent form. Further, if potential willing participant wanted to meet face-to-face to ask questions or further discuss the study, a meeting was arranged. If athletes, did not want to participate, they were asked to dispose of the forms privately.

After receiving completed forms in the mail, the research assistant contacted participants to schedule their DXA scan and to complete the electronic survey (LEAF-Q, DXA Screener, EAT-26).

Screening Procedures

Participants completed the HHQ (Appendix B) and the PAR-Q (Appendix C) by paper and pencil. Participants were asked to complete these forms so researchers know that the participants are healthy and physically able to safely participate in the study. The HHQ (Appendix B) is also a means to collect data on possible supplements and medications that could impact bone health. This information is important for the researchers to know so the participant could be reminded to not take any supplements the day of the DXA scan. Additionally, the DXA screener (Appendix F) identifed those unable to safely participate in a DXA scan.

Study Questionnaires

To distribute the electronic survey (Appendix H), Qualtrics (Provo, UT) was used to provide the athlete with a URL. The URL provided a link to instructions to complete the survey (Appendix H). Questions on the electronic survey included the LEAF-Q (Appendix D); the DXA

screen (Appendix F); and the EAT-26 (Appendix G), combined and headings deleted to streamline Appendix H. The survey (Appendix H) began with demographic information such as age, sex, and education level. Participants were asked to provide current height (inches and feet) and weight (pounds) in addition to the highest weight and lowest weight experienced at their present height. History of tobacco use, medication use, and the number of hours spent training during the week were also asked. Next, the athlete's history of missed practices due to injury, illness, evaluation of adverse gastrointestinal symptoms, and use of contraceptives were documented. Lastly, the survey asked questions regarding current and past menstrual function. Together these questions completed the LEAF-Q (Appendix D) and the DXA screen (Appendix F). If the participant was a male, questions regarding contraceptives and menstrual function were bypassed and the survey moved to the second part.

The second part of the survey asked questions from the Eating Attitudes Test (EAT-26) (Appendix G). Questions evaluated the athlete's eating behaviors, thoughts, and feelings towards food. The EAT-26 cannot diagnose eating disorders but it is a reliable and valid test used to identify those needing further evaluation of eating disorders. The EAT-26 consisted of 26 questions.

If athletes screen positive for eating disorders or problems with bone health (scans will be read by the study Medical Doctor), subjects were advised to see their physician for a complete health screening and follow-up.

Food Diary

Participants were asked to complete a three-day food diary (Appendix I) keeping a detailed record of all food and beverage intake for two week days and one weekend day (or two "normal days" and one "abnormal eating" day). The form also required participants to list all

supplements and drugs, i.e. anything that goes in the mouth. To estimate total energy intake, participants were guided by a registered dietitian nutritionist to complete the three days, regarding what they ate and how much they ate. Additional details of food brand or providing food wrappers was also encouraged. The three-day food diary was provided by the research team as a stapled packet. Participants were provided detailed written instructions with examples, and in-person instructions explaining the proper way to record food intake. Participants were provided with contact information for any questions or concerns regarding the food diary.

DXA Scan

A DXA scan was performed on the athletes after completion of the three-day food diary, exercise log, and electronic survey. The DXA scan was used to determine bone mineral density (BMD) of the athletes to study relationships between energy and other nutrient intake, exercise levels, and estimated level of BMD. Additionally, it was used to assess body composition such as fat mass and lean mass. Participants were asked to come for DXA scans in the morning during a rested state with no exercise prior. Participants need to be in a euhydrated state to improve accuracy of the scan (Gibson et al., 2019, p. 244). Female participants were also be asked to undergo a pregnancy test before the DXA scan as it is not recommended that pregnant individuals be exposed to radiation. Before measurement the DXA machine was calibrated. Athletes were asked to void the bladder and remove all jewelry. Wearing minimal clothing (shorts and a t-shirt) participants were measured for height and weight. Next, participants were in a supine position and asked to remain still during the measurement that took about 20 minutes. The registered dietitian nutritionist, under the supervision of a trained DXA certified technician then completed the DXA scan. Additionally, a manual heart rate measurement to determine participants resting heart rate after the DXA scan was taken. This was completed by finding a

pulse in the participants wrist and counting the beats for one minute. After the results were obtained, they were analyzed and forwarded to a radiologist to screen for medical concerns. Participants received results from the DXA scan and the nutritional analysis, including the EAT-26 score, at the end of the study. Participants are able to use the information provided for their own benefit, whether they choose to seek further guidance or not.

Exercise Log

Exercise energy expenditure was self-reported using a three-day exercise log. This log included type of exercise, duration, and intensity (Appendix I). Participants were asked to log exercise the same days as the food log. Participants used rate of perceived exertion to determine the intensity of the exercise training. An instructional sheet was provided in combination with the exercise log to assist participants in identifying the intensity of the exercise ("Rated Perceived Exertion (RPE) Scale," n.d.)(Appendix J). Rate of perceived exertion (RPE) can be classified on a scale of six to twenty, 06 (no exertion at all), 7 (extremely light), 9 (very light), 11 (light), 13 (somewhat hard), 15 (hard, heavy), 19 (extremely hard), 20 (maximum exertion)("Rated Perceived Exertion (RPE) Scale," n.d.). Participants were asked to provide a rate of perceived exertion for each exercise during a training session as well as the duration. Same as the three-day food diary, participants who had questions on how to properly record exercise were given contact information to discuss questions with a researcher.

Athletes at Risk for Health Problems

Participants were given a number (DX1, DX2,…Etc.) associated with their results to maintain confidentiality. If a participant scored high for eating disorders on the EAT-26, had problems with bone health (scans will be read by study MD), or energy deficient, they were advised to see their physician for a complete health screening and follow-up. No information was

provided to coaches regarding individual results. Participant were given a “participant report card” (Figure 1) that displayed their individual results from the study including calorie intake, exercise expenditure, protein intake, calcium intake, vitamin D intake, EA, total BMD, z-score, EAT-26 score and LEAF-Q score if they were not at risk. The report card provided a recommended calorie range for the individual calculated using the Total Daily Energy Expenditure (TDEE) equation (Rosenbloom, 2018). Recommended protein intake was also provided. This was calculated by multiplying the participants weight in kilograms by 1.2-1.7 (Karpinski & Rosenbloom, 2017). A small description of each category and the significance of the test and results were provided as well. These report cards were emailed to the participants after completion of the study.

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Introduction

The purpose of this study was to investigate RED-S in female and male collegiate athletes and determine if there is an association with bone health. Relative energy deficiency in sport (RED-S) is a condition in which low energy availability (LEA) occurs in athletes and results in negative effects on health and performance in both females and males (Mountjoy et al., 2014). Relative energy deficiency in sport (RED-S), defined as a syndrome that impairs physiological function, and includes but is not limited to impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis, and cardiovascular health. Relative energy deficiency in sport is commonly found in athletes and those training for recreational activities in which the individual is expending more energy than being consumed (Mountjoy et al., 2014). A lack of knowledge regarding RED-S exists among coaches, athletic trainers and athletes (Mountjoy, Costa, et al., 2018).

In an editorial by Mountjoy et al. (2018), it is suggested that the impact of RED-S is highly underestimated (Mountjoy, Burke, Stellingwerff, & Sundgot-Borgen, 2018). The authors compare the magnitude of concussion research to the importance of awareness for RED-S and the impact of future research. The information from the comparison of the International Sport Federation surveys from 2012 to 2016 states that nutrition and RED-S are given a low priority by programs. However, to improve athlete health and performance and decrease injury, Mountjoy et al. (2018) suggests including an increase in RED-S education in a more comprehensive manner to achieve an all-encompassing approach to athlete health, for injury prevention and treatment. In the conclusion of the editorial the authors stated that RED-S should be better known as cause for injury in sport and that knowledge and awareness is important to maintaining overall athlete

health (Mountjoy, Costa, et al., 2018). In combination with this, coaches, athletic trainers, and athletes should have a higher awareness of the negative effects RED-S has on health and performance and the long-term health consequences.

This study sought to answer the following research questions. Do female and male long-distance runners who screen positive for RED-S have bone mineral density below recommended levels? Are female and male runners who screen positive for RED-S more susceptible to bone injuries? Do female and male runners who screen positive for RED-S have low energy availability? Are female and male runners who have low energy availability become more susceptible to bone injuries? Are female and male distance runners who are at risk for eating disorders also at risk for RED-S and low energy availability? Are female and male distance runners who are at risk for eating disorders more susceptible to bone injury? Information gathered from this study may benefit coaches, athletes, and health care professionals in understanding the impact nutrition has on performance physically and psychologically. Potentially preventing bone injuries and eating disorders from occurring

Methods

A cross-sectional study was implemented to recruit collegiate female and male competitive long-distance runners from North Dakota State University. Participants were required to be eighteen to twenty-five years old, not injured, and currently participating as part of a collegiate competitive team. Additionally, they must have been training a minimum of five hours per week, during recruitment and during testing week of the study. Athletes meeting study requirements were invited to an informational meeting to learn about the study; and (1) the benefits of logging both food and exercise and (2) the benefits of bone health for performance and long-term health. Each male and female athlete was invited to participate in an electronic

survey (Appendix H). The electronic survey combined a dual energy x-ray absorptiometry (DXA) screener survey, to screen for DXA safety, the EAT-26 survey to screen for eating disorders (D. M. Garner et al., 1982), the LEAF-Q, to screen for RED-s (Melin et al., 2014), a three-day food diary and exercise log.

Study procedures were clearly presented on the informed consent per approval by the university Institutional Review Board. Each athlete was given the following: (1) the informed consent form (2) a health history screening form (HHQ) (3) physical activity readiness questionnaire (PAR-Q) (Warburton, Gledhill, Jamnik, & McKenzie, 2019) (4) and a SASE (self-addressed stamped envelope). If interested in participating in the study, athletes were asked to return the completed forms, food diary and exercise log within one week. After receiving completed forms, the research assistant contacted participants to schedule their DXA scan and to complete the electronic survey.

A URL was used to distribute the electronic survey administered with Qualtrics (Provo, UT). The URL provided a link to instructions to complete the survey. The survey began with demographic information including age, sex, and education level. Participants were asked to provide current height (inches and feet) and weight (pounds) in addition to the highest weight and lowest weight experienced at their present height. History of tobacco use, medication use, and the number of hours spent training during the week was also collected. Next, the athlete's history of missed practices due to injury, illness, evaluation of adverse gastrointestinal symptoms, and use of contraceptives was documented. Lastly, the survey included questions regarding current and past menstrual function. For male participants, questions regarding contraceptives and menstrual function were bypassed and the survey moved to the second part. The second part of the survey included questions from the Eating Attitudes Test (EAT-26).

These questions evaluated the athlete's eating behaviors, thoughts, and feelings towards food. The EAT-26 cannot diagnose eating disorders but it is a reliable and valid test that can be used to identify individuals needing further evaluation for eating disorders (Garner et al., 1982). The EAT-26 consisted of 26 questions.

For the three-day food diary, participants were asked to keep a detailed record of all food and beverage intake for two week days and one weekend day (or two "normal days" and one "abnormal eating" day). To estimate total energy intake, participants were guided by a registered dietitian nutritionist to complete the three days, regarding what they ate and how much they ate. The three-day food diary was provided by the research team as a stapled packet. Participants were provided detailed written instructions with examples, and an in-person instructional session explaining the proper way to record food intake. Exercise energy expenditure was self-reported using a three-day exercise log. This log included type of exercise, duration, and intensity. Participants were asked to log exercise the same days as the food log. Participants used rate of perceived exertion to determine the intensity of the exercise training. An instructional sheet was provided in combination with the exercise log to assist participants in identifying the intensity of the exercise ("Rated Perceived Exertion (RPE) Scale," n.d.)(Appendix J). Rate of perceived exertion (RPE) can be classified on a scale of six to twenty, 06 (no exertion at all), 7 (extremely light), 9 (very light), 11 (light), 13 (somewhat hard), 15 (hard, heavy), 19 (extremely hard), 20 (maximum exertion)("Rated Perceived Exertion (RPE) Scale," n.d.). Participants were asked to provide a rate of perceived exertion for each exercise during a training session as well as the duration.

The DXA scan was performed on the athletes after completion of the three-day food diary, exercise log, and electronic survey. The DXA scan was used to determine bone mineral

density (BMD) of the athletes so that the study could include relationships between energy and other nutrient intake, exercise levels, and estimated level of BMD. Additionally, the DXA was used to assess body composition such as fat mass and lean mass. Participants were asked to come for DXA scans in the morning during a rested state with no exercise prior. Participants needed to be in a euhydrated state to improve accuracy of the scan (Gibson et al., 2019, p.244). Female participants were also asked to undergo a pregnancy test before the DXA scan because it is not recommended that pregnant individuals be exposed to any radiation. Additionally, a manual heart rate measurement was collected as a low heart rate indicates risk of LEA (O'Donnell et al., 2015). After the results were gathered, they were analyzed and forwarded to a radiologist to screen for medical concerns.

Participants received results from the DXA scan and the nutritional analysis, including the EAT-26 score, at the end of the study (see Figure 1). Participants were then be able to use the information provided for their own benefit, whether they choose to seek further guidance or not.

The "participant report card" (Figure 1) displayed individual results from the study including calorie intake, exercise expenditure, protein intake, calcium intake, vitamin D intake, EA, total BMD, z-score, EAT-26 score and LEAF-Q score if they were not at risk. The report card provided a recommended calorie range for the individual calculated using the Total Daily Energy Expenditure (TDEE) equation (Rosenbloom, 2018). Recommended protein intake was also provided. This was calculated by multiplying the participants' weight in kilograms by 1.2-1.7 (Karpinski & Rosenbloom, 2017). A small description of each category and the significance of the test and results was provided as well. These report cards were emailed to the participants after completion of the study.

If a participant scored high for eating disorders on the EAT-26, had problems with bone health, or were energy deficient, they were advised to see their physician for a complete health screening and follow-up. No information was provided to coaches regarding individual results.

Results

A total of 13 North Dakota State University collegiate male (n=3) and female (n=10) long-distance runners participated in this study. All athletes on the men's and women's cross-country team were invited to the information session to participate in the study. There are 16 athletes on the men's cross-country team and 15 on the women's cross-country team. A total of 30 athletes from both teams came to the information session provided. The percent rate of participation was 42% out of the potential population. Participants completed every requirement of the study and there were no drop outs. Each participant received an electronic PDF of a report explaining individual results (Figure 1).

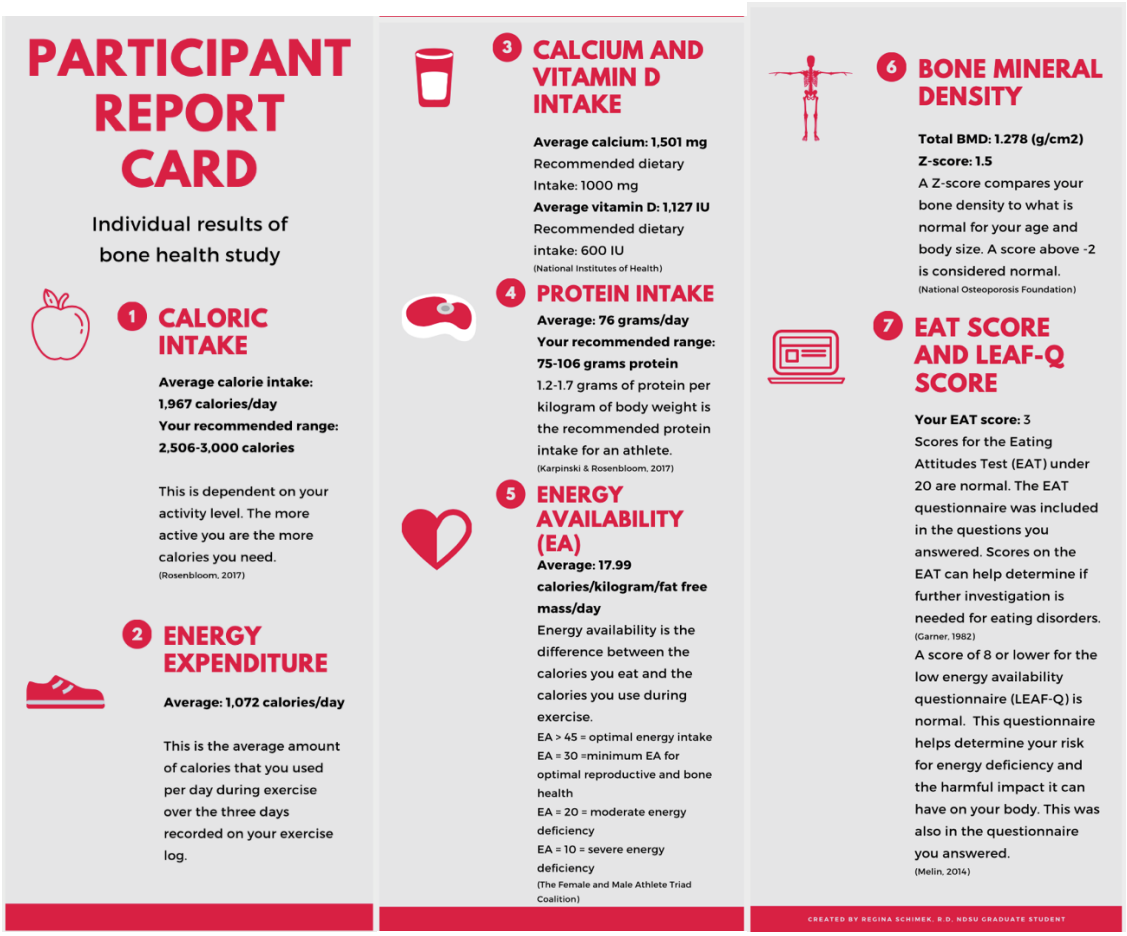

Figure 3. Example of individual participant report card for bone health study among long-distance runners.

Participant ages ranged from 18 to 22 with an average age of 19.8 years old. Data regarding participant demographic information is presented in Table 1. Table 2 displays the anthropometric and body composition measurements for all participants.

Table 1

Demographic characteristics of participants in study of long-distance runners and bone health

	%	n
Sex		
Female	76.9	10
Male	23.1	3
Age female participants		
Mean = 20.2 yrs		10
18	10%	1
19	20%	2
20	30%	3
21	20%	2
22	10%	1
Age male participants		
Mean = 18.6 yrs		3
18	33%	1
19	66%	2
Ethnicity		
White	100%	13

To be at risk for RED-S a female participant had to score an eight or higher on the LEAF-Q. Male participants who scored a 3.2 or higher were considered at risk for RED-S. This was determined by averaging the female's scores of questions on the LEAF-Q that did not pertain only to females, such as questions about gastrointestinal symptoms and injuries, that males could answer as well. Questions used to determine males at risk for RED-S are considered a low energy availability in males' questionnaire (LEAM-Q) for the purposes of this study. Results from LEAF-Q, LEAM-Q and RED-S screens along with comparison of differences are displayed in Table 3 and 4. Overall, seven out of thirteen participants (53%) were at risk for RED-S based on the scoring system for the LEAF-Q and the LEAM-Q.

Table 2

Anthropometric and body composition measurements among thirteen long-distance runners in bone health study

	All (n=13)	Women (n=10)	Men (n=3)
Height (in.)			
Mean	67.44	66.86	70.60
Range	60.75-74.25	60.75-69.5	67.8-74.25
Weight (lbs.)			
Mean	133.0	128.12	149.29
Range	109.86-166.30	109.86-156.60	139.10-166.30
BMI			
Mean	20.59	20.46	21.03
Range	18.23-22.79	18.23-22.79	20.10-21.79
Underweight classification	<18.5	<18.5	<18.5
Total%fat*			
Mean	20.25	21.43	16.33
Range	12.40-24.80	16.70-24.80	12.40-21.50
Essential fat minimum		10-13%	2-5%
Region%fat†			
Mean	19.40	20.54	15.63
Range	11.90-23.90	16.00-23.90	11.90-20.60
Lean mass (lbs.)			
Mean	100.32	96.51	113.01
Range	80.90-139.29	80.90-113.16	83.50-139.29

Note: **Total percentage of fat (total%fat).* Recommended essential fat minimum (Karpinski & Rosenbloom, 2017, pg. 178).†*Regional percentage of fat (region%fat) is a measure of regional body composition*. BMI cut off for underweight classification ("Nutrition - Body mass index - BMI," n.d.).

Table 3

Results from the low energy availability in female's questionnaire (LEAF-Q) and participants determined at risk for RED-S

	All (n=13)	Women (n=10)	Men (n=3)	P value
BMI				
Mean	20.59	20.46	21.03	
Range	18.23-22.79	18.23-22.79	20.10-21.79	
Underweight classification	<18.5	<18.5	<18.5	p<0.208
At risk for RED-s	7.0	5.0	2.0	p<0.007
Not at risk for RED-S	6.0	5.0	1.0	

Note: Cut points for BMI ("Nutrition - Body mass index - BMI," n.d.).

Table 4

Eating attitudes test (EAT), low energy availability in female's questionnaire (LEAF-Q), low energy availability in male's questionnaire (LEAM-Q), and illness scores among 13 long-distance runners in a bone health study

	All (n=13)	Women (n=10)	Men (n=3)
EAT score			
Mean	6.53	7.4	3.6
Range	0-45.0	0-45.0	3.0-4.0
Scores >20 need further assessment by a qualified professional			
LEAF-Q score			
Mean	xxx	9.5	xxx
Range		3.0-17.0	
Scores ≥8 classify a participant at risk of RED-S			
LEAM-Q score			
Mean	xxx	xxx	4.67
Range			3.0-7.0
Scores ≥3.2 classify a participant at risk of RED-S			
Illness score			
Mean	0.76	0.80	0.67
Range	0-2.0	0-2.0	0-2.0

An independent samples *t*-test was used to compare the BMI of those at risk for RED-S and those not at risk of RED-S ($p<0.208$). A Chi square test for independence was used to determine if female and male runners who screened positive for RED-S have low energy availability ($p<0.457$). Table 5 displays the mean and range of EA for all participants. An independent samples *t*-test comparing EA levels of those at risk for RED-S and those not at risk for RED-S was non-significant ($p<0.429$). Pearson's correlation was used to determine if female and male distance runners who are at risk for disordered eating are also at risk for RED-S and low energy availability**.** This correlation using the participants EAT score ($p<0.508$, $p<0.375$),

EA levels ($p<0.508$, $p<0.28$), and whether they were at risk for RED-S or not ($p<0.375$, $p<0.28$) was non-significant for all comparisons. Average EAT score of all participants was 6.538. Scores greater than 20 indicate a need for further investigation by a qualified professional (Garner et al., 1982). However, this average was skewed due to a high score of 45 for one participant.

Table 5

Average energy intake (EI), exercise energy expenditure (EEE), energy availability (EA), and resting metabolic rate (RMR) among thirteen long-distance runners in bone health study

	All (n=13)	**Women (n=10)**	**Men (n=3)**
EI (kcal)			
Mean	2,487	2,359	2,912
Range	1606-3,640	1606-3,640	2,217-3,574
EEE (kcal)			
Mean	669	643	759
Range	301-1,072	301-1,072	624-1,001
EA (kcal·kg FFM·day)			
Mean	32.36	31.81	34.21
Range	17.99-47.82	17.99-47.82	22.23-42.83
RMR (kcal/day)			
Mean	1,359	1,323	1,479
Range	1,176-1,726	1,176-1,479	1,204-1,769
Resting heart rate (bpm)*			
Mean	51.53	53.0	46.66
Range	44-62	44-62	44-48
Norm	60-100	60-100	60-100

Note: *The generally accepted range for resting heart rate is 60 to 100 beats per minute. The "red flag" range for cardiac abnormality is the lower threshold of 50 beats per minute (Karpinski & Rosenbloom, 2017, p. 398).

Linear regression was used to determine if female and male distance runners who are at risk for disordered eating become more susceptible to bone injury ($p<0.269$). This test used the participants EAT score and if they had experienced injury in those at risk for RED-S and those not at risk for RED-S. An independent samples *t*-test of all participants EAT scores in

comparison to whether they were at risk for RED-S or not was non-significant ($p<0.079$). Though the p-value appears marginally significant this is skewed due to an outlier score of 45.

To determine if female and male long-distance runners who screen positive for RED-S have bone mineral density below recommended levels an independent sample *t*-test using the participants LEAF-Q score and whether or not they were determined to be at risk for RED-S or not at risk for RED-S. Using the LEAF-Q scores, those at risk for RED-S had a mean score of 11.43 and those not at risk for RED-S had a mean score of 4.83 ($p<0.007$).

Table 6 includes the mean and ranges of total BMD, Z-scores, pelvis, and spine measurements taken by the DXA scan for all participants, women and men. Z-scores of those at risk of RED-S (mean = 0.5714) compared to those not at risk of RED-S (mean = 1.2167) had a marginally significant p-value of 0.063. The World Health Organization states that a Z-score of -2.0 indicates risk for low BMD. Women and men's BMD of those at risk for RED-S (mean 1.2001) and those not at risk for RED-S (mean 1.2587) with a range of 1.375 to 1.856 ($g \cdot cm^{-2}$) was non-significant ($p<0.178$).

Table 6

Bone mineral density findings for all participants and separated into women and men. This table includes the averages and the ranges of the results provided by the DXA scan on bone health.

	All (n=13)	Women (n=10)	Men (n=3)
Total BMD (g/cm^2)			
Mean	1.22	1.21	1.28
Range	1.08-1.37	1.08-1.30	1.20-1.37
Spine BMD (g/cm^2)			
Mean	1.02	1.01	1.06
Range	0.91-1.16	0.91-1.11	1.00-1.16
Pelvis BMD (g/cm^2)			
Mean	1.16	1.13	1.25
Range	0.98-1.38	0.98-1.31	1.16-1.38
Z-Score			
Mean	0.86	0.93	0.66
Range	-0.2 -2.0	-0.2-2.0	-0.1- 1.4
(-2) indicates risk for low BMD			

Note: Cut points for Z-scores ("Bone Mass Measurement" n.d.).

Using the data gathered we sought to determine if female and male runners who screen positive for RED-S were more susceptible to bone injuries. A comparison of the number of injuries for those at risk of RED-S and not at risk of RED-S had mean numbers of injuries being 2.77 and 0.33 number of injuries ($p<0.022$). Seven out of thirteen participants reported experiencing some type of injury in the LEAF-Q. Researchers added additional questions into the LEAF-Q regarding illness using a similar context to solicit more information regarding experienced illness. The illness score was determined by allocating the same point totals to the illness response as injury questions. Chi-square test for independence was used to compare the illness score of those at risk for RED-S and those not at risk for RED-S was non-significant ($p<0.135$). However, those at risk for RED-S had a higher number of participants who reported illness in the questionnaire. Injuries reported included plantar fasciitis, stress reactions, tendinitis, overuse of hamstring, quad pull, strain in SI joint, and stress fracture in femur.

The survey included several open-ended questions. These questions included what type of activities they participated in, types of injuries if any, and if they avoid any particular types of food. Only one participant reported any food avoidances: dairy products such as milk and cheese. This participant did not report any history of injury. An independent samples *t*-test was used to compare calcium consumption of those at risk for RED-S (mean 1,615 mg) to those not at risk for RED-S (mean 1,721 mg) ($p<0.533$).

Linear regression was used to determine if female and male runners who have low energy availability become more susceptible to bone injuries. When comparing those at risk of RED-S to those not at risk of RED-S with their energy availability levels as a predictor and whether or not they reported experiencing injuries as a dependent variable, marginal significance was indicated ($p<0.080$). Average EA reported for all participants was 32.36. Energy availability for all participants was determined using the EA calculator ("Energy Availability Calculator - The Female and Male Athlete Triad Coalition," n.d.). Average EI, EEE, EA, and RMR for all participants, and separately for women and men are provided in Table 5. An independent samples *t*-test was used to compare resting heart rate of those at risk of RED-S (mean 52 bpm) and those not at risk of RED-S (mean 51 bpm) ($p<0.654$). An independent samples *t*-test was used to compare RMR of those at risk for RED-S (mean 1,322) and those not at risk for RED-S (mean 1,401.66) was found non-significant ($p<0.419$).

Discussion

Researchers aimed to investigate RED-S in female and male collegiate athletes and determine if there is an association with bone health. For this purpose, researchers sought to answer questions regarding RED-S impact on athlete's health. Screening tools used were the

LEAF-Q, LEAM-Q, and EAT-26 along with a three-day food diary and exercise log for EI and EEE. This data was used to determine athlete's EA and if those with LEA had lower BMD.

Low energy availability can be indicated by a BMI of less than 17.5 $kg \cdot m^{-2}$ (De Souza, Williams, et al., 2014). Using percentiles like BMI for age to calculate BMI are recommended for an individual until the age of 20 and further assessment is needed to determine if an individual has LEA ("About Child & Teen BMI," n.d.). The mean age for all participants in the study was 19.8 years old. The participants for this study had a mean BMI of 20.59 $kg \cdot m^{-2}$. There was no significance between participant BMI of those who were at risk for RED-S and those not at risk for RED-S. Therefore, BMI for this group did not identify risk for RED-S unlike the results of Tornberg et al. (2017). The Tornberg et al study was structured to determine if there was a link between athletes with secondary functional hypothalamic amenorrhea and neuromuscular performance. Athletes with secondary functional hypothalamic amenorrhea had lower BMI's ($P<0.05$) compared to athletes with eumenorrhea (Tornberg et al., 2017). Amenorrhea is one of the indicators for RED-S (Sygo et al., 2018).

The LEAF-Q is a validated 25-item questionnaire assessing physiological symptoms related to energy deficiency such as menstrual dysfunction, history of injury, and gastrointestinal function (Melin et al., 2014). A score of 8 or higher on the LEAF-Q can help determine if a female participant is at risk for RED-S. Questions used to determine males at risk for RED-S are considered a low energy availability in males' questionnaire (LEAM-Q) for the purposes of this study. A score of 3.2 or higher on the LEAM-Q was used to determine male participants at risk for RED-S. This score was obtained by averaging the female's scores of questions on the LEAF-Q that would be relevant for both males and females, such as questions about gastrointestinal symptoms and injuries. The methods for determining male scores on the LEAM-Q was based on

methods used in a study investigating LEA in athletes (Slater, 2015). For the current study, seven out of thirteen participants were at risk for RED-S by scoring an ≥8 or ≥3.2 on the LEAF-Q and LEAM-Q, respectively. Overall, the average EA was 32.36 kcal·kg·FFM·day^{-1}. Optimal EA is considered ≥ 45 kcal·kg·FFM·day^{-1}; when EA drops below 30 kcal·kg·FFM·day^{-1} or less, negative physiological changes begin to occur (Loucks et al., 2011). Based on the 3-day food diary, six of the thirteen participants had EA below 30 kcal·kg·FFM·day^{-1}. Six participants had estimated EA between 30 and 45 kcal·kg·FFM·day^{-1}. A similar study found that females with amenorrhea and males with lower testosterone had a significant difference in scores on the LEAF-Q and the Triad tool than females with eumenorrhea and males with low compared to moderate EA (Heikura et al., 2018; Joy et al., 2014). Though there was no significance between participants who are at risk for RED-S and LEA ($p<0.429$), only one participant had what is considered optimal EA of 45 kcal·kg·FFM·day^{-1}. Evidence suggests that some individuals may be more sensitive to LEA than others and may be more likely to experience negative physiological symptoms (Cialdella-Kam et al., 2014). Injuries reported by participants with an EA below 45 kcal·kg·FFM·day^{-1} included stress reactions in the shins, tendinitis, plantar fasciitis, Achilles tendinitis, IT band injury, mild overuse injury of the hamstring, strain in an SI joint, and stress fracture in the femur.

When comparing the EA levels of those at risk for RED-S and those not at risk for RED-S the test was non-significant ($P<0.429$). As mentioned, optimal EA is considered to be 45 kcal·kg·FFM·day^{-1} and negative impacts from LEA is seen in those that report 30 kcal·kg·FFM·day^{-1} or lower depending on the individual, as some are more sensitive to different levels of LEA. The Female and Male Athlete Triad Coalition provides a calculator to determine EA based on body weight, body fat percentage, EEE, exercise duration, and caloric intake

("Energy Availability Calculator - The Female and Male Athlete Triad Coalition," n.d.). The Female and Male Athlete Triad coalition categorizes EA into four different levels: EA>45 kcal·kg·FFM·day^{-1} is energy replete, EA=30 kcal·kg·FFM·day^{-1} is the minimum EA for optimal reproductive and bone health, EA=20 kcal·kg·FFM·day^{-1} is moderate energy deficiency, and EA=10 kcal·kg·FFM·day^{-1} is severe energy deficiency ("Energy Availability Calculator - The Female and Male Athlete Triad Coalition," n.d.). According to this categorization only one participant met the criteria for optimal energy intake (47.82 kcal·kg·FFM·day^{-1}). Six participants had EA between 30-40 kcal·kg·FFM·day^{-1}, four participants had EA between 20-30 kcal·kg·FFM·day^{-1}, and two participants had EA between 10-20 kcal·kg·FFM·day^{-1}. The mean EA level for all participants was 32.36 kcal·kg·FFM·day^{-1}, close to the minimum EA for optimal reproductive and bone health.

The correlation between participant EAT-26 scores ($p<0.508$, $p<0.375$), EA levels ($p<0.508$, $p<0.28$), and whether or not they scored to be at risk for RED-S was non-significant ($p<0.375$, $p<0.28$). All but one participant scored under 20 for the EAT-26. A score of 20 or higher indicates a potential presence of an eating disorder or disordered eating and warrants further investigation by a professional. Only one participant scored high on the EAT-26 (45). This same participant also had LEA and was at risk for RED-S based on the LEAF-Q score (23.61 EA kcal·kg·FFM·day^{-1}, 15 LEAF-Q score). A study investigated whether the presence of dieting behaviors, disordered eating, and level of body dissatisfaction for female athletes was impacted by the level of sport such as elite, recreational, or noncompetitive. The EAT-26 was used to identify if the participants were at risk for an eating disorder or disordered eating that warranted further investigation by a professional (Kong & Harris, 2015). As a result, more disordered eating patterns were noticed in leanness-important sport and elite athletes.

Participants in the current study are in what is considered a leanness focused or weight sensitive sport and are at a collegiate level (i.e. cross-country competitive running). However, the study by Kong & Harris had 320 participants, while the current study included only 13 participants. All athletes on the men's and women's cross-country team were invited to the information session to participate in the study. There are 16 athletes on the men's cross-country team and 15 on the women's cross-country team at North Dakota State University. A total of 30 athletes from both teams came to the information session provided. The percent rate of participation was 42% out of the potential population. This study was not able to find any correlations between disordered eating, LEA, and RED-S. A larger sample size might have revealed different relationships between risk factors for disordered eating, eating disorders, and how those factors might impact bone health and EA.

There was no significance between female and male distance runners who were at risk for disordered eating by taking the EAT-26 test and scoring 20 or higher and susceptibility to bone injury. Athletes are potentially at higher risk for eating disorders than non-athletes due to the pressure and focus put on the body for performance, or particularly for sports that have more focus on aesthetic characteristics (Martinsen & Sundgot-Borgen, 2013). These eating disorders and disordered eating may lead to LEA due to a lack of energy intake and in turn can impact bone health. In an acute interventional study, Papageorgiou et al. (2017) found that five days of LEA, at 15 kcal·kg·LBM·day^{-1}, decreased bone formation and increased bone resorption among women (Papageorgiou et al., 2017).

When comparing participants EAT-26 scores and whether or not they were at risk for RED-S by scoring ≥8 or ≥3.2 on the LEAF-Q and LEAM-Q, respectively, the test was non-significant. Psychological impact is one of the components listed on Figure 1 showing the ten

health consequences of RED-S. Additionally, there is an association between negative psychological factors and LEA (Melin et al., 2014). The LEAF-Q is meant to compliment disordered eating/eating disorder screening tools. These screening tools are meant to determine if athletes are at risk for LEA which is often linked to eating disorders and amenorrhea (Melin et al., 2014).

A test comparing the scores of the LEAF-Q and LEAM-Q of those at risk for RED-S and those not at risk for RED-S was significant. Those with RED-S had a higher mean score (11.43) than those not at risk for RED-S (4.83). This test tells us that those at risk for RED-S score higher than those who are not at risk for RED-S. The LEAF-Q is a brief 25 item questionnaire assessing physiological symptoms related to energy deficiency such as menstrual dysfunction, history of injury, and gastrointestinal function. Questions involve categories such as occupation, type of sport, age, height, weight, training, dizziness, gastrointestinal function, menstrual function, physical activity, contraceptive use and illness and injury in the past year. Answers are self-reported on dichotomous, nominal and Likert-type ordinal scales (Melin et al., 2014). Seven out of the thirteen participants in this study scored high enough to be considered at risk for RED-S.

There was a marginal siginificance between Z-scores of participants at risk for RED-S and participants not at risk for RED-S. Those at risk for RED-S had a lower mean Z-score than those not at risk for RED-S. A bone mineral density (BMD) score of <-1.0 calls for further assessment and *Z*-scores below -2.0 are considered low BMD. The American College of Sports Medicine (ACSM) defines low BMD as 'history of hypoestrogenism, nutritional deficiencies, stress fractures, along with other secondary risk factures for fracture and a BMD Z-score of -1.0 to – 2.0' (Khan et al., 2004; Nattiv et al., 2007; Writing Group for the ISCD Position

Development Conference, 2004). No participants had Z-scores below -1.0 and all DXA results were evaluated by a medical professional (i.e. radiologist). The study physician recommended no further assessment of bone health for any of the participants. However, this could have been due to their young ages. Peak bone mass is reached between the ages of 25 to 30. For women, 95% of bone mass is formed by the age of 20 ("Bone Mass Measurement" n.d.). It's important to correct any problem with bone health at the age of 19 as it is a vital time in peak bone mass formation. Impaired bone health is one of the ten health consequences impacted by RED-S as presented by the RED-S health consequences in figure 1 (Mountjoy, Ackerman, et al., 2018). The Triad, a condition influencing three components of female athlete health including energy availability, menstrual function and bone health, is a condition that is closely related to RED-S (Nattiv et al., 2007). Relative energy deficiency in sport was developed as an "expansion" on the Triad to provide a broader term for the symptoms experienced by LEA or relative energy deficiency, for women and men (Mountjoy et al., 2014). The International Society for Clinical Densitometry (ISCD) recommends *Z*-scores be used to determine BMD in the adolescent and premenopausal populations (Leib, 2005; Leslie et al., 2006; Writing Group for the ISCD Position Development Conference, 2004). The mean age for participants in the current study is 19.8 years old. However, when comparing total BMD for those at risk for RED-S and those not at risk for RED-S there was no significance and the mean scores were very similar. The test comparing the number of injuries between those at risk for RED-S and those not at risk for RED-S was significant ($p<0.022$) showing that participants risk for RED-S had a higher number of reported injuries. Six out of the seven participants who were at risk for RED-S reported experiencing at least one injury. Only two of the six participants who were not at risk for RED-S reported experiencing at least one injury. An increased risk for injury is one of the components on Figure

2 showing the possible negative performance effects of RED-S (Mountjoy et al., 2014). A study by Heikura et al. (2018) concluded that low reproductive hormones on injury revealed that amenorrheic and low testosterone athletes had 4.5 times higher rate of absences in training due to bone injury. Overall, these results showed that higher risk scores on RED-S and Triad were associated with lower testosterone concentrations and higher occurrence of all-time fractures for both males and females (Heikura et al., 2018).

Immunological impairment is one of the ten health consequences displayed in Figure 1. Athletes who endure intense training are susceptible to a weakened immune system and can experience conditions such as an upper respiratory tract infection (Shimizu et al., 2012). Two studies conducted on Olympic athletes concluded that being female, and having LEA was one of the most significant factors for risk of illness (Drew et al., 2017; Drew et al., 2018). In the current study it was concluded from the results that the illness scores of those at risk for RED-S compared to those not at risk for RED-S was non-significant ($p<0.135$). Researchers added questions to the LEAF-Q and LEAM-Q about illness that were written and scored in a similar fashion at injury questions and scores. These questions were added to search for a link between illness and RED-S. Non-significant results of this study were similar to those of Ackerman et al. (2018) that had participants take a series of surveys to determine if they had LEA. Results of these studies were used to determine the likeliness of participants with LEA compared to those without LEA to the ten IOC health consequences of RED-S. Participants with LEA had a higher prevalence of all the health consequences except growth and development and immunological function (Ackerman et al., 2018).

The comparison of calcium consumption of those at risk for RED-S and those not at risk for RED-S was non-significant ($p<0.533$). The mean calcium intake for participants in the study

was 1,625 mg with a range of 1,001 mg to 3,054 mg. Dietary restriction from special diets such as vegetarian, vegan diets, or LEA can compromise intake of some nutrients like calcium (Cialdella-Kam et al., 2016; Lis et al., 2019). Additionally, RED-S can cause a decrease in endogenous estrogen and can lead to low BMD through the disruption of calcium deposition into the bone (Elliot-Sale et al., 2018). Calcium along with vitamin D are important minerals that support bone building and maintenance (Nattiv et al., 2007).

There was a marginal significance found when comparing EA levels and whether or not they had reported experiencing an injury between those at risk for RED-S and those not at risk for RED-S ($p<0.080$). Therefore, EA of those at risk for RED-S was marginally significant when predicting if a participant had experienced an injury. As mentioned previously, RED-S is an expansion of the female athlete triad. The Triad is condition that affects females when one or more of the three components occur: LEA with or without disordered eating, menstrual dysfunction, and low BMD (Nattiv et al., 2007). Injuries or bone injuries, and LEA are components that are often paired together. Also, as highlighted earlier Figure 2 shows that increased injury risk is part of the possible negative performance effects of RED-S.

When comparing resting heart rate of those at risk of RED-S and those not at risk of RED-S there was no significance ($p<0.654$). Cardiovascular impact is one of the ten health consequences of RED-S shown in Figure 1. The mean resting heart rate for all participants in this study was 51.5 beat per minute. The generally accepted healthy range for resting heart rate is 60 to 100 beats per minute and the “red flag” range for cardiac abnormality is the lower threshold of 50 beats per minute (Karpinski & Rosenbloom, 2017, p. 398). It is found that female athletes who become amenorrheic commonly due to LEA, develop endothelial dysfunction (Rickenlund et al., 2005). Menstrual dysfunction that is correlated with hypoestrogenemia can have a negative

impact on cardiovascular function. In severe cases of energy deficiency, such as anorexia nervosa, cardiovascular health is increasingly impacted (Spaulding-Barclay et al., 2016). Negative effects are a decrease in left ventricular mass, pericardial effusion, bradycardia, and valve abnormalities like mitral valve prolapse (Spaulding-Barclay et al., 2016). In a study investigating hypoestrogenemia on the blood pressure of premenopausal women it was found that women with amenorrhea had lower blood pressure and heart rate at rest and during orthostatic stress (O'Donnell et al., 2015). The average heart rate in this study was lower, however it was not correlated with LEA. The lower heart rate could be due to the level of physical fitness of the participants.

Resting metabolic rate was compared between those at risk for RED-S and those not at risk for RED-S and was found to be non-significant ($p<0.419$). Change in metabolic rate is one of the ten health consequences of RED-S shown in Figure 1. In a study observing EA and reproductive function on energy metabolism in female endurance athletes 63% of female subjects in this study presented with low or reduced EA. Researchers found a negative impact of LEA on RMR, menstrual function, and bone health. Resting metabolic rate was 7% higher in athletes with optimal EA. Low RMR was found in 53% of participants and Athletes with low or reduced EA and/or menstrual dysfunction had lower RMR than athletes with optimal EA (De Souza, Lee, et al., 2007).

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SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate RED-S in female and male collegiate athletes and determine if there is an association with bone health. Participants completed electronic surveys consisting of the LEAF-Q and EAT-26 to determine their risk for RED-S, eating disorders, and disordered eating. They also underwent a DXA scan to examine bone health and determine bone mineral density. In addition, participants completed a three-day food diary and exercise log for EI and EEE to be analyzed and determine individual EA. Data from these tests was used to find relationships between RED-S, LEA, eating disorders, disordered eating, and bone health.

A sample size of 13 participants was used to compile results for the research questions we sought to answer. Overall, this study determined that both male and female athletes who are at risk for RED-S have a greater chance of experiencing a higher number of injuries than athletes not at risk for RED-S. In comparison to bone health, though Z-scores of participants were not at risk for low bone mineral density, those who were at risk for RED-S had lower Z-scores than those not at risk for RED-S. A small sample size may explain why many of the tests were non-significant. However, it is important to note that over half of the participants were at risk for RED-S. With a larger sample size additional results may be found for the relationship between RED-S and bone health in male and female athletes. Further research is recommended on both male and female athletes to determine the true extend of RED-S impact on overall athlete health and performance in variety of sports.

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APPENDIX A. INSTITUTIONAL REVIEW BOARD APPROVAL/INFORMED CONSENT

NDSU NORTH DAKOTA STATE UNIVERSITY

September 23, 2019

Dr. Sherri Stastny
Department of Health, Nutrition & Exercise Sciences

IRB Approval of Protocol #HE20044, "Diet and Bone Health Among Long Distance Runners"
Co-investigator(s) and research team: Regina Schimek and Dr. Steven Mitchell

Approval expires: September 12, 2020 Continuing Review Report Due: 8/1/2020

Research site(s): NDSU Funding agency: n/a
Review Type: Full Board, meeting date – 9/13/2019
Risk Level: No more than minimal risk
IRB approval is based on original submission, with revised: protocol and consent form (received 9/20/2019).

Additional approval is required:
o prior to implementation of any proposed changes to the protocol (Protocol Amendment Request Form).
o for continuation of the project beyond the approval period (Continuing Review/Completion Report Form). A reminder is typically sent two months prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:
o any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (Report of Unanticipated Problem or Serious Adverse Event Form).
o any significant new findings that may affect risks to participants.
o closure of the project (Continuing Review/Completion Report Form).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,

Kristy Shirley

Kristy Shirley, CIP
Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult https://www.ndsu.edu/research/for_researchers/research_integrity_and_compliance/institutional_review_board_irb/. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

INSTITUTIONAL REVIEW BOARD
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Diet and Bone Health Among Long Distance Runners

This study is being conducted by: Sherri Stastny, PhD, RD, CSSD, LRD, Professor in the department of Health, Nutrition, and Exercise Sciences at North Dakota State University; and Regina Schimek, M.S., RD and graduate student in the same department at North Dakota State University.

Key Information about this study:

This consent form is designed to inform you about the study you are being asked to participate in. Here you will find a brief summary about the study; however, you can find more detailed information later on in the form.

You are invited to participate in this research study to investigate relative calorie intake and bone health among long distance male and female runners who compete at a collegiate level. You will be asked to complete a set of electronic questionnaires, food diaries, exercise logs, and to take a bone mineral density (BMD) test. The time commitment involves 2 study related meetings:

A. Listen to study overview and informational/educational meeting in your normal team meeting room/building (about 1 hour).
B. You will also be asked to fill out health questionnaires, electronic survey, food & exercise diaries and logs in-between and on your own time (about 1-2 hours total time).
C. Complete the BMD test in our lab on campus at NDSU (BBFH 1, Room 16) (about ½ hour).

List of procedures:

A. Informational sessions with information of how to fill out food diary and exercise log: You will be invited to an informational meeting to learn about the study. Your coach nor your coaching staff will be part of this meeting. During this meeting we will go over study details and instructions on how to fill out a 3-day food diary and exercise log. We will also distribute health forms and an informed consent form for your optional participation in the study.

B. **Paper/Pencil Food Intake Journal (or Diary), Health Screening Forms and Physical Activity Measurement/Log:** You will be asked to read an informed consent form to learn about the study. ***At any time, you can contact the research team for questions.***

If you decide to be in the study, you can fill out health screening forms in a private area away from others. If you do not wish to participate in the study, do not sign the informed consent. If you chose to participate, you will be instructed to return the completed forms in the provided stamped envelope (informed consent, food diary, exercise log, and health screening forms). On your own time, you will complete the health history questionnaires, food diary and exercise log and bring them along to a subsequent study meeting time. If you chose not to participate, you can dispose of the health forms, food diary and exercise log forms (no need to return the forms to us). For all participants, the research team will look at your forms for completeness and eligibility before reaching out to you to participate in the study. Forms will be viewed in a private area by a trained research assistant (we will be checking to be sure you are healthy and safe for the bone density scan).

You will be asked to write down all of the food and beverages you consume for 3 days of your choosing during the next week; and on another form, to estimate all the exercise you completed during the same 3 days. You will be asked to report your current medications, supplements, and any health concerns that you may have. This activity should take 1 hour or less.

C. Electronic Questionnaires & Bone Mineral Density (BMD) Test: After receiving your forms, a research assistant will contact you and ask you to completed 2 electronic links:

a. Electronic survey-include questions regarding your exercise performance, how you feel before, during and after exercise, and other nutrition/exercise/health related questions. The survey will take about 20 minutes
b. A link to schedule a time to complete a bone density scan in the Bentson Bunker Field House on NDSU campus.

The bone mineral density (BMD) test will be completed while you relax on a table. Strong bones are important for your health. A BMD test is the best way to measure your bone health. It compares your bone density, or mass, to that of a healthy person who is the same age and sex as you are. The most widely recognized BMD test is called a central dual-energy x-ray absorptiometry, or central DXA test. A BMD test can provide a snapshot of your bone health. The test can identify low bone mass or osteoporosis, determine your risk for fractures (broken bones), and measure your response to osteoporosis treatment.

After arriving for the bone mineral density test, females will be asked to provide a urine sample for a pregnancy screen before the DXA procedure as DXA is not recommended for pregnant individuals. The urine will be collected in our lab just before we measure your height and weight.

We ask that you dress in shorts and a T-shirt. For this procedure, two velcro straps will be placed on your lower limbs to help keep the lower body in correct position during the scan. Once positioning is complete the trained researchers will guide you through the procedure. You will be asked to remain still as the scanning arm moves from the top of your head to the feet and back to the head. This takes approximately 5-12 minutes depending on your height and weight. When the scan is complete, the velcro straps will be removed and you will be assisted off the DXA table. The total procedure together will take 30 minutes or less.

The DXA procedure is supervised by a licensed radiologic technician for bone densitometry (Dr. Stastny).

Compensation. There is no compensation for this study.

Who can participate? You are being asked to participate because you are an English-speaking female or male collegiate athlete *currently* participating in collegiate-sanctioned long-distance running and are between the ages of 18 and 25 years. In addition, you currently train at least 5 hours per week. We are asking for 40 volunteer participants for our study. Females who are pregnant (or who may think they are pregnant) cannot participate in this study.

Where is the study going to take place, and how long will it take? This study will take place in the Sanford Health and Athletic Complex at NDSU and in the Bentson Bunker Field House Room 16 on NDSU campus. Here you will attend an informational lecture regarding the study requirements and how to properly record food intake and exercise. The instruction will be led by a licensed, registered dietitian. This will take approximately 1 hour. Afterward, you will record all food intake and exercise expenditure for the three days in the upcoming 1 week window of time, plus fill out health forms and an electronic survey (together this will take about 1 hour). The DXA scan will take approximately 30 minutes or less in Bentson Bunker Field House, Room 16.

What are the risks and discomforts? The study team has minimized the known risks by studying a group of healthy participants. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known risks to you**.**

- Breach of privacy and/or confidentialiy of health information (low risk of occuring).
- Discomfort during assessments.
- Low dose radiation exposure. The full body DXA scan is not capable of producing high doses of radiation. For example, if you had 625 full body DXA scans in one year you would still only be exposed to ~25% of the limit for radiation exposures are in the United States from natural sources that are in the environment. However, it is still considered good practice to test for pregnancy for all females of child bearing age, before a scan is completed as it is not recommended that pregnant individuals receive a DXA scan.

Therefore, all subjects will be asked to provide a urine sample/pregnancy screen test upon arrival for testing. Any positive pregnancy test would deem any further testing halted.

- Loss of health information from questionnaires (low risk of issue).
    - All assessments will be completed with only the study team present in rooms with closed doors and windows. We will keep health information confidential and locked in an office. We will also shred all personal health information once the study is completed.

What are the benefits to other people? We will develop educational materials intended for the general public after learning more about relationships of nutrition, bone health, and long distance running at the conclusion of this study. These educational materials will include physical activity interactions with dietary intake. Additionally, participants will gain knowledge regarding their dietary intake and exercise expenditure. You will also receive results from the BMD test. DXA can provide information on both BMD and body composition (how much body fat and lean mass you have). Lastly, this data collected can be used to improve diet and exercise knowledge and to increase awareness for athletes to prevent injury and improve performance.

Do I have to participate in the study? Whether you participate in this research is your choice. If you decide to participate in the study, you may change your mind or stop participating at any time without penalty or loss of benefits to which you are already entitled.

What are the alternatives to being in this research study? Instead of being in this research study, you can choose not to participate.

Who will have access to my information? How will it be presented*?* We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under password protection. If you withdraw before the research is over, your information will be retained in the research record and we will not collect additional information about them.

All personal information will be kept in a secure filing system. Each participant will be coded with an assigned number so that information cannot be linked to a particular participant. No individual information will be shared. After all research volunteers are tested, average and range of results will be compiled for reporting purposes. No names will be used to report ANY information associated with this study. Your confidentiality and privacy is assured. However, your BMD may be shared with your personal physician—that is your choice.

a. Identifiers such as names will not be a part of any measurements; the information from this study will not be used for further research studies or distributed to another investigator for future research studies without additional informed consent from the subject or the legally authorized representative.

Can my participation in the study end early? If you fail to participate in all scheduled sessions, you may be removed from the study.

What if I have questions?

Before you decide whether you'd like to participate in this study, please ask any questions that come to mind now. Later, if you have questions about the study, you can contact Sherri Stastny at 701 231 7479 or sherri.stastny@ndsu.edu or Regina Schimek at 507 525 3309 or regina.schimek@ndsu.edu.

What are my rights as a research participant?

You have rights as a research participant. All research with human participants is reviewed by a committee called the *Institutional Review Board (IRB)* which works to protect your rights and welfare. If you have questions about your rights, an unresolved question, a concern or complaint about this research you may contact the IRB office at 701.231.8995, toll-free at 855-800-6717 or via email (ndsu.irb@ndsu.edu).

Documentation of Informed Consent:

You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

_____ _____

Your signature Date

_____ _____

Your printed name Date

You understand that the bone mineral density (DXA) examination is an X-Ray procedure that involves radiological isotopes _____ **(initials)**

_____ _____

Signature of researcher explaining study Date

_____

Printed name of researcher explaining study

APPENDIX B. HEALTH HISTORY QUESTIONNAIRE

Health History Questionnaire

Please answer the following questions to the best of your ability. For the following questions, unless otherwise indicated, circle the single best choice for each question. As is customary, all of your responses are completely confidential and may only be used in group summaries and/or reports. All information collected is subject to the Privacy Act of 1974. If you have any physical handicaps or limitations that would require special assistance with this questionnaire, please let your trainer know. This form is in accordance with the American College of Sports Medicine guidelines for risk stratification when followed correctly by your trainer. Your trainer should be certified with a national organization in order to use these forms correctly.

Name:_____ Ht.:_____ Wt.:_____

Gender:_____ Age:_____ Birthdate:_____

Address: _____

City:_____ State:_____ ZIP:_____ Phone:_____

Emergency Contact:_____ Phone: _____

Personal Physician:_____ Phone:_____

E-mail:_____

1. Have you ever had a definite or suspected heart attack or stroke? .....Yes No
2. Have you ever had coronary bypass surgery or any other type of heart surgery? .....Yes No
3. Do you have any other cardiovascular or pulmonary (lung) disease (***other than*** asthma, allergies, or mitral valve prolapse)? .....Yes No
4. Do you have a history of: diabetes, thyroid, kidney, liver disease. .....Yes No **(circle all that apply)**
5. Have you ever been told by a health professional that you have had an abnormal resting or exercise (treadmill) electrocardiogram (EKG)? .....Yes No
6. If you answered YES to any of Questions 1 through 5, please describe:

_____

_____

_____

_____

7. Do you currently have any of the following:

a. pain or discomfort in the chest or surrounding areas that occurs when you engage in physical activity? ..... Yes No

b. shortness of breath ..... Yes No

c. unexplained dizziness or fainting ..... Yes No

d. difficulty breathing at night except in upright position ..... Yes No

e. swelling of the ankles (recurrent and unrelated to injury) ..... Yes No

f. heart palpitations (irregularity or racing of the heart on more than one occasion) ..... Yes No

g. pain in the legs that causes you to stop walking (claudication) ..... Yes No

h. known heart murmur ..... Yes No

Have you discussed any of the above with your personal physician? ..... Yes No

8. Are you pregnant or is it likely that you could be pregnant at this time? ..... Yes No
If yes, what is your expected due date?_____

9. Have you had surgery or been diagnosed with any disease in the past 3 months? ..... Yes No
If yes, please list date_____ and surgery/disease_____

10. Have you had high blood cholesterol or abnormal lipids within the past 12 months or are you taking medication to control your lipids? ..... Yes No

11. Do you currently smoke cigarettes or have quit within the past 6 months? ..... Yes No

12. Have your father or brother(s) had heart disease prior to age 55 OR mother or sister(s) had heart disease prior to age 65? ..... Yes No

13. Within the past 12 months, has a health professional told you that you have high blood pressure (systolic ≥ 140 OR diastolic ≥ 90)? ..... Yes No

14. Currently, do you have high blood pressure or within the past 12 months, have you taken any medicines to control your blood pressure? ..... Yes No

15. Have you ever been told by a health professional that you have a fasting blood glucose greater than or equal to 110 mg/dl? ..... Yes No

16. Describe your regular physical activity or exercise program:

type:_____

frequency: _____ days per week

duration: _____ minutes

Intensity: *low* *moderate* *high* **(circle one)**

BMI: _____

17. If you have answered YES to any of questions 7-16, please describe:

_____

_____

_____

18. Are you currently under any treatment for any blood clots? .....Yes No

19. Do you have problems with bones, joints, or muscles that may be aggravated with exercise? .....Yes No

20. Do you have any back/neck problems? .....Yes No

21 Have you been told by a health professional that you should not exercise? .....Yes No

22. Are you currently being treated for any other medical condition by a physician? .....Yes No

23. Are there any other conditions (mitral valve prolapse, epilepsy, history of rheumatic fever, asthma, cancer, anemia, hepatitis, etc.) that may ***hinder*** your ability to exercise? .....Yes No

24. During the past six months, have you experienced any ***unexplained*** weight loss or gain (greater than ten pounds for no known reason)? .....Yes No

25. If you have answered YES to any of questions 18-24, please describe:

_____

_____

_____

26. Please list below all prescription and over-the-counter medications you are currently taking:

Medicine:	Reason for taking:	Dosage:	Amount/Frequency:

27. Are there any medicines that your physician has prescribed to you in the past 12 months which you are currently not taking? .....Yes No
If so, please list:

_____

_____

_____

I have answered the Health History Questionnaire questions accurately and completely. I understand that my medical history is a very important factor in the development of my fitness/wellness program. I understand that certain medical or physical conditions which are known to me, but that I do not disclose to my trainer, may result in serious injury to me. If any of the above conditions change, I will immediately inform my trainer of those changes. I, knowingly and willingly, assume all risks of injury resulting from my failure to disclose accurate, complete, and updated information in accordance with the attached questionnaire. I also understand that in order to properly risk stratify my Health History Questionnaire, my trainer should have a minimum of a national certification as a personal trainer. My trainer also verbally explained this statement to me to my understanding.

Client's Signature:_____ Date:_____

Trainer's Signature:_____ Date:_____

APPENDIX C. PAR-Q

2019 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

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FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

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6. **Do you have any Mental Health Problems or Learning Difficulties?** This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** This includes Tetraplegia and Paraplegia

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
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The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

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11-01-2018

APPENDIX D. LOW ENERGY AVAILABILITY IN FEMALES QUESTIONNAIRE

(LEAF-Q)

October 30, 2013 [THE LEAF-Q]

(Supplemental Digital Content 1)
The LEAF-Q
A questionnaire for female athletes

Department of Nutrition, Exercise and Sports

Life Science

University of Copenhagen

Denmark

Contact: Anna Melin, aot@life.ku.dk

October 30, 2013 [THE LEAF-Q]

The low energy availability in females questionnaire (LEAF –Q), focuses on physiological symptoms of insufficient energy intake. The following pages contain questions regarding injuries, gastrointestinal and reproductive function. We appreciate you taking the time to fill out the LEAF-Q and the reply will be treated as confidential.

Name: Address:

E-mail:

Cell:

Profession:

Education:

Age: (years)

Height: (cm)

Your highest weight with your present height: (excluding pregnancy)

Your lowest weight with your present height:

Do you smoke? Yes □ No □

Weight:

(kg)

Do you use any medication (excluding oral contraceptives)? Yes □

If yes, what kind of medication?

No □

Your normal amount of training (average) – number of hours per week and what kind of exercise, such as running, swimming, bicycling, strength training, technique training etc.:

Comments or further information regarding exercise:

October 30, 2013 [THE LEAF-Q]

1. Injuries
A: Have you had absences from your training, or participation in competitions during the last year due

to injuries?
No, not at all Yes, once or twice Yes, three or four times Yes, five times or more

A1: If yes, for how many days absence from training or participation in competition due to injuries have you had in the last year?

1-7 days 8-14 days 15-21 days 22 days or more A2: If yes, what kind of injuries have you had in the last year?

Comments or further information regarding injuries:

Mark the response that most accurately describes your situation

2. Gastro intestinal function

1. A: Do you feel gaseous or bloated in the abdomen, also when you do not have your period?

   Yes, several times a day Yes, several times a week

   Yes, once or twice a week or more seldom Rarely or never

2. B: Do you get cramps or stomach ache which cannot be related to your menstruation?

   Yes, several times a day Yes, several times a week

   Yes, once or twice a week or more seldom Rarely or never

C: How often do you have bowel movements on average?

Several times a day Once a day Every second day

Twice a week Once a week or more rarely D: How would you describe your normal stool?

Normal (soft) Diarrhoea-like (watery) Hard and dry Comments regarding gastrointestinal function:

3. Menstrual function and use of contraceptives

3.1 Contraceptives Mark the response that most accurately describes your situation

A: Do you use oral contraceptives? Yes No

A1: If yes, why do you use oral contraceptives?
Contraception Reduction of menstruation pains Reduction of bleeding To regulate the menstrual cycle in relation to performances etc..
Otherwise menstruation stops
Other

A2: If no, have you used oral contraceptives earlier? Yes No

A2:1 If yes, when and for how long?

B: Do you use any other kind of hormonal contraceptives? (e.g. hormonal implant or coil) Yes No

B1: If yes, what kind?
Hormonal patches Hormonal ring Hormonal coil Hormonal implant Other

3.2 Menstrual function Mark the response that most accurately describes your situation A: How old were when you had your first period?

11 years or younger 12-14 years 15 years or older I don't remember

I have never menstruated (If you have answered "I have never menstruated" there are no further questions to answer)

B: Did your first menstruation come naturally (by itself)? Yes No I don't remember

B1: If no, what kind of treatment was used to start your menstrual cycle? Hormonal treatment
Weight gain
Reduced amount of exercise Other

C: Do you have normal menstruation?
Yes No (go to question C6) I don't know (go to question C6)

C1: If yes, when was your last period?
0-4 weeks ago 1-2 months ago 3-4 months ago 5 months ago or more

2. C2: If yes, are your periods regular? (Every 28th to 34th day) Yes, most of the time No, mostly not
3. C3: If yes, for how many days do you normally bleed?
   1-2 days 3-4 days 5-6 days 7-8 days 9 days or more

C4: If yes, have you ever had problems with heavy menstrual bleeding? Yes No

C5: If yes, how many periods have you had during the last year?
12 or more 9-11 6-8 3-5 0-2

3.2 Menstrual function Mark the response that most accurately describes your situation

C6: If no or "I don't remember", when did you have your last period?
2-3 months ago 4-5 months ago 6 months ago or more I'm pregnant and therefore do not menstruate

D: Have your periods ever stopped for 3 consecutive months or longer (besides pregnancy)?
No, never Yes, it has happened before Yes, that's the situation now

E: Do you experience that your menstruation changes when you increase your exercise intensity, frequency or duration?

Yes No
E1: If yes, how? (Check one or more options)

I bleed less I bleed fewer days My menstruations stops I bleed more I bleed more days

October 30, 2013 [THE LEAF-Q]

(Supplemental Digital Content 2)

The LEAF-Q Scoring key

A total score ≥8 is to be considered at risk for the Triad

Department of Nutrition, Exercise and Sports Life Science
University of Copenhagen

Denmark

Contact: Anna Melin, aot@life.ku.dk

1. A: 0 No, not at all, 1 Yes, once or twice, 2 Yes, three or four times, 3 Yes, five times or more
1. A1: 1 1-7 days, 2 8-14 days, 3 15-21 days, 4 22 days or more
2. A: 3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom,
0 Rarely or never
2. B: 3 Yes, several times a day, 2 Yes, several times a week, 1 Yes, once or twice a week or more seldom,
0 Rarely or never
2. C: 1 Several times a day, 0 Once a day, 2 Every second day, 3 Twice a week, 4 Once a week or more rarely
2. D: 0 Normal, 1 Diarrhoea-like, 2 Hard and dry

3.1 A1: 0 Contraception, 0 Reduction of menstruation pains, 0 Reduction of bleeding,
0 To regulate the menstrual cycle in relation to performances etc.., 1 Otherwise menstruation stops
3.2 A: 0 11 years or younger, 0 12-14 years, 1 15 years or older, 0 I don't remember,

8 I have never menstruated

3.2 B: 0Yes, 1 No, 1 I don't remember
3.2 B1: 1 Hormonal treatment, 1 Weight gain, 1 Reduced amount of exercise, 1 Other
3.2 C: 0 Yes, 2 No (go to question 3.2 C6), 1 I don't know (go to question 3.2 C6)

3.2 C1: 0 0-4 weeks ago, 1 1-2 months ago, 2 3-4 months ago, 3 5 months ago or more 3.2 C2: 0 Yes, most of the time, 1 No, mostly not
3.2 C3: 1 1-2 days, 0 3-4 days, 0 5-6 days, 0 7-8 days, 0 9 days or more 3.2
C4: 0 Yes, 0 No

3.2 C5: 0 12 or more, 1 9-11, 2 6-8, 3 3-5, 4 0-2

3.2 C6: 1 2-3 months ago, 2 4-5 months ago, 3 6 months ago or more
0 I'm pregnant and therefore do not menstruate
3.2 D: 0 No, never, 1 Yes, it has happened before, 2 Yes, that's the situation now

3.2 E: 1 Yes, 0 No

3.2 E1: 1 I bleed less, 1 I bleed fewer days, 2 My menstruations stops, 0 I bleed more, 0 I bleed more days

APPENDIX F. DXA SCREENER

Developed by Diane Thériault for the Canadian Panel, International Society for Clinical Densitometry, April, 2004

Patient Questionnaire

Name (print):_____ Date: _____

Is there a chance that you are pregnant? Yes No
Have you had a barium X-ray in the last 2 weeks? Yes No
Have you had a nuclear medicine scan or injection of an X-ray dye in the last week? Yes No
Have you had hyperparathyroidism or a high calcium level in your blood? Yes No

If you answered yes to any of the above, speak to our receptionist right away.

1. Your: Age: ____ Sex: Male Female

2. Your ethnicity (check one):
__Caucasian (White) __Black __Aboriginal __Asian __Hispanic __Other
Your country of birth: _____

3. Have you ever had a bone density test? Yes No
If YES, when and where? _____

4. Have you had a recent weight change? Yes No
If YES, tell us about it: _____

5. Your tallest height (late teens or young adult): _____

6. Have you ever broken a bone? Yes No

Bone broken	Simple fall?	If not a simple fall, please describe the circumstances	Age when this occurred

7. Has a parent or sibling had a broken hip from a simple fall or bump? Yes No

8. Has a parent or sibling had any other type of broken bone from a simple fall or bump? Yes No

9. How many times have you fallen in the last year? _____

10. Have you ever had surgery of the spine, hips, legs or arms? Yes No
If YES, describe what type of surgery you had and which side was affected
_____
_____

11. Are you currently receiving or have you previously received prednisone pills (cortisone)?
Yes, currently ____ Yes, previously _____ No ____
If YES, for how long? _____ What is your dose? _____mg or _____ pills each day

12. List any chronic medical conditions that you have:
_____ _____
_____ _____
_____ _____

/Users/ginaschimek/Desktop/Research articles/Appendix A DEXA screening (1).doc

13. Are you currently receiving or have you previously received any of the following medications?

	No	Yes	For how long?
Medication for seizures or epilepsy			
Chemotherapy for cancer			
Medication for prostate cancer			
Medication to prevent organ transplant rejection			

14. Have you been treated with any of the following medications?

Medication	Ever?	Currently?	If current, how long?
Hormone replacement therapy (Estrogen)			
Tamoxifen			
Raloxifene (Evista)			
Testosterone			
Etidronate (Didronel/Didrocal)			
Alendronate (Fosamax)			
Risedronate (Actonel)			
Intravenous pamidronate (Aredia)			
Clodronate (Bonefos, Ostac)			
Calcitonin (Miacalcin nasal spray)			
PTH (Forteo)			
Zoledronic acid (Zometa)			
Sodium fluoride (Fluotic)			

15. How many servings of the following do you eat/drink per day (on average)?

	Milk (full cup)	Orange juice fortified with calcium (full cup)	Yogurt (small container or ½ cup)	Cheese
Number of servings				

16. Do you take any calcium supplements (including TUMS)? Yes No

17. Do you take any vitamin D supplements (including multivitamins and halibut liver oil)? Yes No

18. Do you smoke? Yes No

For women only…

19. Are you still having menstrual periods? Yes No

20. Before menopause, have you ever missed your periods for 6 months or more, besides during pregnancy? Yes No

21. Have you had your menopause? Yes No
If yes, at what age? _____

22. Have you had a hysterectomy? Yes No
If YES, at what age? _____
Have you had both of your ovaries removed? Yes No
If YES, at what age? _____

APPENDIX G. EAT-26

The Eating Attitudes Test (EAT), Garner, 1979

Please circle the number under the column which applies best for you.

	Never	Rarely	Sometimes	Often	Usually	Always
1. I am terrified about being overweight.	0	1	2	3	4	5
2. I avoid eating when I am hungry.	0	1	2	3	4	5
3. I find myself preoccupied with food.	0	1	2	3	4	5
4. I have gone on eating binges where I feel that I may not be able to stop .	0	1	2	3	4	5
5. I cut my food into small pieces.	0	1	2	3	4	5
6. I am aware of the calorie content of foods that I eat.	0	1	2	3	4	5
7. I particularly avoid foods with a high carbohydrate content (i.e. bread, rice, potatoes, etc.).	0	1	2	3	4	5
8. I feel that others would prefer if I ate more.	0	1	2	3	4	5
9. I vomit after I have eaten.	0	1	2	3	4	5
10. I feel extremely guilty after eating.	0	1	2	3	4	5
11. I am preoccupied with a desire to be thinner.	0	1	2	3	4	5
12. I think about burning up calories when I exercise.	0	1	2	3	4	5

The Eating Attitudes Test (EAT), Garner, 1979

	Never	Rarely	Sometimes	Often	Usually	Always
13. Other people think that I am too thin.	0	1	2	3	4	5
14. I am preoccupied with the thought of having fat on my body.	0	1	2	3	4	5
15. I take longer than others to eat my meals.	0	1	2	3	4	5
16. I avoid foods with sugar in them.	0	1	2	3	4	5
17. I eat diet foods.	0	1	2	3	4	5
18. I feel that food controls my life.	0	1	2	3	4	5
19. I display self-control around food.	0	1	2	3	4	5
20. I feel that others pressure me to eat.	0	1	2	3	4	5
21. I give too much time and thought to food.	0	1	2	3	4	5
22. I feel uncomfortable after eating sweets.	0	1	2	3	4	5
23. I engage in dieting behavior.	0	1	2	3	4	5
24. I like my stomach to be empty.	0	1	2	3	4	5
25. I enjoy trying new rich foods.	0	1	2	3	4	5
26. I have the impulse to vomit after meals.	0	1	2	3	4	5

APPENDIX H. ELECTRONIC SURVEY

We are very excited for you to participate in this study. This study is designed to determine bone health in relation to dietary habits of competitive, collegiate male and female long-distance runners. Your participation in this study will help improve knowledge on the relationship between energy intake, physical activity, and bone health. This information can be used to provide education and recommendations to athletes in order to improve their health and performance while training. The results you will receive individually will also provide you with information on bone health and body composition. The information can be used to improve diet, exercise, performance and prevent injury.

This survey will take approximately 15 minutes of your time. Please read and answer all the questions as thoroughly as possible.

Please provide in comments or suggestions that you feel would improve your experience while taking this survey.

1. Sex: □ Male □ Female

2. Your ethnicity (check one):

□ White □ Black □ American Indian/Alaskan Native □ Asian □ Hispanic □ Other
Your country of birth: _____

3. Age: (years)
4. Height: (inches)
5. Weight: (pounds)
6. Your highest weight with your present height: (excluding pregnancy)
7. Your lowest weight with your present height:
8. Do you smoke or use any form of tobacco? Yes □ No
9. What is your normal number of training (average) hours per week?

□ 0-1 □ 2-3 □ 4-5 □ 5-6 □ 6 hours or more

10. Please indicate types of activities in which you participate at least once per week:

Running

Swimming

Bicycling

strength training or resistance training

Other_____

Comments or further information regarding exercise:

Injuries
Mark the response that most accurately describes your situation

11. Have you had absences from your training, or participation in competitions during the last year due to illness?
□ No, not at all □ Yes, once or twice □ Yes, three or four times □ Yes, five times or more

12. If yes, for how many days absence from training or participation in competition due to illness have you had in the last year?

□ 1-7 days □ 8-14 days □ 15-21 days □ 22 days or more

13. If yes, what kind of illness have you had in the last year?

Comments or further information regarding illness:

14. Have you had absences from your training, or participation in competitions during the last year due to injuries?
□ No, not at all □ Yes, once or twice □ Yes, three or four times □ Yes, five times or more

15. If yes, for how many days absence from training or participation in competition due to injuries have you had in the last year?

□ 1-7 days □ 8-14 days □ 15-21 days □ 22 days or more

16. If yes, what kind of injuries have you had in the last year?

Comments or further information regarding injuries:

17. Do you feel gaseous or bloated in the abdomen, during your period?

□ Yes, several times a day □ Yes, several times a week □ Yes, once or twice a week or more seldom □ Rarely or never

18. Do you feel gaseous or bloated in the abdomen, when you do not have your period?

□ Yes, several times a day □ Yes, several times a week □ Yes, once or twice a week or more seldom □ Rarely or never

19. Do you get cramps or stomach ache which cannot be related to your menstruation?

□ Yes, several times a day □ Yes, several times a week □ Yes, once or twice a week or more seldom □ Rarely or never

20. How often do you have bowel movements on average?

□ Several times a day □ Once a day □ Every second day □ Twice a week □ Once a week or more rarely

21. How would you describe your normal stool?

□ Normal (soft) □ Diarrhea-like (watery) □ Hard and dry

Break here—some questions only for females in yellow

22. Do you currently use oral contraceptives?

□ Yes □ No

23. If yes, why do you use oral contraceptives?

□ Contraception □ Reduction of menstruation pains □ Reduction of bleeding □ To regulate the menstrual cycle in relation to performances etc. □ Otherwise menstruation stops □ Other

24. If no, have you previously used oral contraceptives?

□ Yes □ No

25. If yes, when and for how long?

□ 0-6 months □ 6-12 months □ 1 year □ 2 years □ 3 or more years

26. Do you use any other kind of hormonal contraceptives? (e.g. hormonal implant or coil)

□ Yes □ No

27. If yes, what kind?

□ Hormonal patches □ Hormonal ring □ Hormonal coil □ Hormonal implant □ Other

28. How old were when you had your first period?

□ 11 years or younger □ 12-14 years □ 15 years or older □ I don't remember

□ I have never menstruated (If you have answered "I have never menstruated" there are no further questions to answer)

29. Did your first menstruation come naturally (by itself)?

□ Yes □ No □ I don't remember

30. If no, what kind of treatment was used to start your menstrual cycle?

□ Hormonal treatment □ Weight gain □ Reduced amount of exercise □ Other

31. Do you have normal menstruation? (normal is when your period is about the same length every month — or somewhat irregular, and your period might be light or heavy, painful or pain-free, long or short, and still be considered normal. Within a broad range, "normal" is what's normal for you.)

□ Yes □ No (go to question C6) □ I don't know (go to question C6)

32. If yes, when was your last period?

□ 0-4 weeks ago □ 1-2 months ago □ 3-4 months ago □ 5 months ago or more

33. If yes, are your periods regular? (Every 28^{the} to 34th day)

□ Yes, most of the time □ No, mostly not

34. If yes, for how many days do you normally bleed?

□ 1-2 days □ 3-4 days □ 5-6 days □ 7-8 days □ 9 days or more

35. If yes, have you ever had problems with heavy menstrual bleeding?

□ Yes □ No

36. If yes, how many periods have you had during the last year?

□ 12 or more □ 9-11 □ 6-8 □ 3-5 □ 0-2

37. If no or "I don't remember", when did you have your last period?

□ 2-3 months ago □ 4-5 months ago □ 6 months ago or more □ I'm pregnant and therefore do not menstruate

38. Have your periods ever stopped for 3 consecutive months or longer (besides pregnancy)?

□ No, never □ Yes, previous □ Yes, currently

39. Do you experience that your menstruation changes when you increase your exercise intensity, frequency or duration?

□ Yes □ No

40. If yes, how? (Check one or more options)

□ I bleed less □ I bleed fewer days □ My menstruations stops □ I bleed more □ I bleed more days

41. Is there a chance that you are pregnant?
□ Yes □ No

42. Have you had a barium X-ray in the last 2 weeks?
□ Yes □ No

43. Have you had a nuclear medicine scan or injection of an X-ray dye in the last week?
□ Yes □ No

44. Have you had hyperparathyroidism or a high calcium level in your blood?
□ Yes □ No

45. Have you ever had a bone density test?
□ Yes □ No

46. If YES, when and where?
_____

47. Have you had a recent weight change?
□ Yes □ No

48. If YES, tell us about it:
_____

49. Have you ever broken a bone?
□ Yes □ No

Bone broken	Simple fall?	If not a simple fall, please describe the circumstances	Age when this occurred

50. Has a parent or sibling had a broken hip from a simple fall or bump?
□ Yes □ No

51. Has a parent or sibling had any other type of broken bone from a simple fall or bump?
□ Yes □ No

52. How many times have you fallen in the last year? _____

53. Have you ever had surgery of the spine, hips, legs or arms?
□ Yes □ No

54. If YES, describe what type of surgery you had and which side was affected
_____
_____

55. Are you currently receiving or have you previously received prednisone pills (cortisone)?
□ Yes, currently □ Yes, previously □ No

56. If YES, for how long? _____

57. What is your dose? _____mg or _____ pills each day

List any chronic medical conditions that you have:

_____ _____
_____ _____
_____ _____

Are you currently receiving or have you previously received any of the following medications?

	No	Yes	For how long?
Medication for seizures or epilepsy			
Chemotherapy for cancer			
Medication for prostate cancer			
Medication to prevent organ transplant rejection			

Have you been treated with any of the following medications?

Medication	Ever?	Currently?	If current, how long?
Hormone replacement therapy (Estrogen)			
Tamoxifen			
Raloxifene (Evista)			
Testosterone			
Etidronate (Didronel/Didrocal)			
Alendronate (Fosamax)			
Risedronate (Actonel)			
Intravenous pamidronate (Aredia)			
Clodronate (Bonefos, Ostac)			
Calcitonin (Miacalcin nasal spray)			
PTH (Forteo)			
Zoledronic acid (Zometa)			
Sodium fluoride (Fluotic)			

How many servings of the following do you eat/drink per day (on average)?

	Milk (full cup)	Orange juice fortified with calcium (full cup)	Yogurt (small container or ½ cup)	Cheese
Number of servings				

58. Do you take any calcium supplements (including TUMS)?

□ Yes □ No

59. Do you take any vitamin D supplements (including multivitamins and halibut liver oil)?

□ Yes □ No

60. Do you take any other dietary supplements?

□ Yes □ No

61. How would you describe your eating habits?

□ Good □ Fair □ Poor

62. How many times a day do you eat?

□ 1 □ 2 □ 3 □ 4 □ 5 or more

63. Do you avoid any of the following foods? (check/circle all that apply)

□ Beef □ Pork □ Chicken □ Turkey □ Fish □ Fruits □ Breads □ Grains (pasta, rice)

□ Sweets (candy, desserts) □ Fried foods □ Alcohol □ Dairy (milk, cheese)

□ Vegetables □ Fast foods □ Fast/oils (mayo, etc.) □ Other

64. During the past 7 days how many meals did you eat with people?

□ I eat alone □ 1-2 times □ 3-4 times □ 5-6 times □ 7 times □ More than 7 times

65. Do you live…

□ Alone □ With family □ With friends □ Other, please specify…

66. Overall, I feel my health is…

□ Excellent □ Very good □ Good □ Fair □ Poor

Please circle the number under the column which applies best for you.

	Never	Rarely	Sometimes	Often	Usually	Always
67. I am terrified about being overweight.	0	1	2	3	4	5
68. I avoid eating when I am hungry.	0	1	2	3	4	5
69. I find myself preoccupied with food.	0	1	2	3	4	5
70. I have gone on eating binges where I feel that I may not be able to stop.	0	1	2	3	4	5
71. I cut my food into small pieces.	0	1	2	3	4	5
72. I am aware of the calorie content of foods that I eat.	0	1	2	3	4	5
73. I particularly avoid foods with a high carbohydrate	0	1	2	3	4	5

content (i.e. bread, rice, potatoes, etc.).						
74. I feel that others would prefer if I ate more.	0	1	2	3	4	5
75. I vomit after I have eaten.	0	1	2	3	4	5
76. I feel extremely guilty after eating.	0	1	2	3	4	5
77. I am preoccupied with a desire to be thinner.	0	1	2	3	4	5
78. I think about burning up calories when I exercise.	0	1	2	3	4	5

	Never	Rarely	Sometimes	Often	Usually	Always
79. Other people think that I am too thin.	0	1	2	3	4	5
80. I am preoccupied with the thought of having fat on my body.	0	1	2	3	4	5
81. I take longer than others to eat my meals.	0	1	2	3	4	5
82. I avoid foods with sugar in them.	0	1	2	3	4	5
83. I eat diet foods.	0	1	2	3	4	5
84. I feel that food controls my life.	0	1	2	3	4	5
85. I display self-control around food.	0	1	2	3	4	5
86. I feel that others pressure me to eat.	0	1	2	3	4	5

87. I give too much time and thought to food.	0	1	2	3	4	5
88. I feel uncomfortable after eating sweets.	0	1	2	3	4	5
89. I engage in dieting behavior.	0	1	2	3	4	5
90. I like my stomach to be empty.	0	1	2	3	4	5
91. I enjoy trying new rich foods.	0	1	2	3	4	5
92. I have the impulse to Vomit after meals.	0	1	2	3	4	5

Please provide in comments or suggestions that you feel would improve your experience while taking this survey.

Thank you for taking the time to fill out this survey!

APPENDIX I. THREE-DAY FOOD DIARY AND EXERCISE LOG

Latest Version 9 – 2017 NDBC

3-day Food Diary and Nutrition Screening Form

Subject #: _____ Date: _____

Please **circle** preferred method of communication: Voice Text Email

Is it OK to send reminders regarding completion of study forms? Yes No

For the following questions, please circle or check best answer:

1. Which one of the following best describes your ethnic background? **(Check one)**

   a. _____ White/Caucasian d._____ Asian or Pacific Islander

   b. _____Black/African-American e._____ American Indian/Alaska Native

   c._____ Hispanic f._____ Other; specify_____

2. How would you describe your eating habits? **(Check One)**

   a.____ Good b._____ Fair c._____ Poor

3. How many times a day do you eat? a._____ How many foods/meal average? b._____

4. How often do you eat out? _____(number of times per week)

5. When you go out to eat, what are the three most common places you go?

   a. _____
   b. _____
   c. _____

6. Do you **avoid** any of the following foods? **(Check/circle all that apply)**

a._____ Beef	f._____ Breads	g. _____ Vegetables
b._____ Pork	h. ____ Grains (pasta, rice)	e. _____ Fast foods
c._____ Chicken	i. ____ Sweets (candy, desserts)	j. _____Fats/oils (mayo, etc.)
d._____ Turkey	k. ____ Fried foods	m. ____ Other _____
e._____ Fish	g._____ Alcohol	
f. _____ Fruits	l. _____ Dairy (milk, cheese)	

7. Do you currently take any dietary supplements? ____Yes ____ No

   If yes, which ones? **(Check all that apply)**

   a. ____ Creatine e. ____Vitamins i. ____ Pyruvate

b. ____ Protein shakes
c. ____ Amino Acids
d. ____ HMB
f. ____ Minerals
g. ____ Herbs
h. ____ "Andro"/ DHEA
j. ____ Energy boosters (eg, preworkout)
k. ____ Other; Specify

8. Please estimate your daily needs for calories and protein, even if you are unsure (fill in numbers):

_____ calories per day

_____ grams of protein per day

9. Please tell us your current occupation _____

10. How much did you earn, before taxes and other deductions, during the past 12 months?

(Circle One)

a. Less than $5,000

b. $5,000 through $24,999

c. $25,000 through $49,999

d. $50,000 through $74,999

e. $75,000 through $99,999

f. $100,000 and greater

g. Don't know

h. No response

11.What is the highest level of education you have completed or the highest degree you have received? **(Circle One)**

a. Less than high school
b. Some high school
c. High school or equivalent (e.g., GED)
d. Some college, but no degree
e. Associate's degree
f. College (Bachelor's degree)
g. Some graduate school, but no degree
h. Graduate school (e.g., Master's degree or Doctor of Philosophy)
i. Not sure
j. Decline to answer

12. During the past 7 days how many meals did you eat with people?

a. I eat alone
b. 1 -2 times
c. 3 - 4 times
d. 5 - 6 times
e. 7 times
f. More than 7 times

13. Do you live…

a. Alone
b. With family
c. With friends
d. Other, please specify _____

14. Overall, I feel my health is…

a. Excellent
b. Very Good
c. Good
d. Fair
e. Poor

Latest Version 9 – 2017 NDBC

FOOD DIARY (FOOD LOG)

	EXAMPLE Day Date:_____	
Time	Food Item and Method of Preparation	Amount Eaten
6:00 am	Pink Lady apple	One small apple sliced
	Country Hearth 100% whole wheat bread	2 slices
	Jiff Creamy Peanut Butter	2 Tablespoons
9:00 am	Caribou white mocha with whip	16 oz
noon	Lean Cuisine Favorites Alfredo Pasta with Chicken & Broccoli	1 container
3:00 pm	Diet Coke	1 can
	Navel Orange	1 Large

	YOUR Day 1 Date_____ Day of week_____	
Time	Food Item and Method of Preparation	Amount Eaten

	Your Day 2 Date:_____ Day of week:_____	
Time	Food Item and Method of Preparation	Amount Eaten

	YOUR Day 3 Date_____ Day of week_____	
Time	Food Item and Method of Preparation	Amount Eaten

APPENDIX J. RATE OF PERCEIVED EXERTION

Rate of Perceived Exertion Scale

6 No exertion at all

7 Extremely light

8

9 Very light

10

11

12

13 Somewhat hard

14

15 Hard (heavy)

16

17

18

19 Extremely hard

20 Maximal exertion

APPENDIX K. TRIAD: CUMULATIVE RISK ASSESSMENT

Risk Factors	Magnitude of Risk Low Risk = 0 points each	Moderate Risk = 1 point each	High Risk = 2 points each
Low EA with or without DE/ED	☐ No dietary restriction	☐ Some dietary restriction‡; current/past history of DE;	☐ Meets DSM V criteria for ED*
Low BMI	☐ BMI ≥ 18.5 **or** ≥ 90% EW** or weight stable	☐ BMI 17.5 < 18.5 **or** < 90% EW **or** 5 to < 10% weight loss/month	☐ BMI ≤17.5 **or** < 85% EW **or** ≥ 10% weight loss/month
Delayed Menarche	☐ Menarche < 15 years	☐ Menarche 15 to < 16 years	☐ Menarche ≥16 years
Oligomenorrhea and/or Amenorrhea	☐ > 9 menses in 12 months*	☐ 6-9 menses in 12 months*	☐ < 6 menses in 12 months*
Low BMD	☐ Z-score ≥ -1.0	☐ Z-score -1.0*** < - 2.0	☐ Z-score ≤ -2.0
Stress Reaction/Fracture	☐ None	☐ 1	☐ ≥ 2; ≥ 1 high risk or of trabecular bone sites†
Cumulative Risk (total each column, then add for total score)	**_____ points +**	**_____ points +**	**_____ points = _____Total Score**

APPENDIX L. TRIAD: CLEARANCE AND RTP GUIDELINES BY MEDICAL RISK STRATIFICATION

	Cumulative Risk Score*	Low Risk	Moderate Risk	High Risk
Full Clearance	0 – 1 point	☐		
Provisional/Limited Clearance	2 – 5 points		☐ Provisional Clearance ☐ Limited Clearance	
Restricted from Training and Competition	≥ 6 points			☐ Restricted from Training/ Competition-Provisional ☐ Disqualified

APPENDIX M. TRIAD: RED-S CAT

RED-S CAT™

Relative Energy Deficiency in Sport (RED-S) Clinical Assessment Tool (CAT)

For use by medical professionals only

Name Date: Examiner:

What is the RED-S CAT?

The RED-S CAT is a clinical assessment tool for the evaluation of athletes/active individuals suspected of having relative energy deficiency and for guiding return to play decisions. The RED-S CAT is designed for use by a medical professional in the clinical evaluation and management of athletes with this syndrome. The RED-S CAT is based on the IOC Consensus Statement on RED-S, 2014.[1]

This tool may be freely copied in its current form for use by sport organizations and the athlete medical team entourage. Alterations to the tool or reproduction for publication purposes require permission from the International Olympic Committee.

NOTE: The diagnosis of RED-S is a medical diagnosis to be made by a trained health care professional. Clinical management and return to play decisions for athletes with RED-S should occur under the guidance of an experienced sports medicine team.

What is Relative Energy Deficiency in Sport?

The syndrome of RED-S refers to impaired physiological functioning caused by relative energy deficiency, and includes but is not limited to impairments of metabolic rate, menstrual function, bone health, immunity, protein synthesis, and cardiovascular health.

The cause of RED-S is the scenario termed "low energy availability", where an individual's dietary energy intake is insufficient to support the energy expenditure required for health, function, and daily living, once the cost of exercise and sporting activities is taken into account.

The potential health consequences of RED-S are depicted in the RED-S conceptual model (See Figure 1). Psychological problems can be both the result of and the cause of RED-S.

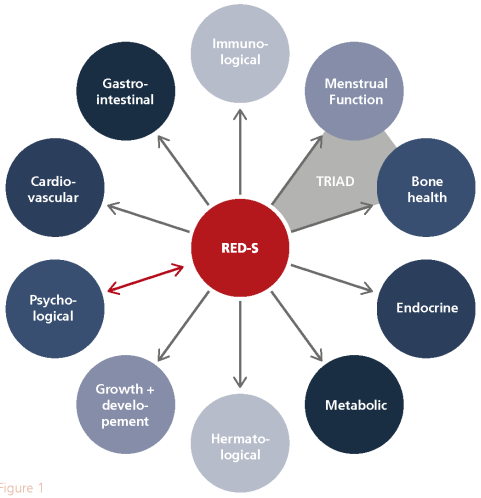

Figure 1

RED-S may also affect athlete sport performance. The potential effects of RED-S on sport performance are illustrated in Figure 2:

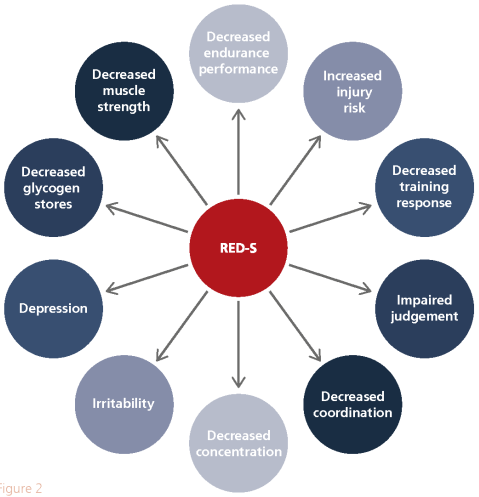

Figure 2

Screening for RED-S

The screening and diagnosis of RED-S is challenging, as symptomatology can be subtle. A special focus on the athlete at risk is needed. Although any athlete can suffer from RED-S, those at particular risk are those in judged sports with an emphasis on the aesthetic or appearance, weight category sports, and endurance sports. Early detection is of importance to maintain and improve performance and prevent long-term health consequences.

Screening for RED-S can be undertaken as part of an annual Periodic Health Examination and when an athlete presents with Disordered Eating (DE)/Eating Disorders (ED), weight loss, lack of normal growth and development, endocrine dysfunction, recurrent injuries and illnesses, decreased performance/performance variability or mood changes.

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RED-S Risk Assessment Model for sport participation

This model can be incorporated into the Periodic Health Examination. Depending on the findings on history and physical examination, the athlete is classified into one of the 3 following categories: **"Red Light":** High risk, **"Yellow Light":** Moderate risk, **"Green Light":** Low Risk.

HIGH RISK: NO START RED LIGHT	MODERATE RISK: CAUTION YELLOW LIGHT	LOW RISK: GREEN LIGHT
- Anorexia nervosa and other serious eating disorders - Other serious medical (psychological and physiological) conditions related to low energy availability - Use of extreme weight loss techniques leading to dehydration induced hemodynamic instability and other life threatening conditions.	- Prolonged abnormally low % body fat measured by DXA* or anthropometry - Substantial weight loss (5–10 % body mass in one month) - Attenuation of expected growth and development in adolescent athlete	- Appropriate physique that is managed without undue stress or unhealthy diet/exercise strategies
	- Low **EA of prolonged and/or severe nature	- Healthy eating habits with appropriate EA
	- Abnormal menstrual cycle: functional hypothalamic amenorrhea >3 months - No menarche by age 15y in females	- Healthy functioning endocrine system
	- Reduced bone mineral density (either in comparison to prior DXA or Z-score <-1 SD). - History of 1 or more stress fractures associated with hormonal/menstrual dysfunction and/or low EA	- Healthy bone mineral density as expected for sport, age and ethnicity - Healthy musculoskeletal system
- Severe ECG abnormalities (i.e. bradycardia)	- Athletes with physical/psychological complications related to low EA+/-disordered eating; - Diagnostic testing abnormalities related to low EA +/-disordered eating	
	- Prolonged relative energy deficiency - Disordered eating behavior negatively affecting other team members - Lack of progress in treatment and/or non-compliance	

* dual energy X-ray absorptiometry

**EA: Energy availability = Energy intake – Energy cost of exercise (additional energy expended in undertaking exercise).

NOTES on diagnostic tools for Low EA:
Although low EA is a key factor in RED-S, at the present time there is no standardised protocol for undertaking an assessment of EA in free-living athletes. Some sports nutrition experts may have developed tools to monitor EA in which they have confidence, and may use these to screen for problems or guide dietary counselling. However, a universal recommendation to measure EA is unwise in the absence of a protocol that is sensitive, reliable, time-efficient and cost-effective.

Sport Participation based on Risk Category

"High Risk - Red Light": no clearance for sport participation.
Due to the severity of his/her clinical presentation, sport participation may pose serious jeopardy to his/her health and may also distract the athlete from devoting the attention needed for treatment and recovery.
"Moderate Risk -Yellow Light": cleared for sport participation only with supervised participation and a medical treatment plan.
Re-evaluation of the athlete's risk assessment should occur at regular intervals of 1–3 months depending on the clinical scenario to assess compliance and to detect changes in clinical status.
"Low Risk – Green Light": full sport participation.

Treatment of Relative Energy Deficiency in Sport (RED-S)

Athletes categorized in the red light and yellow light zones should receive medical evaluation and treatment. The treatment of RED-S should be undertaken by a team of health professionals including a sports medicine physician, sports dietician, exercise physiologist, athletic therapist or trainer, sports psychologist/sports psychiatrist as needed. Patient confidentiality must be maintained. Treatment should focus on correcting the relative energy deficit through increasing energy intake and/or decreasing energy output. Intake of nutrients and other vitamins should follow established guidelines. Repeat assessment of BMD should occur at intervals of 6–12 months, depending on clinical presentation and initial values.

The use of an athlete contract is also recommended. (*See Appendix*)

Relative Energy Deficiency in Sport (RED-S) risk assessment decision making steps for determining readiness for returning to play

Prior to returning an athlete to sport/physical activity following time away for RED-S treatment, an assessment of the athlete's health and the requirements of his/her sport should be undertaken following the step-wise approach:

STEPS	RISK MODIFIERS	CRITERIA	RED-S SPECIFIC CRITERIA
STEP 1 Evaluation of Health Status	MEDICAL FACTORS	- Patient Demographics - Symptoms - Medical History - Signs - Diagnostic Tests - Psychological Health - Potential Seriousness	- Age, sex - See Yellow Light column in RED-S Risk assessment model - Recurrent dieting, menstrual health, bone health - Weight loss/fluctuations, weakness - Hormones, electrolytes, electrocardiogram, DXA - Depression, anxiety, disordered eating/eating disorder - Abnormal hormonal and metabolic function - Cardiac arrhythmia - Stress fracture
STEP 2 Evaluation of Participation Risk	SPORT RISK MODIFIERS	- Type of Sport - Position Played - Competitive Level	- Weight sensitive, leanness sport - Individual vs. team sport - Elite vs. recreational
STEP 3 Decision Modification	DECISION MODIFIERS	- Timing and Season - Pressure from Athlete - External Pressure - Conflict of Interest - Fear of Litigation	- In/out of season, travel, environmental factors - Mental readiness to compete - Coach, team owner, athlete family, sponsors support - If restricted from competition

Return to Play Model

Following clinical reassessment utilizing the 3 step evaluation outlined above, athletes can be re-classified into the "High Risk – Red Light", "Moderate Risk – Yellow Light" or "Low Risk – Green Light" categories. The RED-S Risk Assessment Model is adapted to aid clinicians' decision making for determining an athlete's readiness to return to sport/physical activity.

The RED-S **Return to Play Model** outlines the sport activity recommended for each risk category.

HIGH RISK RED LIGHT	MODERATE RISK YELLOW LIGHT	LOW RISK GREEN LIGHT
- No competition - No training - Use of written contract	- May train as long as he/she is following the treatment plan - May compete once medically cleared under supervision	- Full sport participation

APPENDIX

Relative Energy Deficiency in Sport (RED-S) Treatment Contract

RED-S Treatment Contract for _____

Multidisciplinary Team:

- (Physician) _____
- (Psychotherapist/Psychiatrist) _____
- (Exercise physiologist) _____
- (Dietitian) _____
- (Other) _____

Requirements

Meet with:

- The psychotherapist at intervals recommended by the health professional treatment team
- The dietitian at intervals recommended by the health professional treatment team
- The physician at intervals recommended by the health professional treatment team
- Follow daily meal plan developed by the health professional treatment team
- Follow the adapted training plan developed by the health professional treatment team
- If underweight, weight gain expected to be _____ kg per week/weight stable within week _____
- If underweight, must achieve minimal acceptable body weight/fat of _____ kg/percent by _____
- Regular weigh-in at the following time intervals of _____ week (s)
- After this date, _____ (dd/mm/yyyy), must maintain weight and % fat at or above minimal acceptable body weight/fat mass of _____ (kg/%)
- Other _____

If **ALL** requirements are met and the eating behavior (and other severe conditions) are normalized the Team Physician will decide if cleared for competition.

I, _____ have read this contract and all of my questions were answered.

Athlete Name	Athlete Signature	Date
Team Physician Name	Team Physician Signature	Date

References

Mountjoy M, Sundgot-Borgen J, Burke L, et al. IOC Consensus Statement. Beyond the Triad – RED-S in sport. Br J Sports Med. 2014; 48: 491-7.

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