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THE IMPACT OF ENERGETIC FACTORS ON THE PERFORMANCE OF FEMALE
DISTANCE RUNNERS

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ABSTRACT

Introduction: Female distance runners often do not consume adequate calories to match their energy expended during exercise and for physiological processes. The low energy availability can lead to an energy deficiency which has been shown to negatively impact performance. The purpose of this study was to investigate how energetic factors in competitive female long distance runners' change across a season and determine the impact the change has on running performance. Methods: Competitive female distance runners' performance was measured based on a 5km time trial pre- and post-season. Other testing included VO₂ max, RMR, TT3, and DXA. The runners were grouped based on their pre-season resting metabolic rate as severe energy deficiency, moderate energy deficiency, or energy replete. Results: The pre-season race times for the severe energy deficiency group, moderate energy deficiency group, and energy replete group were 22.8± 0.8, 19.62± 0.59, and 21.25± 0.71, respectively. The post-season average race time for the severe energy deficiency group, the moderate energy deficiency group, and the energy replete group were 22.68± 0.77, 19.49± 0.58, and 21.02± 0.41. The pre-season mean REE/ LBM measured in kcal/day * kg LBM for the severe energy deficiency group, moderate energy deficiency group, and energy replete group were 28.2± 0.63, 30.76± 0.69, and 33.34± 0.97. The post-season mean REE/ LBM for the severe energy deficiency group, moderate energy deficiency group, and energy replete group were 28.35±1.04, 29.72± 0.9, and 33.05± 0.04. The mean pre-season TT3 concentration measured in ng/dl for the severe energy deficiency group, the moderate energy deficiency group, and the energy replete groups were 1.91± 0.02, 1.99± 0.02, and 1.97± 0.02. The mean post-season TT3 for the severe energy deficiency group, the moderate energy deficiency group, and the energy replete groups were 1.94

± 0.02 , 1.97 ± 0.02 , and 1.98 ± 0.02 . Conclusions: This study illustrates that a severe energy deficit is associated with a poor sports performance specifically test race time and race velocity. Female distance runners should aim to consume adequate calories to account for their energy expenditure to avoid an energy deficit and conditions associated with the Female Athlete Triad.

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Chapter 1

Introduction

1.1 Introduction: Female distance runners are particularly susceptible to development of the medical conditions associated with the Female Athlete Triad [1, 2]. These conditions include low energy availability (EA) with or without disordered eating, menstrual dysfunction, and low bone mineral density and can be experienced alone or in combination [3, 4]. There is well-documented evidence of the presence of Triad conditions in exercising women participating in recreational- and elite-level sports [5-8]. The prevalence of Triad conditions is highest in women competing in leanness sports where the focus is on low body fat, weight control, and aesthetic appearance, such as distance running, gymnastics, and dance [6, 8]. Participants in leanness sports have been shown to be at a higher risk for having a low EA, which is characterized by a reduced dietary intake relative to exercise energy expenditure (EEE) [7]. There are several reasons why athletes participating in leanness sports may reduce EA including: (1) intentionally to modify body size and composition to improve performance; (2) compulsively as a result of a pathological weight control or disordered eating behavior; or (3) inadvertently by failing to match energy intake (EI) to EEE and other body energy requirements [9]. Numerous reports exist in the literature on low EI in female distance runners [10-12]. The adoption of a restrictive eating behavior in female distance runners is completed to reduce body weight or fat mass in an effort to achieve optimal distance running performance. However, the impact of low EA on distance running performance is unclear.

In order to preserve energy stores for more vital physiological processes, such as thermoregulation, cellular maintenance, and locomotion, low EA has a suppressive effect on both metabolic and reproductive function [13]. Low EA often induces an energy deficiency, which subsequently promotes adaptations in resting energy expenditure (REE) and metabolic hormones, such as total triiodothyronine (TT3), ghrelin, peptide YY (PYY), and insulin-like growth factor-1 (IGF-1). Research shows low EA to have a suppressive effect on fasting TT3 concentrations [14], luteinizing hormone (LH) pulsatility [15, 16], and menstrual cyclicity [17, 18]. The observed suppression of LH pulsatility has been prevented and reversed by dietary supplementation [16, 17, 19]. These findings demonstrate that exercise alone does not have a suppressive effect on TT3 or LH pulsatility beyond the impact of its energy cost on energy availability [19]. In young women with normal weight, basal ghrelin levels have been shown to be responsive to energy-deficit-induced decreases in body weight, fat mass, and resting metabolic rate [20]. Amenorrheic athletes present elevated ghrelin [21] and PYY concentrations [22], thus ghrelin and PYY may represent key markers of a chronic energy deficit and functional hypothalamic amenorrhea. Chronic energy deficiency may induce subclinical (luteal phase defects and anovulation) and clinical menstrual disturbances (oligomenorrhea and functional hypothalamic amenorrhea), detrimental musculoskeletal injury, and low bone mineral density [4].

The reduced EA in female distance runners is a result of not consuming an adequate amount of calories to match the energy expenditure associated with exercise and other body energy requirements [23]. The development of a chronic energy deficit in women has been associated with negative effects on reproductive and skeletal health [16]. Some studies have shown a relationship between a chronic energy deficiency and decrements in physical

performance tests [24-26]. For example, Van Heest et al. [26] demonstrated junior elite athletes with chronic ovarian suppression, secondary to an energy deficit (determined by suppressed TT3 and a REE measured below 85% of predicted REE), was associated with significant decrements in performance during a 400m freestyle time trial across a 12 week competitive season. However, there is limited evidence to show a direct association between energy deficiency and performance variables in other athletes, to include competitive female distance runners.

1.2 Objective: The overall objective of this study is to investigate how energetic factors in competitive female long distance runners' change across a competitive season and to determine the impact of a change in energetic factors on distance running performance during a competitive time trial (test race time in minutes) in these women. The energetic factors that will be studied include EA, REE, and fasting concentrations of TT3. Performance will be assessed by a competitive time trial (test race time in minutes).

1.3 Specific Aim 1: To examine changes in REE, EA, and fasting concentrations of TT3, a hormone that reflects energetic status, from pre-season to post-season in competitive female distance runners.

The following hypothesis will be tested:

Hypothesis 1A: Resting energy expenditure (kcal/kg LBM) will be significantly greater in competitive female distance runners pre-season compared to post-season.

Hypothesis 1B: Energy availability (kcal/day) will be significantly greater in competitive female distance runners pre-season compared to post-season.

Hypothesis 1C: Fasting concentrations of TT3 (ng/dL) will be significantly greater in competitive female distance runners pre-season compared to post-season.

1.4 Specific Aim 2: To investigate how REE, EA, and fasting concentrations of TT3 influence distance running performance (test race time measured in minutes) across a competitive season in female distance runners.

The following hypothesis will be tested:

Hypothesis 2A: Resting energy expenditure will decrease across a competitive season such that there will be a decrement in performance (test race time measured in minutes will increase) from pre-season to post-season.

Hypothesis 2B: Energy availability will decrease across a competitive season such that there will be an observed decrement in performance (test race time measured in minutes will increase) from pre-season to post-season.

Hypothesis 2C: Fasting concentrations of TT3 will decrease across a competitive season such that there will be a decrement in performance (test race time measured in minutes will increase) pre-season to post-season.

1.5 Statistical Analyses: The data will be analyzed by running a paired t test comparison of pre- and post-season measures of REE, fasting concentrations of TT3, and EA. We will also perform a repeated measures ANOVA with time (pre- and post-season) and group (severe energy deficiency, vs moderate energy deficiency, vs energy replete) factors to compare measures of REE, fasting concentrations of TT3, and EA across the competitive season.

1.6 Experimental Design Overview: This experiment is a prospective repeated measures experiment designed to investigate change in energetic factors (EA, REE, and fasting concentrations of TT3) following a competitive season (pre- vs. post-season) in competitive female distance runners. This study design also allows the determination of the above-mentioned energetic factors on distance running performance in these women. Running performance in this study will be measured based on a 5km competitive time trial measured in minutes.

1.7 Participants: Participants eligible to participate will include 1) women, 2) between the ages of 18-25, 3) in good health, 4) actively participating in competitive distance running on the Pennsylvania State University varsity or club cross-country teams, 5) non-smoker, 6) not pregnant, breast-feeding or lactating.

1.8 Rationale: The relationship between optimal nutrition and successful athletic performance is notably critical in female athletes. [23]. During the course of training, female distance runners, in particular, are at risk of developing a chronic energy deficiency or low EA due to insufficient EI in association with a high amount of energy expenditure during training and competition. Obtaining and maintaining proper energy reserves in female distance runners is a means to provide the fuel essential for vital physiological processes (i.e., immune function, thermoregulation, and cellular maintenance) [13], as well as for performance. Reproductive [15, 18] and skeletal health [27, 28] of female athletes have been negatively affected by a chronic energy deficit. Detrimental ramifications have also been observed on performance and recovery from training in female distance runners [10-12]. In exercising girls and women, a chronic energy deficiency or low EA is a key factor leading to the development of the Triad (low EA,

menstrual irregularities, and low bone mass) [4] and may have implications for distance running performance.

To date, the association between chronic energy deficiency and distance running performance, test race time in minutes, in female distance runners is unclear and serves as the rationale for conducting this work. An association between an energy deficiency and decrements in performance has been established in the literature [24, 25, 29]; however, to date there is limited evidence of this association in female distance runners. This research may provide further insight into the most appropriate structure for nutritional and exercise training programs for this sub-set of female athletes to avoid development of an energy deficit and the clinical sequelae of the Triad, and decrements in performance. Performance for the purposes of this study will be defined as test race time measured in minutes and will be assessed by a competitive 5km time trial run. Prevention and treatment of an energy deficit may be the key step in prevention of reproductive dysfunction, bone stress injury and avoidance of detrimental bone mineral density loss.

1.9 Applications: We anticipate that female distance runners with an initial severe energy deficit will have a decrease in performance (specifically, an increase in test race time in minutes) across a competitive season. Most runners are under the impression that a low body weight is associated with a faster racing time. In an attempt to achieve peak performance during the important end of the season races (regionals and nationals), female distance runners often train to achieve their lowest weight at the end of the competitive season. We therefore predict that EA will be lower in competitive female distance runners post-season compared to pre-season. In terms of energetic factors we suggest that REE would be suppressed in the post-season compared to the pre-season. Additionally, key metabolic markers of energy status, suppressed TT3

concentrations, would be indicative of an energy deficit in the post-season in competitive female distance runners.

We expect our results will provide critical evidence on the role of EA and energy deficiency on distance running performance in competitive female distance runners. Female distance runners often do not meet their basic daily caloric intake requirements, which can lead to an energy deficit [23] and subsequent negative health outcomes including the Triad. Educating female distance runners about the negative impact an energy deficit can have on running performance (test race time in minutes) would allow a greater emphasis to be placed on the importance of meeting nutritional requirements. We expect our results to provide critical evidence of the role of EA and energy deficiency on distance running performance in competitive female distance runners. We expect our results will aid competitive female distance runners in preventing development of an energy deficit to maintain or improve performance across the competitive season.

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Chapter 2

Literature Review

The Impact of Energetic Factors on the Female Athlete Triad and Performance in Athletic

Women: A Focus on Female Long-Distance Runners

2.1 Introduction: Optimal energy intake (EI) is associated with successful sports performance and is notably critical in female athletes [1-3]. Performance is defined as the execution of a sport in a competitive environment, which includes the complex interaction between physiological, tactical, technical, and psychological factors [4, 5]. This definition varies in the research setting where performance is indirectly measured through evaluations of exercise capacity (i.e., maximal oxygen uptake (VO_{2max}), blood lactate concentrations during and at maximal exercise, and muscle mass or muscle fiber composition), which are commonly assumed to correlate with competitive athletic performance [4, 6]. Female athletes are often susceptible to developing an energy deficit, compared to their sedentary counterparts, due to the challenges in matching EI with the energy expended with high training volumes and competition [2, 3, 7, 8]. Sports where leanness and low body mass are assumed to be beneficial to performance, such as long distance running (>800m), can lead to athletes not consuming adequate calories in an effort to reduce body fat levels and increase the power to weight ratio [2, 9, 10]. Female long-distance runners often engage in dietary manipulations, typically energy restriction, to maintain or lose weight. Such nutritional strategies are often counterproductive and can compromise recovery and hinder training adaptations [3].

A chronic discrepancy between calories expended and consumed can lead to health complications including the Female Athlete Triad (Triad). The Triad is a medical syndrome observed in young female athletes and is composed of three components: (1) low energy availability (EA) with or without disordered eating, (2) menstrual dysfunction, and (3) low bone mineral density (BMD) [11]. Female athletes are particularly susceptible to developing one or all three components of the Triad, especially when there is pressure to maintain a low body weight [12].

Despite some female distance runners' perception that to attain maximum performance, a low body weight and a lean body composition must be maintained, resulting in an energy deficiency, an energy deficit can lead to impaired growth, health and performance [2, 13]. Energy deficiency, that leads to a decrease in lean body mass, may negatively influence muscular endurance, strength and power [14]. Beyond performance-related decrements, a chronic energy deficit may impair general health, to include negative effects on metabolism [8, 15-21], reproduction [2, 7, 11, 22-24], and bone health [7, 25]. In addition, while the runners are seeking to maintain a low body weight, an energy deficit could increase their risk of stress fractures, an injury that has the potential to halt practice and competition for weeks to months, due to reduced BMD [26]. The prevalence of stress fractures in female athletes is reported to be up to 10% [27] and as high as 22% in female track and field athletes [28]. Athletes need to become informed of the health risks and performance decrements associated with an energy deficiency and become educated on how to maintain an energy balance.

The energetic factors that affect performance in female long-distance runners at risk of developing the Triad are unclear. In this athletic population, previous reports are inconclusive on whether an energy deficit is detrimental or advantageous. During period of high intensity and/or

long duration training, adequate energy intake to maintain body weight and health and optimize training effects is recommended [3]. Recovery and replenishment of fuel stores are dependent on the proper energy consumption and compensation to replace energy losses during habitual exercise training. Failure to replace energy or utilization of harmful dietary strategies places female long-distance runners at a significantly greater risk of the Triad clinical sequelae independent or in combination with decrements in performance [3, 29]. The purpose of this literature review is to summarize our current understanding of the effects of energetic factors on performance in female distance runners and on clinical outcomes of the Female Athlete Triad that result from an energy deficiency. The energetic factors that will be examined include EA, resting energy expenditure (REE), and metabolic hormones. Components of the Triad will be reviewed including low EA with or without disordered eating, menstrual dysfunction, and low BMD.

2.2 Energy Deficiency: Energy balance (EB) is defined as an inadequate dietary EI relative to the energy expended through physiological processes that include cellular maintenance, thermoregulation, growth, reproduction, immunity, and locomotion [17, 30]. In 2007 the American College of Sports Medicine proposed that an energy deficiency can develop in one of three ways [29]. The first is through a clinically diagnosed eating disorder. The second is through intentional attempts to maintain a low body weight to improve performance, but being unaware of the risks and mismanaging calories. The third is a failure to increase caloric intake despite energy expended in exercise [30]. In many female athletes, an energy deficiency often develops in the presence of inadequate dietary intake relative to total energy expenditure [12].

The environment of competitive sports is a unique context with considerable pressure to achieve successful performance. Female athletes may consciously or inadvertently under eat and/or participate in excessive volumes of training in an attempt to achieve an ideal body composition for performance [2]. Such practices result in initiation of energy conservation mechanisms and an acute effect on energy expenditure including: (1) suppression of REE [20, 31], (2) reduced total triiodothyronine (TT3) [20, 32-34], insulin like growth factor-1 (IGF-1)/IGF binding protein-1 [35], leptin [36], an insulin concentrations [36], (3) elevated cortisol [35, 37, 38], growth hormone [35] and ghrelin concentrations [16]. Additional signs of low EA in athletes include a body mass index (BMI) $<17.5 \text{ kg/m}^2$, weight loss, and a ratio of measured REE:predicted REE (pREE) less than 0.90 [11]. The magnitude of daily energy deficit is a greater predictor of chronic energy deficiency than a loss in body mass, therefore an individual may not be losing weight but could still be in an energy deficit [32]. Less critical physiological processes, such as growth and reproductive function [39], may additionally be compromised to maintain energetic partitioning to vital physiological processes, such as thermoregulation and cellular maintenance, and result in serious clinical consequences.

It is evident, through investigations of links between an energy deficiency and disruptions in luteinizing hormone (LH) pulsatility [40] and menstrual cycles [24], that the induction of menstrual disturbances is an unfavorable outcome of the energy deficiency paradigm. Menstrual disturbances occur across a continuum from subclinical (luteal phase defects and anovulation) to severe, clinical (amenorrhea and oligomenorrhea) perturbations in female athletes [41]. The induction of menstrual dysfunction has been postulated to be due to an energy deficiency, thus the resumption of menses should hypothetically occur when an adequate energy status is restored. The suppressed estrogen exposure associated with both subclinical and clinical

menstrual disturbances in female athletes promotes pathological bone loss [29] and other clinical sequelae, such as endothelial dysfunction [42, 43].

2.3 Female Athlete Triad: Physically active women are prone to developing the Triad. The presence of the Triad and related clinical outcomes are well documented in female athletes, notably female long-distance runners. The three components of the Triad include (1) low EA with or without disordered eating, (2) menstrual dysfunction, and (3) low BMD [11, 29].

Disordered eating and inadequate food intake is one component of the triad that involves caloric restraint often due to diet and weight concerns. Menstrual dysfunctions are a second component and dysfunction ranges on a continuum from luteal phase defect cycles to the most severe, amenorrhea. Low bone mass, the third component, involves bone loss, osteopenia, or osteoporosis [12]. Female distance runners are susceptible to all three components of the Triad. Whether an athlete experiences one component or a combination of the three requires medical attention [12].

Previous reports indicate up to 70% of elite female athletes present with clinical eating disorders and/or disordered eating behavior, particularly those athletes participating in sports focused on leanness, aesthetic appearance, and weight control [10]. The prevalence of menstrual disturbances is high among female long-distance runners, ranging from 3-38% in cross-country runners [7, 44-51] and 6-19% in marathon runners [52, 53]. With regard to bone health, osteopenia is observed with a prevalence of 1-50% in amenorrheic runners [44, 54, 55], with the incidence of osteoporosis ranging from 0-33% [44, 47, 54, 55]. The prevalence of low EA in female long-distance runners is not well documented and further investigation will lend valuable insight.

Pollock et al. [47] published the only report to date on the prevalence of all three Triad conditions occurring simultaneously in female long-distance runners. The investigators found that menstrual dysfunction, disordered eating, and low BMD were coexistent in 15.9% of the female athletes [47]. The findings reported by Pollock et al. [47] are notably higher compared to data on the prevalence of the Triad in samples of other athletic groups (mean: 1.3%) [42, 56-62]. Several studies have investigated the associations between combinations of two Triad components in female long-distance runners: bone health and menstrual function [25, 47, 49, 63], eating behavior and menstrual function [7, 44, 47], and eating behavior and bone health [7, 44, 47]. Further examination of these specific combinations of Triad conditions and all three Triad conditions occurring simultaneously in female long-distance runners would provide a more accurate depiction of the scope of the condition and implications of the Triad in this specific athletic population.

2.3A Relationship between Energy Availability, Metabolism, and Reproductive Function

The conceptual model of the Triad defines low EA (with or without disordered eating) as an etiological factor in the development of the Triad in female athletes [11, 29]. Energy availability is defined as dietary EI minus exercise energy expenditure (EEE) adjusted for lean body mass (LBM) measured in kilograms ($EA = EI - EEE/kg \text{ LBM}$) [30]. Energy balance, on the other hand, is a measure of energy status commonly used by clinicians and dietitians to indicate whether an individual is energy replete or in an energy surplus or deficit. Energy balance is defined as the amount of dietary EI minus total energy expenditure (TEE) ($EB = EI - TEE$) [30]. The amount of energy available after all the necessary physiological processes of the body have taken place is the EB [30]. It has been suggested that the calculation of EA is superior to any calculation of 24 hour EB due to the measurement of only one component of 24 hour energy

expenditure (EE) vs. measuring all the components of 24 hour EE (i.e., thermogenesis of non-exercise activity and the thermic effect of food) [30]. EA was used in the Triad model because it focuses specifically on the energy expended during exercise, as opposed to EB [29].

Nonetheless, estimating ones' individual energy requirements remains a challenge. The effect of EA on performance has yet to be performed. The EA threshold of 30 kcal/kg LBM [22] has not been examined as a cut off below which negative effects are translated to markers of performance at the whole body, i.e., running velocity or efficiency, or cellular level, i.e., muscle protein turnover, lactate removal, or muscle glycogen content.

Preservation of energy stores for the most vital physiologic processes, such as thermoregulation, cellular maintenance, and locomotion, with low EA is a result of a suppressive effect on both metabolic and reproductive function [39]. Evidence suggesting EA is a relevant factor affecting metabolism and reproduction is provided from short-term studies in sedentary regularly menstruating women [22, 33, 34, 40] and longitudinal experiments in female cynomolgus monkeys [23, 24]. The suppressive effects of low EA on fasting TT3 [34], LH [22, 40], and menstrual cyclicity [24] are well elucidated in the literature.

Using TT3 as the proposed marker of metabolic status, Loucks et al. [33, 34] examined the effect of manipulating EA, though exercise and dietary intake, on metabolism in healthy, young, regularly menstruating, sedentary women. A suppression of TT3 was observed in these participants as a result of an exercise induced energy deficit, which was prevented with appropriate increases in dietary EI [33]. The effects of an exercise induced energy deficit on TT3 were independent of the exercise intensity rather it was the energy cost of exercise on EA that impacted TT3 concentrations. In a follow up investigation, Loucks et al. [34] revealed that reductions in TT3 were induced abruptly between measured EA of 19 and 25 kcal/kg LBM.

Subsequently, these researchers became interested in the effect of the EA manipulations being used in their metabolism research on reproductive function, specifically LH pulsatility. It was known that disruptions in LH pulsatility occurred and presumably translated to exercise associated amenorrhea, however it was unclear whether exercise stress was the mechanism by which the disruptions occurred. Loucks et al. [40] disproved the hypothesis that LH pulsatility was disrupted by exercise stress but proposed that changes in EA induced concomitant changes in LH pulsatility. This subsequent hypothesis was then investigated in 29 regularly menstruating, sedentary women [22]. The results of the investigation of the dependence of LH pulsatility on varying EA conditions (balanced, 45 kcal/kg LBM vs restricted, 30, 20, and 10 kcal/kg LBM) demonstrated that LH pulsatility is disrupted below an EA threshold of 30 kcal/kg LBM [22]. The disruption is observed as a decreased LH pulse frequency and an increase in the amplitude of the LH pulse [22].

The role of low EA on the development and reversal of exercise-induced amenorrhea was evaluated with prospective experiments on female cynomolgus monkeys [23, 24]. In these two prospective studies the researchers gradually increased the daily exercise (12 km/day over a 7 to 24 month period) in eight adult female monkeys without any changes in food intake. This research protocol resulted in the development of amenorrhea in the eight adult female monkeys [23]. The monkeys were observed to have increased menstrual cycle lengths and a 28% decrease in TT3 concentrations, despite a lack of significant weight loss [23, 24]. Four of the eight amenorrheic monkeys were provided the appropriate dietary energy to reverse the exercise induced energy deficit (138-181% of EI) without changes to the daily exercise routine. Initially, all four monkeys presented with increases in their reproductive hormone concentrations (LH, FSH, estradiol, and progesterone), with the eventual restoration of ovulatory cycles in less than

one year [24]. The findings of Williams et al. [23, 24] support the premise that metabolic and reproductive adaptations occur as an energy conservation mechanism, without significant changes in body weight. Additionally, increases in EA may appropriately compensate for the underlying energy deficit associated with amenorrhea in exercising monkeys and restore regular menstrual function.

2.3B Low Energy Availability in Female Long-Distance Runners

The reasons for the reduced EA observed in female long-distance runners and other female athletes include: 1) intentional, i.e., to modify body size and composition to improve performance; 2) compulsive, i.e., the result of pathological weight control or disordered eating behavior; or 3) inadvertent, i.e., failing to match EI to EEE [30]. Intentional restrictive eating behaviors are often adopted by female long-distance runners as a means to maintain or reduce their body weight or fat mass to achieve an optimal distance running performance. Female distance-runners are highly susceptible to the induction of an energy deficit by failure to compensate for increases in their energy expending activities. Despite the high volume of energy expenditure, runners may not appropriately increase their EI compared to control groups [64-66]. The prevalence of low EA has been reported to be 6% in female high school athletes [58] and ranges from 12-33% in female college athletes [67].

Numerous reports of low EI, ranging from 1,272-2,937 kcal/d, in female long-distance runners exist in the literature [20, 64-66, 68, 69]. When athletes are categorized by menstrual status (eumenorrheic vs. amenorrheic), the eumenorrheic athletes demonstrate a higher EI compared to their amenorrheic counterparts (1,690-2,937 kcal/d vs. 1,272-1,950 kcal/day) [64, 66, 70, 71]. In 1993, Edwards et al. [68] reported a 32% greater TEE measured by doubly-labeled water compared to EI measured by diet logs. Similarly, in 1995, Beidleman et al. [64]

demonstrated self-reported EI was lower than energy expenditure in female runners by a larger deficit (-987 kcal) compared with controls (-395 kcal). The observed reductions in EI are often linked to abnormal eating attitudes/behaviors and disturbed body image [20, 68]. It is also suggested that findings with respect to self-reported EI and energy expenditure are difficult to interpret.

Energy availability has been reported by or data was provided from which EA could be calculated in five investigations of female long-distance runners [20, 64, 66, 71, 72]. In these investigations EA values ranged from 19-50 kcal/kg LBM. While in two studies in which EB was determined, the female long-distance runners presented in a negative EB [64, 65]. In 2007, Loucks [13] reviewed 25 studies of eumenorrheic female runners and 12 studies of amenorrheic female runners. Energy availability was reported to range from 12-29 kcal/kg FFM in these studies [13]. An EA threshold of 30 kcal/kg FFM has been reported to be a discriminating factor between women that are energy replete and regularly menstruating (greater or equal to 30 kcal/kg FFM) and women with energy conservation and reproductive dysfunction (below 30 kcal/kg FFM). Future research is necessary to demonstrate the utility of the EA threshold in a field setting and on measures of performance.

2.4 The Relationship between Energy Status and Resting Energy Expenditure: Resting energy expenditure is a major determinant of total daily energy expenditure and is the total amount of calories the body requires at rest just to maintain basic bodily functions [20, 30]. The body has to expend energy in the form of calories for cellular maintenance, thermoregulation, growth, reproduction, immunity, and locomotion [30]. The Harris-Benedict equation is commonly used to predict REE and in addition assists in determining if an individual is in an

energy deficit or is energy replete. The equation takes into account the age, sex, body weight, and height of the individual to predict the amount of heat production in 24 hours per square meter of body surface [73]. The Harris-Benedict equation for women is heat production/24 hours = $655.0955 + 9.5634 * \text{weight} + 1.8496 * \text{stature} - 4.6756 * \text{age}$ [73]. In the equation, heat production is calculated as kJ/day, weight is measured in kg, stature measured in cm, and age reported in years [73].

In most studies, REE is measured in subjects by having them rest supine for a minimum of thirty minutes in the early morning following a 12 hour fast, light or no exercise the previous day, and no caffeine for 24 hours. Then, using a ventilated hood, indirect calorimetry is conducted using a metabolic cart to measure the volume of oxygen consumed (VO_2) and volume of carbon dioxide produced (VCO_2) for a minimum of 30 minutes [74]. REE is calculated using the Weir Equation, which takes into account VCO_2 and VO_2 . The Weir Equation is $\text{kcal/day} = (3.94 * \text{VO}_2 + 1.11 * \text{VCO}_2) * 1.44$ [75], which is subsequently converted to kJ.

A suppressed REE is an energetic adaptation to an energy deficit [12, 17, 20, 32, 76]. When an energy deficit occurs the body does not have adequate calories to fulfill the requirements for cellular maintenance, thermoregulation, growth, reproduction, immunity, and locomotion and shuts off the “negotiable” areas such as growth and reproduction. Comparisons of laboratory-assessed REE with the pREE are an estimate of how much each individual’s measured REE deviates from the Harris-Benedict pREE. In anorexic women, during periods of low body weight, prior to the initiation of refeeding, a reduced REE:pREE of 0.60-0.80 is often reported [77-79]. In addition, a low REE has been associated with reproductive suppression in exercising women who maintain a normal weight [20, 31, 80]. In female athletes a suppressed metabolism is operationally defined as a REE:pREE less than 0.90 [11, 17, 31, 32]. The

operational definition of a 0.90 REE:pREE has been used by investigators whom show an amenorrheic runners have a significantly lower REE compared to eumenorrheic runners and sedentary controls [20, 76]

Previous reports in exercising women with functional hypothalamic amenorrhea demonstrate an association between a suppression of REE and a chronic energy deficiency. In 2007, De Souza et al. reported decreased REE controlled for fat free mass (FFM) and serum TT3 in exercising women with amenorrhea compared to those with ovulatory cycles whether sedentary or exercising. Similarly, exercising women with subclinical menstrual disturbances (consistent or inconsistent) also presented with lower REE/kg FFM and TT3 compared to sedentary ovulatory women [32]. Further, a dose response relationship exists between reductions in REE and TT3, such that increases in energy conservation (a reduction in REE and TT3) occur concomitantly with increases in the severity of observed menstrual dysfunction. Despite similar REE among groups, Myerson et al (1991) similarly found a lower REE in amenorrheic runners compared to their eumenorrheic, sedentary control counterparts. As such, an EB and stable body weight may be maintained in amenorrheic female runners by way of decreases in REE and other accompanying energy conservation responses (i.e., alterations in metabolic hormones) in spite of a significantly lower EI than eumenorrheic runners.

2.5 The Relationship between Energy Status and Metabolic Hormones: A suppressed REE is notably associated with energy deficiency [81] and is often associated with changes in metabolic hormones such as TT3, ghrelin, and peptide YY (PYY). Specifically, an energy deficit translates to a cascade of adaptations, including reduction in REE, and simultaneous alterations in fasting circulating hormone concentrations to restore homeostasis [31, 32]. Triiodothyronine is the most

active form of thyroid hormone and it plays a significant role in the precise control of several physiologic functions, such as growth, metabolism, core temperature, and heart rate [82]. Ghrelin is a hormone secreted in the stomach and gastrointestinal tract from X/A-like cells [83, 84] and is an orexigenic hormone involved with stimulation of food intake [85, 86]. Peptide YY is a hormone secreted from L cells in the gut, where it acts as an anorexigenic hormone involved in slow digestion and increasing time for absorption of nutrients in the ileum [87].

2.5A Triiodothyronine: TT3 is the most active form of thyroid hormone and it has a significant role in regulating cellular metabolism and energy expenditure [8]. The tight relationship between TT3 and metabolism is based on the strong relationship between TT3 and REE, VO_2 , and TEE [88]. Human and animal experiments support the direct and indirect effects of energy and macronutrient intake on thyroid hormone status and REE [89-91]. Thus, TT3 is a key marker of adaptations to energy deficiency and exercise training. A reduction in the circulating TT3 concentration is inferred as a governing metabolic signal that may initiate further energy conservation mechanisms to restore a eumetabolic state [88]. In female anorexia nervosa patients suppressed plasma TT3 concomitant with decreased REE [92]. Weight gain in these anorexic patients resulted in increases in both TT3 concentrations and REE, independent of gains in fat free mass (FFM) [92]. Manipulation of EA for 4-5 days in sedentary, regularly menstruating women demonstrated that low-TT3 syndrome was induced within four days of severe EA restriction [33, 34]. Of note, only partial dietary compensation for EEE may be necessary to prevent reductions in TT3 concentrations [33, 34]. The mechanism for the development of low TT3 may be explained by the energetic cost of exercise, not the stress of exercise, as Loucks et al. [33] prevented reductions in TT3 by increasing dietary EI to match EEE. In the presence of an energy deficit in amenorrheic athletes suppressed TT3 concentrations

are consistently observed [8, 20, 70]. Thus, TT3 has been implicated as a hormone associated with chronic energy deficiency, which is sufficient to cause reproductive dysfunction in exercising women.

2.5B Ghrelin: Ghrelin a hormone released from enteroendocrine cells of the stomach and gastrointestinal tract to stimulate appetite [21]. Ghrelin concentrations rise prior to a meal then fall within 1-2 hours after a meal [85], as well as increase during periods of fasting [86]. Ghrelin is a well-known metabolic signal that is indicative of an energy deficit and is a contributor to the restoration of energy homeostasis [93]. In normal weight young women, ghrelin concentrations have been shown to be responsive to decreases in body weight, fat mass, and REE [19]. There is substantial evidence documenting elevated fasting concentrations of ghrelin in anorexic and amenorrheic women [17, 19, 94-98]. De Souza et al. [16] demonstrated that amenorrheic female athletes exhibit a minimum of an 85% increase in fasting ghrelin concentrations compared to control groups. Despite the elevated fasting ghrelin, amenorrheic female athletes display low EI and a metabolic profile consistent with a chronic energy deficit.

2.5C Peptide YY: Peptide YY is a peptide hormone secreted from L-cells in intestinal mucosa and that suppresses gastric emptying by regulating the release of neuropeptide Y and agouti-related protein in the arcuate nucleus of the hypothalamus [85, 99, 100]. Peak concentrations of PYY are reached 1-2 hours after a meal aiding in meal cessation [101], and thus plays a role in regulating energy homeostasis. Past investigators have found that PYY concentration is effected by the mode, intensity, and duration of exercise [18]. In amenorrheic exercising women [102] and anorexic women [99, 103, 104] fasting PYY is elevated, along with ghrelin, compared to healthy controls. Peptide YY and ghrelin were found to both be elevated following 2-2.5 hours of running [18]. Scheid et al. [102] demonstrated PYY concentrations

were inversely correlated with REE/kg FFM and REE:pREE in amenorrheic exercising women. The disruption of the natural inverse relationship between PYY and ghrelin may be critical in the development of energy deficiency. A chronic elevation of fasting PYY may represent a physiological mechanism that is supportive of energy restriction in exercising women despite the elevation in fasting ghrelin concentrations [104]. Infusions of both ghrelin and PYY have been demonstrated to suppress LH secretions and suppress GnRH secretion, respectively, in animal models [105-108]. Thus, it is hypothesized that the abnormal fasting concentrations of the gut hormones, ghrelin and PYY, may play a role in the etiology of the disordered energy homeostasis and reproductive dysfunction in the population of amenorrheic exercising athletes.

2.6 Relationship between Energy Status and Reproductive Health: Low EA not only has a suppressive effect on metabolic function but additionally causes reproductive dysfunction in the form of menstrual disturbances [2, 24, 32]. Low EA and reproductive dysfunction is often observed in female endurance athletes [2]. Other factors known to influence athletic menstrual dysfunction include percent body fat, energy drain, quality and quantity of diet, irregular nutritional patterns, chronological age, and gynecological age [20].

Menstrual disturbances range on a continuum from less severe (subclinical) to most severe (clinical) and include luteal phase defects, anovulation, oligomenorrhea, and amenorrhea, respectively [12]. Normal, ovulatory menstrual cycles, in exercising women range in length from 26-32 days [12]. Cycles with luteal phase defects are of a regular length and are associated with reduced progesterone during the luteal phase [12]. This can be caused by ovulating later than the typical 12-14 day ovulatory cycle or the corpus luteum is not producing an adequate amount of progesterone [12]. Anovulatory cycles are of a regular length; however, ovulation does not occur

and the cycle is associated with suppressed estrogen and progesterone levels [12].

Oligomenorrhea is defined as irregular menstrual cycles ranging in length from 36 to 90 days [29]. Amenorrhea is the most severe menstrual disturbance and is defined as the absence of menstrual cycles for a minimum of three consecutive months [12]. Primary amenorrhea is defined as the failure for a young woman to menstruate by age 15 [12, 109]. Delayed menarche is defined as the onset of menses at age 14 or older [110]. Secondary amenorrhea occurs in women who have undergone menarche, but have had no menses for at least three consecutive months some duration of time following menarche [12]. Secondary amenorrhea in female athletes is reported in 1-44% compared to 2-5% in sedentary women [2, 20, 23].

Amenorrhea is often seen in athletes competing in aesthetic sports such as such as distance runners, gymnasts, and ballet dancers [15, 23]. Amenorrhea is associated with hypoestrogenism which is linked to other medical disturbances such as stress fractures, premature cardiovascular disease [11, 12, 24, 111-114]. Menstrual irregularities can be reversed provided supplemental EI is consumed to balance EEE. If regular menstrual cycles are maintained thereafter bone health and fertility can potentially be restored [24].

2.7 Relationship between Energy Status and Skeletal Health: Weight bearing exercise activity exposes bone to loading that increases internal forces of stress and deformation [111]. In healthy persons, the increased stress increases the strength of the bone; however in the presence of low EA the stress can accumulate into larger microcracks, which can further develop into a bone stress injury [111]. Sustainment of low EA over a period of weeks to months has been shown to cause suppression of hormones (leptin, estradiol, IGF-1) that cause bone formation and increase secretion of hormones (cortisol) that cause bone resorption [11, 17, 115-118]. The

alteration in hormone concentrations causes an imbalance in bone turnover, a lowered capacity to form new bone [116] and impaired ability to repair microdamage caused by the stress of weight bearing activity [119].

Low EA can lead to a lack of essential bone building micronutrients such as calcium, vitamin D, vitamin K, vitamin C, magnesium, and iron [120, 121]. Energy restriction at a young age can prevent young women from reaching their peak bone mass [116]. A BMD z-score of less than or equal to -2.0 is defined as being below the expected range for the patients' chronological age [11]. Athletes with low BMD, i.e., z-score of < -1.0 , are at a greater risk for stress fractures [11].

Running is a weight bearing sport that can increase bone mineral density at specific regions of greatest mechanical stress; however, prolonged amenorrhea in athletes is associated with a reduction in bone mineral density particularly in the lumbar spine [118, 122]. Female runners with chronic amenorrhea for greater than four years have reduced bone turnover and reduced bone formation when compared to eumenorrheic runners [123]. Bone stress injuries are most commonly observed in the lower extremity areas of the tibia and metatarsals, but have also been reported in the fibula, navicular, sesamoid, sacrum, femur, and ribs [124-126]. The incidence of bone stress injuries reported by investigators with retrospective studies among female endurance runners and track athletes ranged from 8.3-52.0% [26, 124, 127]. In prospective studies of female runners and military recruits the incidence of bone stress injuries ranges from 3.3-28.9% [128-130]. The incidence of osteopenia in amenorrheic runners has been reported to range from 1-50% [54, 55, 129], while the incidence of osteoporosis has been reported to be slightly lower, ranging from 0-33% [44, 47, 54, 55].

Recently Barrack et al. [111] conducted a prospective study evaluating the risk of stress fracture development in association with the presence of triad risk factors. The researchers evaluated 12 Triad-risk factors and found 6 to be the most significant [111]. The six most significant Triad-risk factors associated with bone stress injury were: greater or equal to 12 hours of exercise per week, BMI lower than 21.0 kg/m², oligo-/amenorrhea, high dietary restraint, leanness sport participation, and low BMD (z-score less than -1.0 at the lumbar spine or total body) [111]. Barrack et al. [111] reported a dose-response relationship between the number of Triad-related risk factors reported by participants and the development of a bone stress injury. Girls presenting with 1 significant Triad risk factor had an increased risk of bone stress injury of 15-21%, while the participants with 2 or 3 concurrent risk factors had an increased risk of 21-30% and 29-50%, respectively. The ability for clinicians to quantify injury risk may prove useful in making decisions regarding clearance and return to play among athletes [111].

2.8 The Relationship between Energy Status and Performance: Execution of a sport in a competitive environment is the operational definition of performance [5]. In a research setting the execution of performance is indirectly evaluated through physiologic factors associated with performance in athletes, such as muscle mass and/or fiber composition, blood lactate during exercise and at maximal exercise, and VO₂ during submaximal or maximal exercise. In addition to the energetic factors other factors such as self-motivation, social support, the relationship between athlete and coach and other intrapersonal and interpersonal skill development may impact the performance of female long-distance runners [131]. The psychological stress of competition can have direct and indirect impacts on athletic performance [5, 132, 133]. In a strictly research setting it is a challenge to control the internal and external factors the impact

athletic performance, therefore evaluation of athletes in a specific competitive event wherein all the factors have the potential to predominate. To date, an investigation that evaluates the effect of energy status in performance outcomes (i.e., time trial finishing time or running velocity) in female long-distance runners has yet to be conducted.

The primary goal of competitive long-distance running participation is the achievement of the fastest finishing race time. To achieve this goal female long-distance runners work to achieve a body size and composition that minimizes the resistance of movement against gravity (i.e., a higher muscle:fat ratio) and optimization of energy mobilization from several fuel sources (i.e., muscle and liver glycogen and adipose triglycerides) [2]. For continued performance at an elite-level a proper EA level is suggested to be advantageous in training and competition. Performance may be negatively impacted by chronically low EA, especially in conjunction with drastic weight loss and disruption of endocrine function [134]. The training effects specific to strength and endurance performance are negated by losses of lean body mass and may translate to detrimental effects on immune, endocrine, and musculoskeletal function [135].

Low EA, in particular low carbohydrate consumption, and not the volume of exercise can lead to reproductive disorders such as amenorrhea [2]. Athletes who are amenorrheic have an increased risk of stress fractures compared to those who are eumenorrheic. It is important for female distance runners to stay energy replete in order to reduce their risk of stress fractures [26, 70]. Stress fractures can stop the athletes training and prevent them from running for weeks to months.

Weight loss and energy deficits can impact muscle function. Researchers have shown that decrements in physical performance are associated with losses of FFM [136]. For example, Nindl et al. [136] demonstrated significant decreases in FFM, strength and power-related

performance outcomes in young, lean, and healthy men following an 8-week Army course with a 1000kcal/day caloric deficit. At 5 weeks post course, measures of participants FFM and physical performance returned to initial values. Another example is the work of Roemmich et al. [14] who demonstrated that weight loss in male wrestlers secondary to a caloric restriction associated energy deficiency led to a reduction in muscular strength and power. Studies of energy restriction in women and men suggest that a moderate energy deficit may not negatively impact exercise performance [137, 138]. Zachwieja et al. [138] demonstrated an improved 5 mile time trial time in physically active men and women despite a two week reduction in carbohydrate (5.2 vs 3.7 g/kg/day) and energy (-750 kcal/day) intake. The change in performance was not different compared to the control group [138]. Recently, Van Heest et al. [139] reported a high correlation between sport performance and ovarian steroids (estradiol and progesterone), metabolic hormones (TT3 and IGF-1) and markers of energy status (EA and EI) over a 12-week competitive season in junior elite female swimmers. Sport performance decreased by 9.8% in the ovarian suppressed group, whereas normally menstruating athletes had an 8.2% improvement in performance [139]. Van Heest et al. [139] are the first to demonstrate that athletes in an energy deficit in conjunction with reproductive dysfunction translated into poor sport performance and that the sport performance worsens with a continued energy deficit. In a case study by Dueck et al. [1] to determine the effect of a 15-week diet and exercise intervention program on energy status, hormonal profiles, body composition, and menstrual function in an amenorrheic endurance athlete demonstrated a transition from a negative to positive energy balance. The athlete increased weight and body fat percentage, increased fasting LH and decreased fasting cortisol [1]. The participant continued the program for an additional 3 months and resumed menstruation [1]. From the athlete's point of view the improvement in energy balance increased

her performance ability [1] and points to the importance of energetic status in optimal athletic performance. Despite the evidence that an energy deficit can be detrimental to the health of a distance runner, there is a need to show the effects of an energy deficit on performance factors, such as running time and velocity, in female distance runners across a competitive season.

2.9 Conclusions: The engagement in dietary energy manipulations subsequent to the development of a chronic energy deficit is often observed in female athletes. Athletes participating in leanness-focused sports typically present with a chronic energy deficit. A chronic energy deficit is a key factor in the development of the Triad, an interrelated syndrome of low EA with or without disordered eating, menstrual dysfunction, and low BMD, presenting alone or in combination [11, 29]. Female distance runners are particularly susceptible to development of the medical conditions associated with the Triad [10, 47]; however, the prevalence of low EA/chronic energy deficiency in female long-distance runners is not well established. The definition of energy status in the literature to date has been varied and thus, it is not clear which energetic factor has the greatest impact on Triad related clinical and sport performance related outcomes. The development of a chronic energy deficit in women has been associated with negative effects on reproductive health, skeletal health, and performance [24, 40, 65].

The measurement of competitive performance in a research setting is notably challenging. Controlling for several factors including intrapersonal and interpersonal skills, psychological stress, social support, the athlete coach relationship, and other environmental factors. Acute and chronic energy deficiencies have been shown to have negative consequences on health outcomes and physical performance outcomes in male wrestlers and army rangers and female junior elite swimmers. To our knowledge, investigations examining the association

between energy status and performance in long-distance runners have yet to be conducted.

Metabolic hormone markers of energy status, such as TT3 and ghrelin, serve as markers of adaptations to energy deficiency and exercise training. Characterization of changes in energetic factors (i.e. REE, TT3, ghrelin) across a competitive season in female long-distance runners and determination of the effect of energy status on performance outcomes measured under competitive conditions requires continued research.

2.10 References

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Chapter 3

Methods

3.1 Experimental Design: This study was a prospective investigation examining energetic, metabolic, and performance characteristics in 21 premenopausal female long-distance runners categorized by energy status following a competitive season. Participants were assessed pre- and post-competitive running season (10-12 weeks). Participants qualified for the study if they were currently participating in competitive distance running on a team. Resting energy expenditure (REE) was measured using indirect calorimetry and a ventilated hood system. Metabolic hormone concentrations were determined from a fasting blood sample assayed for total triiodothyronine (TT3). Menstrual status was confirmed by self-report and menstrual history evaluation. Body composition was determined using dual x-ray absorptiometry (DXA). The research staff measured test race time via a competitive 5km time trial on an accurately measured outdoor course. A maximal oxygen uptake (VO_{2max}) test was completed on a treadmill using indirect calorimetry and a modified Astrand protocol. Energy availability (EA) was calculated by measuring energy intake (EI) via three-day nutritional logs, exercise energy expenditure (EEE) using the Ainsworth Compendium [1], and lean body mass obtained from DXA.

3.2 Study Participants: Eligibility criteria for this study included: (1) age 17-25 years; (2) good health as determined by medical exam; (3) active participation in competitive distance running on a team; (4) non-smoker; (5) not pregnant and/or lactating; and (6) no other reasons that would preclude participation (i.e., injury, academic ineligibility). The Institutional Research Board

approved this study and all participants signed an informed consent document if ≥ 18 yr or a parental guardian signed the informed consent if the participant was 17 yr.

3.3 Study Time Period: The study involved up to 4 visits to our laboratory in the pre- and post-season to complete protocol measurements. Participants completed a general information visit wherein study details and protocol requirements were explained and informed consent was obtained. Height and weight were then measured and participants completed the Health, Exercise and Nutrition Survey that included information on demographics, medical, exercise, menstrual, bone health, and nutrition history. Additional pre-season measurements included an REE test, a fasting blood sample, a body composition and bone density DXA scan, and a VO_{2max} test. A competitive 5km time trial was completed to determine test race time and running velocity. Participants were also given logs to continuously record their training and menstrual characteristics on a daily basis across the season.

3.4 Anthropometric and Body Composition Data: Total body mass was measured to the nearest 0.1 kg on at least 1 occasion (each measurement within a 2-4 week period) during the pre- and post-season time periods. The mean of these measurements was presented for each time period during the study. Participants were weighed in shorts and a t-shirt. Height was measured to the nearest 1.0 cm. Body mass index (BMI) was calculated as the average body mass divided by height squared (kg/m^2). Body composition was assessed using DXA during the pre- and post-season. Subjects were scanned on a GE Lunar iDXA (enCORE 2008 software version 12.10.113). The division of soft tissue into fat (g) and lean tissue (g) was based on an attenuation

ratio of high- and low-energy photons or R-value. All women were required to provide a urine sample prior to completion of the DXA and a pregnancy test was performed.

3.5 Resting Energy Expenditure: REE was measured during the pre- and post-season. REE was determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA). Room temperature ($^{\circ}\text{C}$), humidity (% H_2O), and barometric pressure (mmHg) were measured. Participants were instructed not to exercise/only participate in light training or ingest caffeine within 24h, refrain from ingesting food and alcohol within 12h prior to testing, and arrive at the lab within 90 min after awakening. Before conducting the REE analysis, weight (kg), height (cm) and age (yr) were recorded, and predicted REE (pREE; kcal/day) was calculated using the Harris-Benedict equation [2] $655.0955 + 9.5634*\text{weight} + 1.8496*\text{stature} - 4.6756*\text{age}$. REE measurements were performed between 0830 and 1100 h in a lit room at a comfortable temperature setting (20- 24 $^{\circ}\text{C}$). After the volunteers lay quietly for 30-45 min, a transparent canopy was placed over their head. Volunteers were instructed to lie flat on their back and remain awake during the 30-min measurement period. Oxygen consumption (VO_2 ; mL/min) and carbon dioxide production (VCO_2 ; mL/min) were measured every 20s throughout the test. To calculate REE, data for VO_2 and VCO_2 were only used if steady state was attained. Steady state was achieved when the volume of expired air, VO_2 , and respiratory quotient values were not varying by more than 10%. REE was calculated using the Weir equation [3]: $\text{REE (kcal/ day)} = (3.9*\text{VO}_2 + 1.1*\text{VCO}_2)*1.44$. A ratio of the actual REE to pREE (REE:pREE) was calculated once during each monitoring period using the Harris-Benedict equation [2].

3.6 Energy Status: Energy status was defined using an objective laboratory-based measure, REE, to identify individuals who exhibit energetic adaptation to an energy deficiency [4-7]. We expressed REE as a ratio of actual to pREE based on the predicted calculated using the Harris-Benedict equation [2] to estimate how much each individual's actual REE deviated from the pREE. Reductions in REE have been observed in several studies in exercising women with amenorrhea [4, 5]. In anorexia nervosa, most data published compared actual REE to the Harris-Benedict pREE [2]. In anorexia nervosa, investigators compare actual REE to the Harris-Benedict pREE [2] and have reported a suppressed REE:pREE of 0.60-0.80 during period of low body weight and before refeeding in anorexic women [8-10]. Comparing the measured values to the predicted values is ultimately indicative of whether an energy replete or energy deficient status is present in the runners. An REE:pREE score of greater than or equal to 0.90 is typically considered energy replete, whereas an REE:pREE score of less than 0.9 is often considered energy deficient [4-6]. As such we categorized our participants according to tertiles of REE:pREE to appropriately capture whether a dose-response relationship exists with respect to energy status and our primary outcome variables: severe energy deficiency (ED) ($REE:pREE < 0.84$), moderate ED ($0.84 \leq REE:pREE < 0.90$), and energy replete ($REE:pREE \geq 0.90$).

3.7 Blood sampling and storage: Blood samples were collected between 0730 and 1000h on a single occasion during the study, stored and processed as previously described [4]. After allowing clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for fifteen minutes at 4°C. Serum samples were aliquoted into 2mL polyethylene storage tubes and stored in a freezer at -80° C.

3.8 Serum Hormone Measurements: TT3 concentrations were analyzed as previously described [4, 11, 12]. TT3 was analyzed using a chemiluminescence-based immuno assay analyzer. The analytical sensitivity for the TT3 assay was 0.54 nmol/L, and the intra-assay and inter-assay coefficients of variation were 13.2% and 15.6%, respectively.

3.9 Dietary Energy Intake: Dietary EI (kcal/day) was assessed using three-day diet logs recorded for two week days and one weekend day, as previously described [4, 6]. These three-day nutritional logs recording food intake have previously been demonstrated to provide comparable data to seven-day records in women who may underreport their food intake, including lean women [13]. Participants were recommended to weigh (ECKO Kitchen Scale, World Kitchen, LLC, Rosemont, IL, USA) or measure (using standard cups/tools) all food and beverages consumed in detail, as well as record the time and location of every eating episode. Study personnel instructed the participants on how to accurately record EI. The nutrient data from the three-day logs were coded and analyzed using Nutritionist Pro Diet Analysis software v.4.5 (Axxya Systems, Stafford, TX).

3.10 Exercise Energy Expenditure: Participants kept logs of their purposeful exercise training for seven-day occasions throughout the entire duration of the study. These logs provided a measurement of exercise training volume (min/wk) and the information necessary for calculation of EA. The mean of weekly exercising training volume was presented for the pre- and post-season time periods. EEE was assessed using seven-day exercise logs where all purposeful exercise sessions were recorded. Activities included on the sheet include only purposeful exercise during training sessions, but not daily living activities such as house cleaning or walking

a dog. Energy expended during the purposeful exercise sessions was measured using the Ainsworth et al. [1] compendium of physical activities to determine the appropriate metabolic equivalent (MET) level for the exercise performed, which was multiplied by the duration (min) of the exercise session. The MET value additionally includes a resting component. To take only REE into consideration by estimating REE individually, REE (kcal/min) was therefore subtracted from the EEE values. The same member of the research team determined the MET levels from exercise logs.

3.11 Energy Availability: EA (kcal/kg lean body mass (LBM)) was determined as EI minus REE relative to kg LBM [$EA = (EI - REE)/LBM$ (kg)] [14, 15] using dietary EI data assessed from three-day diet logs, REE assessed from exercise training logs, and lean body mass obtained using DXA during the pre- and post-season. These measurements were all determined within the same seven-day period.

3.12 Competitive Time Trial: A competitive 5km time trial was completed on an outdoor course accurately measured by the research staff wherein test race time (min) was determined as an indicator of performance. Running velocity (miles/ hour) was calculated by converting both test race time (min) and distance (km) into the desired units using the website:

<http://www.1728.com/velocity.htm>.

3.13 Maximal Oxygen Consumption: Measurement of VO_{2max} was performed during the pre- and post-season on a treadmill using indirect calorimetry and the modified Astrand protocol [16]. Gas exchanged was monitored continuously by a breath-by-breath system (SensorMedics Vmax

metabolic cart; Yorba Linda, CA). We considered VO_{2max} to be achieved if three of the following four criteria were obtained: (1) attainment of age-predicted maximal heart rate ($208 - (0.7 * age)$); (2) respiratory exchange ratio ≥ 1.1 ; (3) plateau in oxygen consumption despite an increase in exercise workload; (4) attainment of a rating of perceived exercise score ≥ 18 [17].

3.14 Menstrual Status: Menstrual history was determined in all subjects and defined as the number of self-reported menstrual cycles in the past 3, 6, 9, and 12 months. Menstrual status was categorized as amenorrheic (reported no menses for the past 3 months or less than 4 menses in the past year), oligomenorrheic (reported irregular menses at intervals of 36-90 days or between 4-9 menses in the past year) or eumenorrheic (reported regular menses at intervals of 26-35 days or more than 9 menses in the past year). Menstrual logs were completed throughout the study to confirm self-reported menstrual status. Participant use of oral contraceptives was also recorded and categorized separately.

3.15 Statistical Analysis: All data was analyzed using the SPSS for Windows (version 18.0, Chicago, IL) statistical software package. Data was tested for non-normality, homogeneity of variance, and outliers before statistical hypothesis tests were performed. A p-value < 0.05 denoted statistical significance. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percentages for categorical data. Pre-season measurements were examined using one-way analysis of variance analysis (ANOVA). A one-way ANOVA with repeated measures with one within factor (time: pre- and post-season) and one between factor (group: Severe ED vs. Moderate ED vs. Energy Replete) were performed to compare REE, fasting TT3, PYY, ghrelin, EA, and test race time across the competitive

season. Paired *t*-test and one-way ANOVA analyses were performed to confirm time effect and group effects, respectively.

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Chapter 4

Manuscript

The Effect of Pre-season Energy Status on Energetic Factors and Performance

Across a Competitive Season in Female Long-distance Runners

4.1 Introduction: Female athletes who participate in endurance or aesthetic sports (i.e., distance running, gymnastics, ballet, and figure skating), wherein there is a perception that leanness is advantageous for performance, are often considered at higher risk of developing medical conditions associated with the Female Athlete Triad [1, 2]. These conditions can be experienced alone or in combination and include low energy availability (EA) with or without disordered eating, menstrual dysfunction, and low bone mineral density (BMD) [3, 4]. The chronic energy deficiency (ED) observed in female distance runners is a key factor underpinning the Female Athlete Triad [3, 4] and is a result of not consuming an adequate amount of calories to match the energy expenditure associated with exercise [5-7]. Specifically, competitive distance running may represent a high pressure environment for weight control or loss that translates into a chronic ED, which is characterized by energetic and metabolic adaptations which can lead to impaired growth, health and performance [2, 8, 9]. Female athletes may induce a chronic ED for several reasons: 1) intentional (i.e., to improve performance through modification of body size and composition); 2) compulsive (i.e., resulting from a disordered eating or pathological weight control behavior); or 3) inadvertent (i.e., failure to match energy intake (EI) with exercise energy expenditure (EEE)) [10]. However, the relationship between optimal nutrition and successful athletic performance is well-documented and notably critical in female athletes [8].

The primary goal of competitive long-distance running participation is the achievement of the fastest finishing race time. To achieve this goal female long-distance runners work to achieve a body size and composition that minimizes the resistance of movement against gravity (i.e., a higher muscle:fat ratio) and optimization of energy mobilization from several fuel sources (i.e., muscle and liver glycogen and adipose triglycerides) [8]. Athletes are recommended to consume adequate volumes of calories during periods of high-intensity and long duration training to maintain body mass, remain healthy, and optimize training effects [11]. However, the prevalence of low EA in female athletes has been shown to range from 6 to 13% [5, 9, 12], as such the effects of low EA and the effect of the associated energetic and metabolic alterations on performance in female long-distance runners is an important research area to explore.

The human body suppresses metabolic and reproductive function in the face of a chronic ED in order to preserve limited energy stores for the vital physiological process of thermoregulation, cellular maintenance and locomotion [13]. In human and animal experiments adaptations in resting energy expenditure (REE) and metabolic hormones, such as total triiodothyronine (TT3) and ghrelin, occur in order to re-establish an eumetabolic state and translate to a disruption of luteinizing hormone (LH) pulsatility [14, 15] and menstrual cyclicity [16]. A chronic ED is the primary factor associated with the induction of subclinical (luteal phase defects and anovulation) and clinical (oligomenorrhea and amenorrhea) menstrual disturbances, which may result in musculoskeletal injury and low BMD [3, 4]. A direct effect of chronic ED on performance has yet to be established; however, in the presence of weight loss and a disruption of endocrine function there is the potential to observe negative effects on performance [17]. The effects of training specific to strength and endurance performance may be negated by losses of lean body mass (LBM) and can translate to detrimental effects on immune, endocrine,

and musculoskeletal function [18]. Physiological, psychological, and performance outcomes have been shown to be adversely affected by acute energy restriction [19-23]; however, the effects of chronic ED on performance remains unclear. There is evidence from energy restriction studies that a moderate ED may not be unfavorable to exercise performance [23, 24]; while other investigators have shown in female runners and swimmers energy restriction and menstrual dysfunction are not conducive to optimal endurance performance [21, 25].

Optimally, during the highest volume in-season training, competitive female athletes should match EI to EEE to provide enough metabolic fuel to protect LBM and physiological function. The association between chronic ED and performance variables, such as race time in minutes, in female distance runners has yet to be determined in a prospective study design and serves as the rationale for conducting this work. The overall objective of this study was twofold: 1) to investigate how energetic factors (REE, fasting TT3, and EA) change across a competitive season in competitive female long-distance runners categorized by energetic status and 2) determine the impact of energetic factors on distance running performance (during a competitive time trial) from pre- to post-season in competitive female long-distance runners categorized by energetic status. We hypothesized that female long-distance runners with an initial severe energy deficit will demonstrate a greater decrease REE, EA, and fasting TT3 across the competitive season compared to energy replete runners. Additionally, female long-distance runners with a severe ED will demonstrate a greater decrease in performance (specifically, an increase in test race time in minutes) across a competitive season compared to runners with a moderate ED or energy replete status.

4.2 Methods

4.2A Experimental design: This study was a prospective investigation examining energetic, metabolic, and performance characteristics in 25 premenopausal female long-distance runners following a competitive season, categorized by pre-season energy status. Participants were assessed pre- and post-competitive running season (10-12 weeks). Participants qualified for the study if they were currently participating in competitive distance running on a team. Resting energy expenditure was measured using indirect calorimetry and a ventilated hood system. Metabolic hormone concentrations were determined from a fasting blood sample assayed for TT3. Menstrual status was confirmed by self-report and menstrual history evaluation. Body composition was determined using dual-energy x-ray absorptiometry (DXA). The research staff measured test race time via a competitive 5km time trial on an outdoor course. A maximal oxygen uptake ($VO_2\text{max}$) test was completed on a treadmill using indirect calorimetry and a modified Astrand protocol. Energy availability was calculated by measuring EI via three-day nutritional logs, EEE using the Ainsworth Compendium [26], and LBM obtained from DXA.

4.2B Study participants: Eligibility criteria for this study included: (1) age 17-25 years; (2) good health as determined by medical exam; (3) active participation in competitive distance running on a team; (4) non-smoker; (5) not pregnant and/or lactating; and (6) no other reasons that would preclude participation (i.e., injury, academic ineligibility). The Institutional Research Board approved this study and all participants signed an informed consent document if ≥ 18 yr or a parental guardian signed the informed consent if the participant was 17 yr.

4.2C Study time period: The study involved up to 4 visits to our laboratory in the pre- and post-season to complete protocol measurements. Participants completed a general information visit wherein study details and protocol requirements were explained and informed consent was obtained. Height and weight were then measured and participants a Health, Exercise

and Nutrition Survey that included information on demographics, medical, exercise, menstrual, bone health, and nutrition history. Additional pre-season measurements included an REE test, a fasting blood sample, a body composition and bone density DXA scan, and a VO_{2max} test. A competitive 5km time trial was completed to determine test race time and running velocity. Participants were given logs to continuously record their training and menstrual characteristics on a daily basis across the season.

4.2D Anthropometric and body composition data: In shorts and a t-shirt, participants total body mass was measured to the nearest 0.1 kg on at least 1 occasion (each measurement within a 2-4 week period) during the pre- and post-season time periods. The mean of these measurements was presented for each time period during the study. Height was measured to the nearest 1.0 cm. Body mass index (BMI) was calculated as the average body mass divided by height squared (kg/m^2). Body composition was assessed using a GE Lunar iDXA (enCORE 2008 software version 12.10.113) during the pre- and post-season. The division of soft tissue into fat (g) and lean tissue (g) was based on an attenuation ratio of high- and low-energy photons or R-value. All women were required to provide a urine sample prior to completion of the DXA and a pregnancy test was performed.

4.2E Resting Energy Expenditure: Resting energy expenditure was measured by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA) during the pre- and post-season. Room temperature ($^{\circ}C$), humidity (% H_2O), and barometric pressure (mmHg) were measured. Participants were instructed not to exercise/only participate in light training nor ingest caffeine within 24h, refrain from ingesting food and alcohol within 12h prior to testing, and arrive at the lab within 90 min after awakening. Prior to conducting the REE analysis, weight (kg), height (cm), and age (yr) were recorded, and predicted REE (pREE;

kcal/day) was calculated using the Harris-Benedict equation [27] $[655.0955 + 9.5634 * \text{weight} + 1.8496 * \text{height} - 4.6756 * \text{age}]$. Resting energy expenditure measurements were performed between 0830 and 1000h in a lit room at a comfortable temperature setting (20-24°C). After the volunteers lay quietly for 30-45 min, a transparent canopy was placed over their head. Volunteers were instructed to lie flat on their back and remain awake during the 30-min measurement period. Oxygen consumption (VO_2 ; mL/min) and carbon dioxide production (VCO_2 ; mL/min) were measured every 20s. To calculate REE, data for VO_2 , VCO_2 , and respiratory quotient were only used if steady state was attained. Steady state was achieved when VO_2 and VCO_2 values did not vary by more than 10% and the respiratory quotient values did not vary by more than 5%. REE was calculated using the Weir equation [28]: $\text{REE (kcal/ day)} = [3.94 * \text{VO}_2 + 1.11 * \text{VCO}_2] * 1.44$.

4.2F Energy status: Energy status was defined using an objective laboratory-based measure, REE, to identify individuals who exhibit energetic adaptation to an ED [29-32]. Reductions in REE have been observed in several studies in exercising women with amenorrhea [29, 30]. We compared laboratory-assessed REE with Harris-Benedict pREE and expressed the comparison as a ratio (REE:pREE) for each monitoring period to estimate how much each individual's measured REE deviated from the pREE. In anorexia nervosa [33-35], most data published compared measured REE to the Harris-Benedict pREE [27]. In these studies, investigators reported a suppressed REE:pREE ratio of 0.60-0.80 during periods of low body weight and before refeeding in anorexic women [33-35]. Comparing measured to predicted values is ultimately indicative of whether an energy replete or energy deficient status is present in the runners. A REE:pREE score of greater than or equal to 0.90 is typically considered energy replete, whereas an REE:pREE score of less than 0.9 is often considered energy deficient [29-

31]. As such we categorized our participants according to tertiles of REE:pREE measured pre-season to appropriately capture whether a dose-response relationship exists with respect to energy status and our primary outcome variables: severe ED (REE:pREE<0.85), moderate ED ($0.85 \leq \text{REE:pREE} < 0.90$), and energy replete (REE:pREE ≥ 0.90).

4.2G Blood sampling and storage: Blood samples were collected once each study period between 0730 and 1000h following the REE measure, stored and processed as previously described [29]. After allowing clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for fifteen minutes at 4° C. Serum samples were aliquoted into 2mL polyethylene storage tubes and stored in a freezer at -80°C.

4.2H Serum hormone measurements: TT3 concentrations were analyzed as previously described [29]. Briefly, TT3 was analyzed using a chemiluminescence-based immuno assay analyzer. The analytical sensitivity for the TT3 assay was 0.54 nmol/L, and the intra-assay and inter-assay coefficients of variation were 13.2% and 15.6%, respectively.

4.2I Dietary Energy Intake: Dietary EI (kcal/day) was assessed using three-day diet logs recorded for two week days and one weekend day, as previously described [29, 31]. These three-day nutritional logs recording food intake have previously been demonstrated to provide comparable data to seven-day records in women who may underreport their food intake, including lean women [36]. Participants were recommended to weigh (ECKO Kitchen Scale, World Kitchen, LLC, Rosemont, IL, USA) or measure (using standard cups/tools) all food and beverages consumed in detail, as well as record the time and location of every eating episode. Study personnel instructed the participants on how to accurately record EI. The nutrient data from the three-day logs were coded and analyzed using Nutritionist Pro Diet Analysis software v.4.5 (Axxya Systems, Stafford, TX).

4.2J Exercise Energy Expenditure: Participants kept logs of their purposeful exercise training for seven-day occasions throughout the entire duration of the study. Activities included on the sheet include only purposeful exercise during training sessions, but not daily living activities such as house cleaning or walking a dog. These logs provided a measurement of exercise training volume (min/wk) and the information necessary for calculation of EA. The mean of weekly exercising training volume was presented for the pre- and post-season time periods. EEE was assessed using the seven-day exercise logs for the pre- and post-season time periods. Energy expended during the purposeful exercise sessions was measured using the Ainsworth et al. [26] compendium of physical activities to determine the appropriate metabolic equivalent (MET) level for the exercise performed, which was multiplied by the duration (min) of the exercise session. The MET value additionally includes a resting component. To take only EEE into consideration by estimating EEE individually, REE (kcal/min) was therefore subtracted from the EEE values. The same member of the research team determined the MET levels from exercise logs.

4.2K Energy Availability: EA (kcal/kg LBM) was determined as EI minus EEE relative to kg LBM [$EA = (EI - EEE)/LBM$ (kg)] [37, 38] using dietary EI data assessed from three-day diet logs, EEE assessed from exercise training logs, and LBM obtained using DXA during the pre- and post-season. These measurements were all determined within the same seven-day period.

4.2L Competitive Time Trial: A competitive 5km time trial was completed on an outdoor course measured by the research staff wherein test race time (min) was determined as an indicator of performance. Running velocity (miles/ hour) was calculated by converting both test

race time (min) and course distance (km) into the desired units using the website:

<http://www.1728.com/velocity.htm>.

4.2M Maximal Oxygen Consumption: Measurement of $\text{VO}_{2\text{max}}$ was performed during the pre- and post-season on a treadmill using indirect calorimetry and the modified Astrand protocol [39]. Gas exchanged was monitored continuously by a breath-by-breath system (SensorMedics Vmax metabolic cart; Yorba Linda, CA). We considered $\text{VO}_{2\text{max}}$ to be achieved if three of the following four criteria were obtained: (1) attainment of age-predicted maximal heart rate ($208 - (0.7 * \text{age})$); (2) respiratory exchange ratio ≥ 1.1 ; (3) plateau in oxygen consumption despite an increase in exercise workload; (4) attainment of a rating of perceived exercise score ≥ 18 [40].

4.2N Menstrual Status: Menstrual history was determined in all subjects and defined as the number of self-reported menstrual cycles in the past 3, 6, 9, and 12 months. Menstrual status was categorized as amenorrheic (reported no menses for the past 3 months or less than 4 menses in the past year), oligomenorrheic (reported irregular menses at intervals of 36-90 days or between 4-9 menses in the past year) or eumenorrheic (reported regular menses at intervals of 26-35 days or more than 9 menses in the past year). Menstrual logs were completed throughout the study to confirm self-reported menstrual status. Participant use of oral contraceptives was also recorded and categorized separately.

4.2O Statistical analysis: All data were analyzed using the SPSS for Windows (version 21.0, Chicago, IL) statistical software package. Data was tested for non-normality, homogeneity of variance, and outliers before statistical hypothesis tests were performed. A p-value < 0.05 denoted statistical significance. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percentages for categorical data. Pre-season measurements were examined using one-way analysis of variance analysis (ANOVA). A

one-way ANOVA with repeated measures with one within factor (time: pre- and post-season) and one between factor (group: Severe ED vs. Moderate ED vs. Energy Replete) were performed to compare REE, fasting TT3, EA, and test race time across the competitive season. Paired *t*-test and one-way ANOVA analyses were performed to confirm time effect and group effects, respectively.

4.3 Results

4.3A Descriptive Characteristics of Study Participants: Pre-season descriptive characteristics of female long-distance runners are presented in **Table 4.1**. The study participants were 19.6 ± 0.3 yr, had a body mass of 54.6 ± 1.3 kg, were 164.5 ± 1.2 cm tall, had a body fat percentage of 23.0 ± 0.8 % and had 39.7 ± 0.9 kg of LBM. Participants presented with an average age of menarche of 13.5 ± 0.3 yrs, which equals an average gynecologic age of 6.0 ± 0.4 yrs. Pre-season VO_2 max was 61.2 ± 1.3 mL/kg/min. In the 6 months prior to study enrollment, the participants engaged in 472 ± 69 min/wk of purposeful exercise training. Pre-season training volume was 292 ± 30 min/wk (range 83-550 min/wk). Pre-season 5km test race time was 21.1 ± 0.5 min, which equals a mean running velocity of 8.9 ± 0.2 mph. Participants presented with an average REE, REE controlled for LBM (REE/LBM), and REE:pREE of 1216.1 ± 24.3 kcal/d, 30.8 ± 0.6 kcal/kg LBM, 0.87 ± 0.02 REE:pREE, respectively. Of the total participants, 8% were amenorrhoeic, 20% were oligomenorrhoeic, 48% were eumenorrhoeic, and 24% were currently using oral contraceptives.

Table 4.1: Pre-Season Descriptive Characteristics of Female Long-Distance Runners

	Mean±SEM
Demographic Characteristics (N=25)	
Age (yr)	19.6±0.3
Age of menarche (yr)	13.5±0.3
Gynecological age (yr)	6.0±0.4
Anthropometric Characteristics	
Height (cm)	164.5±1.2
Body mass (kg)	54.6±1.3
BMI (kg/m ²)	20.2±0.4
Body Fat (%)	23.0±0.8
Fat Mass (kg)	12.5±0.6
LBM (kg)	39.7±0.9
Fat-Free Mass (kg)	42.0±0.9
Metabolic Characteristics	
REE (kcal/d)	1216.1±24.3
REE/LBM (kcal/kg LBM)	30.8±0.6
REE:pREE	0.87±0.02
Training Characteristics	
VO ₂ max (mL/kg/min)	61.2±1.3
Test Race Time (min:sec)	21.1±0.5
Running Velocity (mph)	8.9±0.2
History of Exercise (min/wk) ^a	471.8±69.3
Exercise Volume (min/wk)	291.5±29.5
Bone Health Characteristics	
Total Body BMD (g/cm ²)	1.11±0.02
L1-L4 BMD (g/cm ²)	1.14±0.03
Total Hip BMD (g/cm ²)	1.09±0.03
Femoral Neck BMD (g/cm ²)	1.09±0.02

BMI = Body mass index; LBM = Lean body mass; REE= Resting energy expenditure;
 REE:pREE = Measured REE compared to Harris-Benedict predicted REE; VO₂max = Maximal
 oxygen uptake; BMD = Body mineral density

^a = History of exercise (min/wk) in past 6 months

4.3B Anthropometric and Body Composition Characteristics across a Competitive

Season: Anthropometric and body composition measures across a competitive season in female long-distance runners grouped by pre-season energy status are shown in **Table 4.2**. There were no differences ($p>0.05$) in body mass, BMI, percent body fat, and LBM among groups at pre- or

post-season. There were no group x time interactions, time effects, or group effects ($p>0.05$) for body mass or BMI. There was a group x time interaction ($p=0.007$) for percent body fat, however, no time effect ($p=0.532$) or group effect ($p=0.377$) were observed. Similarly, there was a group x time interaction ($p=0.014$) for fat mass, however, no time effect ($p=0.319$) or group effect ($p=0.456$) were observed. There was a time effect ($p=0.042$) observed for LBM with a 1% increase in LBM observed post-season, however, no group x time interaction ($p=0.516$) or group effect ($p=0.855$) were observed.

Table 4.2: Anthropometric and body composition characteristics across a competitive season in female long-distance runners grouped by energy status.

	PRE- SEASON Mean±SEM	POST- SEASON Mean±SEM	Group x Time Interaction p-value	Time Effect p-value	Group Effect p-value
Body mass (kg)(N=25)			0.056	0.059	0.910
Severe ED	54.7±2.8	55.9±3.1			
Moderate ED	54.2±2.2	53.9±2.0			
Energy Replete	55.0±1.7	55.5±1.6			
BMI (kg/m²)			0.061	0.067	0.991
Severe ED	20.0±0.5	20.4±0.5			
Moderate ED	20.4±0.7	20.3±0.6			
Energy Replete	20.2±0.9	20.3±0.8			
Percent body fat			0.007	0.532	0.377
Severe ED	24.1±1.4	25.1±1.4			
Moderate ED	22.8±1.3	21.9±1.1			
Energy Replete	22.1±1.3	22.4±1.4			
Fat mass (kg)			0.014	0.319	0.456
Severe ED	13.2±1.2	14.0±1.3			
Moderate ED	12.2±1.1	11.6±0.8			
Energy Replete	12.0±0.8	12.4±0.8			
LBM (kg)			0.516	0.042	0.855
Severe ED	39.3±1.8	39.5±1.8			
Moderate ED	39.5±1.4	40.0±1.5			
Energy Replete	40.4±1.5	40.8±1.6			

BMI = Body mass index; LBM = Lean body mass; ED = Energy deficiency

4.3C REE Characteristics across a Competitive Season: Resting energy expenditure was lower in the severe ED and moderate ED vs. the energy replete group at pre- (1102 ± 35 kcal/d and 1208 ± 22 kcal/d vs. 1340 ± 21 kcal/d, $p<0.001$ and $p=0.006$, respectively) and post-season (1150 ± 44 kcal/d and 1181 ± 32 kcal/d vs. 1339 ± 25 kcal/d, $p=0.003$ and $p=0.011$, respectively). The severe ED runners were 9% ($p=0.028$) and 3% ($p>0.05$) lower than the moderate ED runners at pre-season and post-season, respectively. There was a group effect ($p<0.001$), trend toward a group x time interaction ($p=0.082$) and no time effect ($p>0.5$) for REE. Measured REE/LBM and REE:pREE across a competitive season in female long-distance runners grouped by energy status are shown in **Figure 4.1**. Resting energy expenditure controlled for LBM in the long-distance runners with a severe ED were 9% ($p=0.08$) and 17% lower ($p<0.001$) compared to the moderate ED and energy replete long-distance runners, respectively, at pre-season. As expected, REE:pREE was lower in the severe ED group compared to the moderate ED ($p<0.001$) and energy replete ($p<0.001$) groups at pre-season. Similarly, at post-season REE:pREE was lower in the severe ED runners compared to the energy replete runners ($p=0.01$). Post-season the severe ED group had a 15% ($p=0.003$) lower REE/LBM compared to the energy replete runners; however, REE/LBM ($p>0.05$) and REE:pREE ($p>0.05$) were not significantly different between the severe ED and moderate ED groups at post-season. The REE/LBM in the moderate ED long-distance runners were 8% ($p=0.078$) and 11% ($p=0.076$) lower compared to the energy replete long-distance runners. The moderate ED group had a significantly lower REE:pREE compared to the energy replete group at pre-season ($p<0.001$) and post-season ($p=0.002$). There was a group effect for REE/LBM and REE:pREE ($p<0.002$), but no group x time interaction ($p>0.2$) or time effect ($p>0.3$).

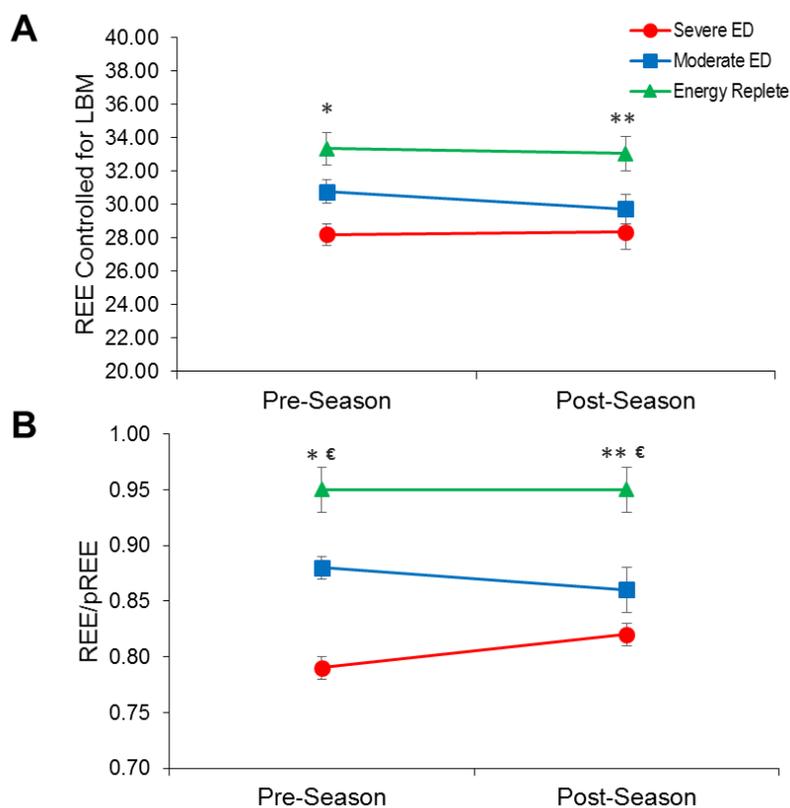


Figure 4.1: REE controlled for lean body mass and measured REE compared to predicted REE

Resting energy expenditure controlled for lean body mass (REE/LBM) and measured REE compared to predicted REE (REE:pREE) across a competitive season in female long-distance runners grouped by energy status. Panel A shows REE/LBM (kcal/kg LBM) across a competitive season in female long-distance runners grouped by pre-season energy status. In panel B REE:pREE across a competitive season in female long-distance runners grouped by pre-season energy status is shown. Severe ED group (REE:pREE < 0.85) (red line), Moderate ED (REE:pREE \geq 0.85 to < 0.90) (blue line), and Energy Replete group (REE:pREE \geq 0.90) (green line). The symbol (*) indicates $p < 0.05$, Severe ED Group vs. the Energy Replete and Moderate ED groups. The symbol (**) indicates $p < 0.05$, Severe ED vs. Energy Replete groups. The symbol (€) indicates $p < 0.05$, Moderate ED vs Energy Replete groups.

4.3D Performance Characteristics following a Competitive Season: Performance

characteristics across the competitive season in female long-distance runners grouped by energy status including measurements of test race time, running velocity, and VO_{2max} , are shown in

Figure 4.2. The severe ED group had a test race time 15% slower (3.2 min) ($p < 0.05$) than the moderate ED group at pre- and post-season. Test race time did not significantly differ between

the severe ED ($p>0.05$) and moderate ED groups ($p>0.05$) vs. the energy replete group at pre- or post-season. Pre-season and post-season running velocity for the moderate ED group was 15% ($p<0.05$) faster compared to the severe ED group. The severe ED, moderate ED and energy replete runners pre-season VO_{2max} values were not significantly different ($p=0.237$); however, the severe ED groups VO_{2max} at post-season was 12% ($p=0.013$) and 12% ($p=0.03$) lower compared to the moderate ED and energy replete groups, respectively. No differences in VO_{2max} were observed between the moderate ED and energy replete group at post-season ($p>0.05$). There were group effects for test race time ($p=0.007$) and running velocity ($p=0.006$), however, there were no group x time interactions ($p>0.9$) or time effects ($p>0.3$) for either variable. There were significant time ($p=0.016$) and group effects ($p=0.039$) for VO_{2max} . However, there was no group x time interaction ($p=0.372$) for VO_{2max} .

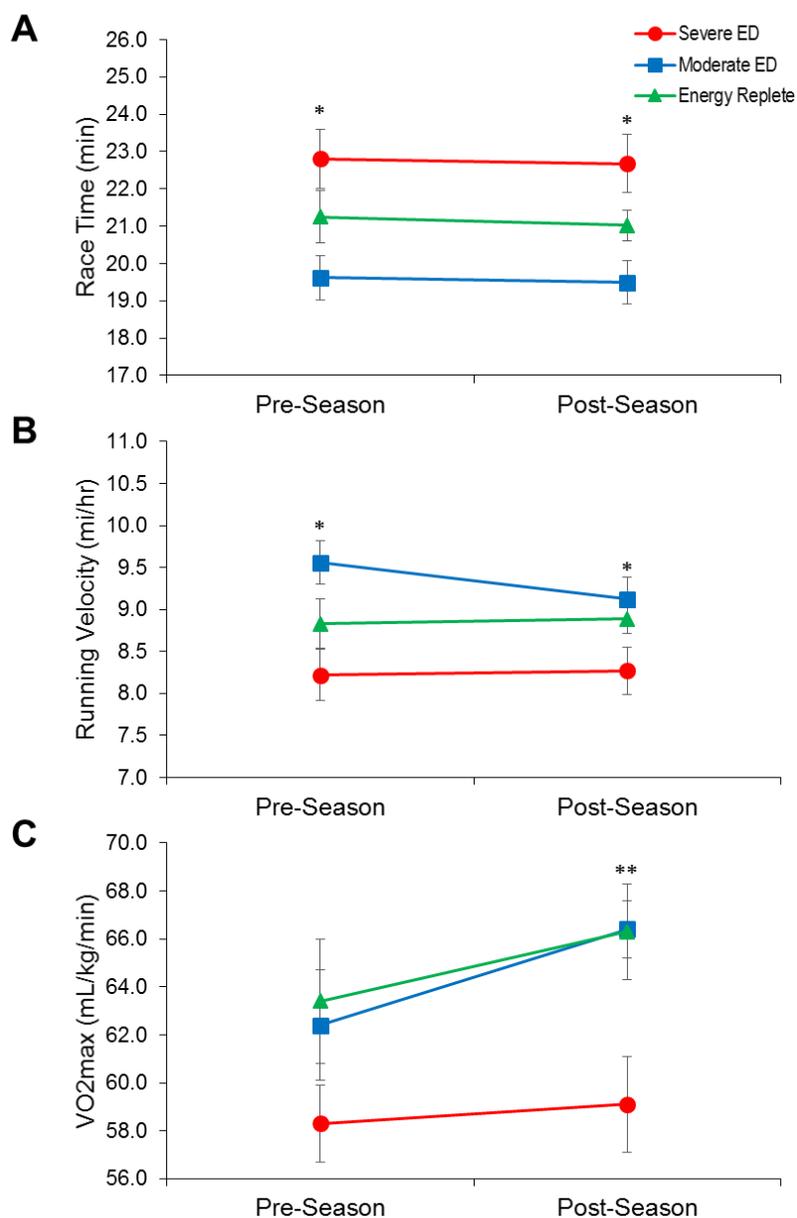


Figure 4.2: Performance Characteristics

Performance Characteristics across the competitive season in female long-distance runners grouped by energy status. Test race time (min) across a competitive season in female long-distance runners grouped by energy status is shown in Panel A. Panel B shows running velocity (mph) across a competitive season in female long-distance runners grouped by energy status. Maximal oxygen uptake (VO₂max) (mL/kg/min) across a competitive season in female long-distance runners grouped by energy status is shown in Panel C. Severe ED group (REE:pREE < 0.84) (red line), Moderate ED group (REE:pREE ≥ 0.84 to < 0.90) (blue line), and Energy Replete group (REE:pREE ≥ 0.90) (green line). The symbol (*) indicates p < 0.05, Severe ED Group vs. the Moderate ED group. The symbol (**) indicates p < 0.05, Severe ED vs. Moderate ED and Energy Replete groups.

4.3E EA Characteristics across a Competitive Season: Measurements of EA, EI, and EEE across a competitive season in female long-distance runners grouped by energy status are shown in **Table 4.3**. EI, EEE, and EA did not differ among groups at pre- ($p>0.1$) or post-season ($p>0.5$). No group x time interaction, time effect, or group effect was observed ($p>0.3$) for EA and EI. There was a group x time interaction ($p=0.05$) for EEE. However, no time ($p=0.56$) and group effects ($p=0.805$) were reported for EEE.

Table 4.3: Energy availability characteristics across a competitive season in female long-distance runners grouped by energy status.

	PRE- SEASON Mean±SEM	POST- SEASON Mean±SEM	Group x Time Interaction p-value	Time Effect p-value	Group Effect p-value
EA (kcal/kg LBM)			0.894	0.955	0.372
Severe ED	36.6±4.0	37.60±4.0			
Moderate ED	45.9±7.9	44.3±4.1			
Energy Replete	45.3±4.5	46.3±6.8			
EI (kcal/d)			0.423	0.640	0.354
Severe ED	1783±158	1891±223			
Moderate ED	2176±226	2042±133			
Energy Replete	2101±177	2265±212			
EEE (kcal/d)			0.050	0.560	0.805
Severe ED	357±58	405±71			
Moderate ED	485±70	388±94			
Energy Replete	416±23	423±40			

EA = Energy availability; EI = Energy intake; EEE = Exercise energy expenditure; LBM = Lean body mass

4.3F Metabolic Hormones across a Competitive Season: Fasting Log TT3 concentrations across a competitive season in female long-distance runners grouped by energy status are shown in **Figure 4.3**. At pre-season, there was a trend to a statistically significantly lower Log TT3 ($p=0.067$) in the severe ED group vs. the moderate ED group. There were no differences in Log TT3 observed between the severe ED vs. the energy replete groups ($p=0.146$) or the moderate ED vs. energy replete groups ($p>0.9$) at pre-season. Log TT3 was similar among

groups at post-season ($p=0.263$). No group \times time interaction ($p=0.175$), time ($p=0.617$), or group effects ($p=0.088$) were observed for Log TT3.

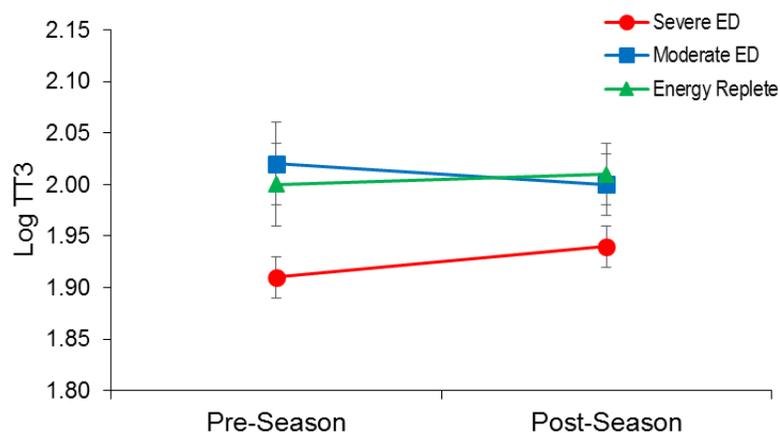


Figure 4.3: Metabolic hormone concentrations

Metabolic hormone concentrations across a competitive season in female long-distance runners grouped by energy status. Fasting total triiodothyronine concentration (ng/dL) across a competitive season in female long-distance runners grouped by energy status (Severe Energy Deficiency (ED) group ($REE:pREE < 0.84$) (red line), Moderate ED group ($REE:pREE \geq 0.84$ to < 0.90) (blue line), and Energy Replete group ($REE:pREE \geq 0.90$) (green line)).

4.4 Discussion: In this prospective study, we investigated the effects of energy status on long-distance running performance in 25 female long-distance runners categorized by pre-season energy status. Additionally, we examined changes in energetic factors (REE, EA, and fasting TT3) across the competitive season. As hypothesized, the female distance runners who presented with an initial severe ED, as a $REE:pREE < 0.85$, demonstrated the slowest test race time and running velocity at pre-season, which was significantly slower than the moderate ED group. The observed performance decrements were also observed in the severe ED group during post-season testing. This is indicative of an association between energy status and long-distance running performance across the competitive season. Notably, during post-season, the severe ED group had a test race time which was 15% (3.2 min) and 7% (1.6 min) slower than the moderate ED and energy replete groups, respectively. Typically, the training goal of long-distance runners is to

reach a performance peak at competitive races. Female distance runners with a severe ED may fail to improve with training across the season, this may lead to negative outcomes in peak training and performance. The test race time difference observed between the severe ED, moderate ED, and energy replete groups is substantial in terms of runner placement in a competitive long-distance race. Identification of pre-season REE as a relevant marker of performance at pre- and post-season indicates REE testing may be appropriate for monitoring energy status with implications for tracking athlete progression of performance toward a peak for competitive races.

We have demonstrated an association between energy status and performance in female-long distance runners; however, a dose-response relationship was not observed. To this end, decrements in performance were only exhibited in the severe ED runners. Contrary to our hypothesis, moderate ED runners (REE:pREE between 0.85-0.89) did not differ in performance from energy replete runners (REE:pREE ≥ 0.90), thus demonstrating comparable test race times and running velocity. Other investigators have also reported a decrease in performance of athletes who were in an energy deficit. Van Heest et al. [25] demonstrated that swimmers with chronic ovarian suppression had a REE:pREE that consistently fell below 0.85 across the season. The ovulatory suppressed swimmers additionally demonstrated a significantly worse swim performance compared to their regularly ovulating counterparts, who exhibited REE:pREE between 0.95-1.04 [25]. The ovulatory swimmers demonstrated an improvement in performance by 8.2% at the end of the competitive season compared to the start of the season, while the ovulatory suppressed swimmers had a 9.8% decline in swim performance from week 0 to week 12 [25]. A number of factors could explain the lack of a dose response association between energy deficiency and competitive performance. We suspect the threshold below which

performance decrements are observed may be lower than the energy deficit threshold sufficient to disrupt metabolic and reproductive function, defined as a REE:pREE of less than 0.90 [29-31, 41]. In fact, a moderate ED may not negatively affect competitive performance. Additionally, a moderate ED may need to be present for a long period of time prior to causing declines in running velocity and overall decrements in performance. Zachwieja et al. [23] demonstrated that a short-term induction of an energy deficit (-750 kcal/day) in physically active men and women did not negatively affect five mile time trial performance. Chronic performance adaptations associated with a moderate ED are unclear and performance impairments may not be observable until a chronic suppression of REE is reached.

The marker of a chronic energy deficiency is an inadequate consumption of metabolic fuel to sustain all major physiologic functions. In the face of a chronic energy deficiency critical functions, such as thermoregulation and cellular maintenance, are protected; whereas, less critical functions, such as reproduction and growth, are suppressed. As demonstrated previously, a suppressed REE and fasting TT3 are observed in amenorrheic exercising women as adaptations to restore an energy replete state, and can be used as markers of an energy deficiency in exercising women [25, 29-31, 41]. As expected, pre-season REE and fasting TT3 were suppressed in the severe ED group relative to the energy replete group. Van Heest et al. [25] demonstrated that sport performance was highly correlated with ovarian steroid and metabolic hormones (including TT3) in ten junior elite female swimmers over a 12-week competitive season. Specifically, ovarian suppressed swimmers had a 12% reduced REE, 27% lower REE:pREE, and a 19% lower TT3 at post-season, where the ovarian suppressed swimmers had a 19% slower 400m swim velocity compared to their ovulatory teammates at week 12 [25]. These findings demonstrate the importance of evaluating menstrual status, REE, and fasting TT3 in

female athletes during pre-participation screenings. Early detection of metabolic suppression would enable athletic medical teams to intervene in athletes presenting with a severe energy deficiency prior to observing poor performance outcomes and potential subclinical or clinical sequelae consistent with an energy deficiency [42, 43].

We hypothesized that EA would decrease across the season; however, we observed no change in EA across the season within or among female distance runners categorized by energy status. The mean EA values among our groups at pre-season ranged from 36.6 to 45.9 kcal/kg LBM and post-season ranged from 37.6 to 46.3 kcal/kg LBM, which are notably higher than the range of EA reported in the literature (12-29 kcal/kg LBM) for amenorrheic runners [9]. Additionally, our findings are in contrast with the report of Van Heest et al. [25] wherein EA and energy intake were significantly lower in the ovarian suppressed swimmers compared to ovulatory swimmers. In a prospective evaluation of collegiate female soccer players, EA values were observed to decline from pre- to mid-season and subsequently increased from mid- to post-season [12]. Investigators have suggested that low EA consistent with development of an energy deficit may vary among young, exercising women [44]; however, accurate measurements of EA in free-living individuals is difficult due to the nature of self-reported diet and exercise training logs [44-47]. More research is required to determine whether EA or energy balance, which considers all components of 24-hr energy expenditure, is a better variable to evaluate the impact of energy status on competitive performance [10]. We used the objective measurements of REE and fasting TT3 to corroborate severe ED in association with performance decrements. To this end we suggest that use of objective measures, such as REE or fasting TT3, are more appropriate to use in defining energy status in exercising women than EA or energy balance.

A strength of our study is the measurement of test race time under competitive conditions to confirm the association between energy status and actual performance across a season, since the commonly used measure of maximal aerobic capacity, VO_{2max} , is not a direct marker of performance. Decrements in VO_{2max} may also be observed in athlete with chronic low EA. We observed a significantly lower VO_{2max} in female long-distance runners with a severe ED, with moderate ED and energy replete runners having higher VO_{2max} values (7 to 12% greater) at pre- and post-season compared to severe ED runners. De Souza et al. [30] demonstrated similar findings of lower VO_{2max} values in energy deficient exercising women compared to their energy replete counterparts. However, numerous investigators have not observed differences in VO_{2max} between exercising women when categorized by menstrual status [48-51].

This study is novel in its investigation of metabolic hormone characteristics prospectively across a competitive season in female long-distance runners categorized by pre-season energy status. Maintenance of energy balance, in the face of a chronic energy deficiency, is suggested to be accomplished by decrements in REE and other accompanying energy conservation responses, such as decreases in TT3. Suppression of REE has been demonstrated to be highly correlated with suppressed fasting TT3 concentrations [52]. In our study there was a trend ($p=0.067$) to a lower fasting TT3 in the severe ED group compared to the moderate ED group. Though this result is contrary to previous reports on energy deficient and amenorrheic exercising women [29, 41] we suggest that the 24% proportion of OC users included in the analysis may mask the effects of energy deficiency on TT3 due to increased thyroxine-binding globulin secretion [53-55]. Since TT3 is indicative of energy status, pre-participation screenings should include evaluation of TT3 if the athlete is not currently using hormonal contraceptives. Further research

is necessary to determine the utility of TT3 as an objective measure of an energy deficit in athletes utilizing hormonal contraceptives.

The present study is the first to use a prospective evaluation of competitive performance outcomes in female long-distance runners across a competitive season. Though competitive performance is difficult to measure, the approach captures the psychological and behavioral factors that impact performance [56, 57]. There were additional notable limitations, specifically in the measurement of EA. It has previously been demonstrated that study participants are prone to under-reporting dietary intake with daily diet logs [47, 58] while over-reporting daily exercise energy expenditure [45], resulting in an overall underestimation of EA. During the competitive season EA may be transient considering the dietary and training changes across a season, thus the use of EA as an indicator of an energy deficiency in free-living research participants is questionable [44]. Due to these limitations in EA calculation and potential variability across the season, we utilized an objective laboratory-based measure, REE, to define energy status and REE:pREE to identify individuals exhibiting an energetic adaptation to an energy deficiency. In prior reports of energetic status in exercising women a REE:pREE score less than 0.90 has been used to define an energy deficiency; however, in the current study we categorized our participants by tertiles of REE:pREE to more accurately capture whether a performance and energy status dose-response relationship exists.

Overall, we demonstrated an association between energy status and performance outcomes in female long-distance runners across a competitive season. We did not observe a dose-response relationship between suppression of REE and decrements in performance, contrary to our hypothesis. A severe ED was, however, associated with decrements in test race time, running velocity, and VO_{2max} in female long-distance runners across the competitive

season. Decreases in REE below 85% of predicted REE are indicative of the presence of sub-optimal energy status, which can be detrimental to competitive performance across a season.

Early detection of an energy deficit would allow for intervention beneficial in the short term for performance outcomes and in the long term for health-related outcomes (i.e., menstrual, skeletal, psychological, and cardiovascular health). Thus, it may be appropriate to implement REE testing throughout the competitive season to monitor energy status and avoid decrements in performance outcomes. Monitoring energy status may assist in the development of more effective dietary and training regimens for each runner, enhancing their ability to achieve peak performance with a concomitant optimal energy status.

4.5 References

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Chapter 5

Summary, Conclusion, and Implications for Practice

Female distance runners are particularly susceptible to development of the medical conditions associated with the Female Athlete Triad as a result of not meeting their daily caloric requirements [1, 2]. These conditions include low energy availability (EA) with or without disordered eating, menstrual dysfunction, and low bone mineral density and can be experienced alone or in combination [3, 4]. Participants in leanness sports, such as distance running, have been shown to be at a higher risk for having a low EA, which is characterized by a reduced dietary intake relative to exercise energy expenditure [5]. Women in an energy deficiency have a suppression of growth and reproduction in order to maintain energy stores for non-negotiable physiological processes, such as thermoregulation, cellular maintenance, and locomotion [6]. Low EA, not consuming adequate calories to compensate for the energy expended during exercise and other physiological requirements, often induces an energy deficiency, which then promotes adaptations in resting energy expenditure (REE) and metabolic hormones, such as total triiodothyronine (TT3).

There is limited literature showing a direct association between energy deficiency and performance variables in competitive female distance runners. The purpose of this thesis was to examine how energetic factors in competitive female long distance runners change across a competitive season and to determine the impact of a change in energetic factors on distance running performance during a competitive time trial (test race time in minutes) in these women. Some investigators have shown a relationship between a chronic energy deficiency and decrements in physical performance tests such as Van Heest et al. in junior elite female swimmers. Sport performance decreased by 9.8% in the ovarian suppressed group and improved

by 8.2% in the normal menstruating group [7-9]. In 1996, Dueck et al. [10] demonstrated in a case study the effect of a 15-week diet and exercise intervention for an amenorrhoeic athlete. After increasing weight and body fat and resuming menstruation the athlete reported an increase in her athletic performance.

This study showed a decrease in performance in female distance runners in the post-season who were severely energy deficient in the pre-season compared to runners who were moderately energy deficient or energy replete. Specifically women in the severe ED group had a significantly greater race time and slower running velocity than the runners in the moderate ED group. Similar reports of decrements in performance have been found in the literature; specifically, Van Heest et al. [9] demonstrated that an energy deficiency in conjunction with reproductive disturbances translated to a poorer sports performance and that the degree of the energy deficit worsened the sport performance. Additionally, Romemich et al. [8] reported that weight loss in male wrestlers, due to a reduction in energy intake and associated with an energy deficiency, led to decreased muscular strength and power. In the current study, runners in the severe ED group also had a significantly lower REE:pREE in the post-season compared to the energy replete group. In 2007, De Souza et al. [11] reported reduced REE in exercising women with amenorrhea compared to those who were ovulating in both exercising and non-exercising women. A dose relationship was found between reduction in REE and menstrual disturbances, with greater reductions being correlated with greater severity of disturbances [11].

Future studies are needed to show a relationship between female distance runners EA and fasting concentrations of TT3 related to energy deficiency. Other investigators have reported low energy intake in female distance runners despite high energy expenditure [12-14]. In 1993, Loucks et al. [15] demonstrated low TT3 could be induced in previously sedentary women in 4

days of energy restriction. Despite the literature supporting the hypothesis of low EA and low TT3 in the female distance runners with a severe energy deficiency, there were no significant differences found between EA or TT3 between the three groups in this study. This could have resulted from study limitations including a low sample size.

Athletes are always looking for an advantage to get ahead of the competition. The female distance runner culture is one that promotes using nutrition and body composition to achieve the fastest racing times, particularly at the end of the season when championship races are held. When a runner wants to shed seconds or even minutes off their race time, one of the first strategies is to expend more calories than the runner intakes in an attempt to reduce body weight and body fat percentage. Runners will often engage in restrictive eating, excessive exercise, or a combination to lose weight or body fat. Coaches will often encourage these tactics too, believing that it will improve the athlete's performance. Most competitive runners and coaches are not aware that by not consuming adequate calories to compensate for calories burned they are putting themselves in an energy deficit, putting them at risk for a disruption in menses, reduced bone health, and poorer athletic performance. The information found in this study should be shared with female distance runners and coaches. A greater emphasis needs to be placed on a diet that includes enough calories to compensate for the calories burned. If coaches and athletes were better educated about the implications of an energy deficit they could turn to alternative techniques to try to better running performance besides weight loss and fat loss.

In conclusion, many distance runners adopt restrictive eating behavior to reduce body weight or fat mass in an effort to achieve optimal distance running performance which can lead to an energy deficit. A chronic energy deficiency or low EA is a key factor leading to the development of the Triad (low energy availability, menstrual irregularities, and low bone mass)

[3, 4] and may have implications for distance running performance. This study found that distance runners in an energy deficit had a poorer performance in the post-season compared to runners who were energy replete. Educating female distance runners about the negative impact an energy deficit can have on running performance (race time in minutes) would allow a greater emphasis to be placed on the importance of meeting nutritional requirements.

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EDUCATION:

The Pennsylvania State University, University Park, PA Expected, May 2015
The Schreyer Honors College
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Academic All Big-Ten for Cross Country, Winter and Spring Track for ten seasons

EXPERIENCE:

Lab Technician for Women's Health and Exercise Laboratory 2012-present
Noll Laboratory, Penn State University

- Prepared research samples and organized and ran numerous lab tests
- Successfully contributed to the Refuel Women's Study, Distance Runner Study
- Committed 10 hours per week while a full time student athlete

Senior Thesis 2012-present
The Impact of Energetic Factors on the Performance in Female Long-Distance Runners

- Recruited and scheduled study participants for testing
- Facilitated testing including time trial, VO₂, and metabolism
- Used statistical analysis to review results and formulate study conclusions

Undergraduate Teaching Assistant 2013
Penn State University, Kinesiology 202: Functional Human Anatomy

- Assisted in lab activities, graded assignments, and provided out of class tutoring to students

Apparel and Shoe Sales, Team Member Summer 2013
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- Provided excellent customer service in a fast paced retail sales environment

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- Led workshops for high school athletes, instructing proper running technique and form drills

ATHLETICS:

Penn State Women's Varsity Cross Country and Track & Field Team 2011- present

- Elected as Captain, and recognized as a capable, responsible leader by my peers
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AWARDS/LEADERSHIP:

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