CHANGES IN ENERGETIC FACTORS AND PERFORMANCE FOLLOWING A COMPETITIVE SEASON IN FEMALE DISTANCE RUNNERS

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ABSTRACT

Female long-distance runners are at a higher risk for chronic energy deficiency, defined as inadequate energy intake relative to energy expenditure, and other medical conditions associated with the Female Athlete Triad, to include low energy availability with or without disordered eating, menstrual disturbances, and low bone mineral density. To date, the relationship between chronic energy deficiency and running performance in female long-distance runners has yet to be determined. **Purpose:** The purpose of this study is two-fold: (1) to characterize the change in energetic factors (REE, fasting concentrations of TT$_3$ and ghrelin, and EA) across a competitive season in female long-distance runners categorized by energy status and (2) to examine distance running performance during a competitive time trial (test race time) from pre- to post-season in female long-distance runners categorized by energy status. **Methods:** This study was a prospective investigation examining energetic, metabolic, and performance characteristics in 18 female long-distance runners (17-25yr) categorized by energy status across a competitive season. Participants were assessed pre- and post-competitive running season (10-12 weeks). REE was measured by indirect calorimetry using a ventilated hood system. A ratio of the actual REE to the Harris Benedict predicted REE (REE/pREE) was calculated once during each monitoring period. We categorized our participants according to tertiles of REE/pREE: severe energy deficiency (ED) (REE/pREE<0.84) (n=6), moderate ED (0.84≤REE/pREE<0.90) (n=6), and energy replete (REE/pREE≥0.90) (n=6). Fasting blood samples were collected and assayed for total triiodothyronine (TT$_3$) using a chemiluminescence-based immunoassay and total ghrelin using radioimmunoassay. Test race time (min) and running velocity (mph) were...
determined as indicators of performance via a competitive 5km time trial on an accurately measured outdoor course. Body composition was measured using dual energy x-ray absorptiometry. Energy intake was assessed using 3-day diet logs. Exercising training volume/expenditure was determined using 7-day training logs. Exercise energy expenditure was measured using the Ainsworth compendium. Energy availability was determined as EI minus EEE relative to kg LBM \( [EA = (EI – EEE)/LBM \text{ (kg)}] \). Maximal oxygen uptake \( (VO_2\text{max}) \) was measured on a treadmill using indirect calorimetry and the modified Astrand protocol. One-way ANOVA with repeated measures were performed to compare REE, fasting TT3 and ghrelin, EA, test race time and running velocity across the competitive season. Paired t-test and one-way ANOVA analyses were performed to confirm time and group effects, respectively. Results: Study participants were 19.5±0.3 yr, weighed 54.6±1.4 kg with 22.9±0.8% body fat (%BF). There were no differences \( (p<0.05) \) in body mass, BMI, and LBM among groups at pre- or post-season. The severe ED group had a higher %BF than the energy replete group \( (p=0.011) \) at pre-season measurements, and a higher % BF \( (p<0.05) \) than both the energy replete and moderate ED groups at post-season. As expected, REE/pREE and REE were lower \( (p<0.05) \) in the severe ED group than energy replete runners at pre-season, as well as lower \( (p<0.05) \) than the energy replete group and the moderate ED group at post-season. The moderate ED group also demonstrated lower REE/pREE \( (p<0.05) \) than the energy replete group at pre- and post-season. At pre-season, the severe ED group had slower test race time and running velocity \( (p<0.05) \) than the moderate ED group. No differences in test race time and running velocity were observed \( (p>0.05) \) between the severe ED and moderate ED groups vs. the energy replete group at pre-season. At post-season, the severe ED group
had a slower test race time and running velocity (p<0.05) than both the moderate ED and energy replete groups. At pre-season, Log TT3 was lower (p=0.043) in the severe ED group vs. the moderate ED group. No changes in EA and fasting TT3 were observed across the season (p=0.005). A time effect was observed for ghrelin within groups (p<0.05). **Conclusion:** In summary, we provide evidence of an association between severe energy deficiency and decrements in distance running performance. Thus, early detection of poor energy status in female distance runners, using REE testing, may be beneficial in for promoting optimal distance running performance across a season. Future research is necessary to confirm this association in a larger sample of female runners.
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Chapter 1

Introduction

Previous research has demonstrated that female distance runners are particularly susceptible to the development of the medical conditions of the Female Athlete Triad alone or in combination, which include low energy availability with or without disordered eating, menstrual dysfunction, and low bone mineral density (Nattiv, Loucks et al. 2007). The Triad conditions are well-documented in exercising women participating in both recreational- and elite-level sports (Torstveit and Sundgot-Borgen 2005; Torstveit and Sundgot-Borgen 2005; Nichols, Rauh et al. 2007; Hoch, Pajewski et al. 2009). The Triad is most prevalent in athletes competing in leanness sports that focus on low body fat, weight control, and aesthetic appearance such as distance running, gymnastics, and dance (Torstveit and Sundgot-Borgen 2005; Nichols, Rauh et al. 2007). Athletes that participate in leanness sports have been shown to be at a higher risk for a low energy availability, characterized by a reduced dietary energy intake relative to exercise energy expenditure (Torstveit and Sundgot-Borgen 2005). Essentially, this reduction in energy availability is a result of not consuming an adequate amount of calories to match the energy expended during exercise. Distance runners and other endurance athletes may reduce energy availability for several reasons: 1) intentionally, i.e., to improve performance by modifying body size and composition; 2) compulsively, i.e., as the result of a disordered eating behavior or pathological weight control; or 3) inadvertently, i.e., failing to match energy intake to exercise-induced energy expenditure (Loucks, Kiens et
Female distance runners typically adopt restrictive eating behaviors as a means to reduce their body weight or fat mass in the effort to achieve optimal distance running performance. Numerous reports on low energy intake in female distance runners exist in the literature (Myerson, Gutin et al. 1991; Edwards, Lindeman et al. 1993; Beidleman, Puhl et al. 1995). To this end, female distance runners are a population of athletes of particular concern for Triad-related disorders. However, the impact of low energy availability on distance running performance is unclear.

Low energy availability has a suppressive effect on both metabolic and reproductive function in order to preserve energy stores for more vital physiological processes, such as thermoregulation, cellular maintenance and locomotion (Wade, Schneider et al. 1996). Low energy availability often induces an energy deficiency. An energy deficiency promotes adaptations in resting energy expenditure (REE) and metabolic hormones, such as triiodothyronine (TT3), ghrelin, leptin, and insulin-like growth factor–1 (IGF-1). Previous research has shown the apparent suppressive effects of low energy availability on fasting TT3 concentrations (Loucks and Heath 1994), luteinizing hormone (LH) (Loucks, Verdun et al. 1998; Loucks and Thuma 2003), and menstrual cyclicity (Williams, Caston-Balderrama et al. 2001; Williams, Helmreich et al. 2001). Restoration of LH pulsatility can be prevented by dietary supplementation (Loucks and Verdun 1998; Williams, Caston-Balderrama et al. 2001). Such findings demonstrate that exercise exclusively has no suppressive effect on TT3 and LH pulsatility beyond the impact of its energy cost on energy availability (Loucks, Verdun et al. 1998). Documented changes in LH, as well as menstrual cyclicity, were also observed in exercise-induced amenorrheic cynomolgus monkeys (Williams, Helmreich et al. 2001).
The gastrointestinal peptide ghrelin also plays a role in long-term energy homeostasis and reproductive function (De Souza, Leidy et al. 2004; Scheid, Williams et al. 2009). Previous research on basal ghrelin levels has shown that it is responsive to energy-deficit-induced decreases in body weight, fat mass, and resting metabolic rate in young women with normal weight (Leidy, Gardner et al. 2004). Furthermore, De Souza and colleagues (2004) indicated that amenorrheic athletes present with elevated ghrelin concentrations and to this end, ghrelin may represent a key marker of a chronic energy deficiency and functional hypothalamic amenorrhea. In terms of clinical implications, chronic energy deficiency may induce subclinical (luteal phase defects and anovulation) and clinical menstrual disturbances (functional hypothalamic amenorrhea and oligomenorrhea) and detrimental musculoskeletal injury and low bone mineral density (Nattiv, Loucks et al. 2007). Particularly for female distance runners, the risk of a bone stress injury may have a deleterious effect on their future running career.

1.1 Objective

The overall objective of this study is to investigate the change in energetic factors (energy availability, REE, and fasting concentrations of TT$_3$ and ghrelin) in competitive female distance runners following a competitive season and further, to determine the impact of the above mentioned energetic factors on distance running performance during a competitive time trial (test race time in minutes) in these women.
1.2 **Specific Aim 1:** To examine energetic factors, to include energy availability, REE, and fasting concentrations of TT₃ and ghrelin, during a competitive season in female distance runners.

The following hypotheses will be tested:

H1: Energy availability (kcal/kg LBM) in competitive female distance runners will be significantly lower during the pre-season compared to the post-season.

H2: Resting energy expenditure (kcal/day) in competitive female distance runners will be significantly lower during the pre-season when compared to post-season.

H3: Fasting concentrations of TT₃ (ng/dl) in competitive female distance runners will be significantly lower during the pre-season when compared to the post-season.

H4: Fasting concentrations of ghrelin (pg/ml) in competitive female distance will be significantly higher during the pre-season when compared to the post-season.

1.3 **Specific Aim 2:** To determine the effect of these energetic factors (energy availability, REE, and fasting concentrations of TT₃ and ghrelin) on distance running performance during a competitive time trial (test race time measured in minutes) following the competitive season in female distance runners.

The following hypotheses will be tested:

H1: Increases in energy availability (kcal/kg LBM) will be associated with improvements in performance (decreases in test race time measured in minutes) from pre-season to post-season.
H2: Increases in resting energy expenditure (kcal/day) will be associated with improvements in performance (decreases in test race time measured in minutes) from pre-season to post-season.

H3: Increases in fasting concentrations of TT$_3$ (ng/dl) will be associated with improvements in performance (decreases in test race time measured in minutes) from pre-season to post-season.

H4: Decreases in fasting concentrations of ghrelin (pg/ml) will be associated with improvements in performance (decreases in test race time measured in minutes) from pre-season to post-season.

1.4 Overview of Experimental Design:

We will use a prospective repeated measures design to investigate the change in energetic factors (energy availability, REE, and fasting concentrations of TT$_3$ and ghrelin) following a competitive season (pre- vs. post-season) in competitive female distance runners. This design will also allow us to determine the impact of the above mentioned energetic factors on distance running performance (test race time in minutes) in these women.

1.5 Participants:

Participants will be included in this study if they are: (1) women, (2) between the ages of 17-25 years, (3) in good health, (4) actively participating in competitive distance running on the Penn State varsity and club cross-country teams, (5) not a smoker, and (6) not pregnant, breast-feeding and or lactating.
1.6 Rationale:

The relationship between optimal nutrition and successful athletic performance is well-documented and notably critical in female athletes (Loucks 2004). In particular, female distance runners are at risk of developing a chronic energy deficiency or low energy availability due to the high amount of energy expended during training and competition in the absence of sufficient energy intake. Emphasis is placed on obtaining and maintaining proper energy reserves in this population as a means to provide the essential fuel for vital physiological processes, i.e., immune function, thermoregulation, and cellular maintenance (Wade, Schneider et al. 1996), as well as for performance. Chronic energy deficiency has been shown to have negative effects on reproductive (Williams, Helmreich et al. 2001; Loucks and Thuma 2003) and skeletal health (Drinkwater, Nilson et al. 1984; Cobb, Bachrach et al. 2003) in female athletes. Additionally, there are potential detrimental ramifications on performance and recovery from training (Myerson, Gutin et al. 1991; Edwards, Lindeman et al. 1993; Beidleman, Puhl et al. 1995) in female distance runners. To this end, a chronic energy deficiency or low energy availability is a key factor leading to the development of the Female Athlete Triad (Triad) (low energy availability, menstrual irregularities, low bone mass) in exercising girls and women (Nattiv, Loucks et al. 2007) and may have relevant implications for distance running performance.

To date, the relationship between low energy availability and distance running performance is unclear in female distance runners and serves as the rationale for conducting this work. Several studies have established the association between an energy deficiency and decrements in performance (Nindl, Friedl et al. 1997; Roemmich and
Sinning 1997; Friedl, Moore et al. 2000). However, to date, there is limited direct evidence on the effects of low energy availability on performance variables (i.e., test race time) in female distance runners. A study demonstrating the association between healthy energy availability and optimal distance running performance is needed in this area of research.

Additionally, this is the first study to explore the changes in important energetic factors, to include REE, TT$_3$ and ghrelin concentrations, following a competitive season in female distance runners. Such findings would provide us with a better understanding of the impact of energetic factors on distance running performance in female distance runners. Overall, this study may offer further insight on how to structure nutritional and exercise training programs for this sub-set of female athletes. Furthermore, prevention and treatment of an energy deficit may represent the key step in prevention of reproductive dysfunction, stress injury and avoidance of detrimental bone loss.

1.7 Expected Findings:

We hypothesize that increases in energy availability, REE, and TT$_3$ with concomitant decreases in ghrelin would promote improvements in distance running performance (specifically, decreases in test race time in minutes) in female distance runners following a competitive season. Since the pre-season typically involves a more rigorous training regimen (i.e., higher training volume and exercise energy expenditure), we predict that energy availability would be lower in competitive female distance runners in pre-season compared to post-season. In terms of energetic factors, we suggest that REE would be suppressed in the pre-season compared to the post-season and
additionally, key metabolic markers of energy status, suppressed TT3 and elevated ghrelin concentrations, would be indicative of an energy deficiency in the pre-season in competitive female distance runners. Improvements in energetic factors (increases in energy availability, REE, and TT3 with concomitant decreases in ghrelin) would be observed following the competitive season in these female athletes.

We anticipate to publish our findings and present at academic meetings in the area of sports medicine and exercise physiology. We expect our results to provide critical evidence on the role of energy availability and energy deficiency on distance running performance in competitive female distance runners.
1.8 References


Chapter 2

Literature Review

Energetic Factors Affecting the Female Athlete Triad and Performance in Athletic Women: A Focus on Female Long-Distance Runners

2.1 Introduction

The association between optimal energy intake and successful sports performance is well-established and notably critical in female athletes (Dueck, Matt et al. 1996; Loucks 2004; Rodriguez, Di Marco et al. 2009). Female athletes are at a greater risk of energy deficiency, compared to their sedentary counterparts, due to the substantial challenges in matching energy intake to the high amount of energy expended during training and competition (Rodriguez, Di Marco et al. 2009). Competitive long-distance running (>800m) is an example of an endurance sport wherein leanness and low body weight are considered advantageous for performance (Sundgot-Borgen and Torstveit 2010). Interestingly, only a small number of long-distance runners actually need to lose body fat for health reasons and therefore, motives for reductions in body fat levels are often associated with increasing power to weight ratio (enhancing movement of body mass against gravity) (Maughan and Burke, 2011). As such, female long-distance runners often engage in dietary energy manipulations, typically energy restriction, in order to maintain or lose weight (particularly following the off-season or while injured, wherein an energy surplus may ensue). However, such nutritional strategies are often counterproductive and a reduction in daily energy intake of 1,000 kilocalories per se can
compromise recovery and hinder training adaptations in female athletes, especially those presenting with low energy availability (EA) (Rodriguez, Di Marco et al. 2009).

Several investigators have linked energy restriction to impairments in markers of performance (Mulligan and Butterfield 1990; Wilmore, Wambsgans et al. 1992; Edwards, Lindeman et al. 1993; Beidleman, Puhl et al. 1995; Nindl, Friedl et al. 1997; Friedl, Moore et al. 2000) and it is hypothesized that decreases in body mass, specifically loss of lean body mass, may negatively influence muscular endurance and strength in athletes. Beyond performance-related decrements, chronic energy deficiency may also impair general health, to include negative effects on reproduction (Williams, Helmreich et al. 2001; Loucks and Thuma 2003) and bone health (Drinkwater, Bruemner et al. 1990; Cobb, Bachrach et al. 2003). To this end, a chronic energy deficiency is suggested as a key factor leading to the development of the Female Athlete Triad (Triad) (low energy availability (EA), menstrual irregularities, low bone mineral density (BMD)) in female athletes (Nattiv, Loucks et al. 2007) (Figure 2.1), with proposed detrimental implications for performance.

2.2 Prevalence of the Female Athlete Triad in Female Long-Distance Runners

The Triad and related clinical outcomes are well-documented in female long-distance runners. The prevalence of menstrual disturbances is high among female long-distance runners, ranging from 3-38% and 6-19% in cross-country (Dale, Gerlach et al. 1979; Lutter and Cusman 1982; Sanborn, Martin et al. 1982; Wakat, Sweeney et al. 1982; Winters, Adams et al. 1996; De Souza, Miller et al. 1998; Cobb, Bachrach et al. 2003; Thompson 2007; Barrack, Rauh et al. 2008; Pollock, Grogan et al. 2010) and marathon
runners (Shangold and Levine 1982; Glass, Deuster et al. 1987), respectively. Previous findings also indicate a higher proportion of elite female athletes, up to 70%, compared to controls, present with clinical eating disorders and/or disordered eating behavior, particularly athletes participating in sports focused on leanness, aesthetic appearance, and weight control, such as long-distance running (Sundgot-Borgen and Torstveit 2010). In terms of bone health, the prevalence of osteopenia in amenorrheic runners ranges from 1-50% (Rutherford 1993; Pettersson, Stalnacke et al. 1999; Barrack, Rauh et al. 2008) the incidence of osteoporosis is slightly lower, 0-33% (Rutherford 1993; Pettersson,
Stalnacke et al. 1999; Barrack, Rauh et al. 2008; Pollock, Grogan et al. 2010). Estimates of low EA in female long-distance runners are not well-documented and further investigation of its prevalence may lend valuable insight.

To date, only one published report in female long-distance runners exists, wherein the prevalence of all 3 Triad conditions occurring simultaneously was determined (Pollock, Grogan et al. 2010). In this study (Pollock, Grogan et al. 2010), the investigators found that menstrual dysfunction, disordered eating, and low BMD were coexistent in 15.9% of the female athletes, which is notably higher compared to data of the prevalence of the Triad in other samples of athletic groups (mean: 1.3%) (Torstveit and Sundgot-Borgen 2005; Vardar, Vardar et al. 2005; Beals and Hill 2006; Nichols, Rauh et al. 2006; Burrows, Shepherd et al. 2007; Hoch, Jurva et al. 2007; Hoch, Pajewski et al. 2009; Schtscherbyna, Soares et al. 2009). Despite the lack of evidence on the prevalence of the “full” Triad syndrome, several other studies have demonstrated associations between bone health and menstrual function (Drinkwater, Nilson et al. 1984; Drinkwater, Bruemner et al. 1990; Cobb, Bachrach et al. 2003; Thompson 2007; Pollock, Grogan et al. 2010); eating behavior and menstrual function (Cobb, Bachrach et al. 2003; Barrack, Rauh et al. 2008; Pollock, Grogan et al. 2010); and eating behavior and bone health (Cobb, Bachrach et al. 2003; Barrack, Rauh et al. 2008; Pollock, Grogan et al. 2010) in female long-distance runners. However, further examination of these specific combinations in female long-distance runners would provide a more accurate depiction of the magnitude and implications of the Triad in this specific athletic population.
2.3 Objective and Rationale for this Review

To date, the energetic factors affecting performance in female long-distance runners at-risk for the Triad are unclear and previous reports are inconclusive on whether an energy deficit in this specific athletic population is detrimental or advantageous. Athletes are recommended to consume adequate energy intake during periods of high-intensity and/or long duration training to maintain body weight and health and optimize training effects (Rodriguez, Di Marco et al. 2009). Energy restriction may promote loss of muscle mass (Koutedakis, Pacy et al. 1994; Koral and Dosseville 2009) and increased risk of fatigue, injury, and illness (Hagmar, Hirschberg et al. 2008), and accordingly, ineffective recovery from training and competition (Burke, Kiens et al. 2004). Recovery and replenishment of fuel stores are dependent on proper energy consumption and compensation, and specifically, sufficient energy, to include macro- and micro-nutrients, fluids, and electrolytes, must replace energy losses during habitual exercise training. Female long-distance runners who fail to replace energy or utilize harmful dietary strategies are at a significantly greater risk of the Triad clinical sequelae independent or in combination with decrements in performance (Nattiv, Loucks et al. 2007; Rodriguez, Di Marco et al. 2009). The purpose of this review is to summarize our current understanding of the effect of energetic factors on Triad-related clinical outcomes and performance in female long-distance runners. We will also briefly discuss the gaps in the literature for which future investigation should be focused with respect to this particular area of research in female long-distance runners.
2.4 Overview of Energy Conservation Mechanisms in Energy-Deficient Female Athletes

In female athletes, an energy deficiency often occurs in the presence of inadequate dietary energy intake relative to energy expenditure (De Souza and Williams 2004). The sport environment represents a unique context wherein there is considerable pressure to achieve successful performance, and female athletes may consciously or inadvertently under eat and/or participate in excessively high volumes of training. As a result, energy conservation mechanisms may be initiated and acutely affect energy expenditure, i.e., suppression of resting energy expenditure (REE) (Myerson, Gutin et al. 1991; De Souza, Lee et al. 2007), and metabolic hormone concentrations, including reduced total triiodothyronine (T3) (Myerson, Gutin et al. 1991; Loucks and Callister 1993; Loucks and Heath 1994; De Souza, Lee et al. 2007), insulin-like growth factor-1 (IGF-1)/IGF binding protein-1 (Laughlin and Yen 1996), leptin (Laughlin and Yen 1997), and insulin concentrations (Laughlin and Yen 1996), and elevated cortisol (Loucks, Mortola et al. 1989; De Souza, Maguire et al. 1991; Laughlin and Yen 1996), growth hormone (Laughlin and Yen 1996), and ghrelin (De Souza, Leidy et al. 2004). Consequently, less critical physiological processes, such as growth and reproductive function (Wade, Schneider et al. 1996), may be compromised to maintain energetic partitioning to vital physiological processes, such as thermoregulation and cellular maintenance, and result in serious clinical consequences.

Furthermore, links between an energy deficiency and disruptions in luteinizing hormone (LH) pulsatility (Loucks, Verdun et al. 1998) and menstrual cyclicity (Williams, Helmreich et al. 2001) have been well investigated, and thus, it is evident that the
induction of menstrual disturbances is an unfavorable outcome of this 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 (De Souza 2003). It has been postulated that an energy deficiency plays an important role in inducing menstrual dysfunction and alternatively, when adequate energy status is restored, the resumption of menses should hypothetically occur. It is specifically the changes in estrogen exposure in female athletes, which when suppressed, promotes pathological bone loss (Nattiv, Loucks et al. 2007) and other clinical sequelae, such as endothelial dysfunction (Hoch, Jurva et al. 2007; O'Donnell, Harvey et al. 2007).

2.5 The Relationship between Energy Availability, Metabolism and Reproductive Function

The most recent conceptual model of the Triad defines low EA (with or without disordered eating) as an etiological factor in the development of the Triad (Nattiv, Loucks et al. 2007) (Figure 2.1). Loucks et al. (2011) define EA as dietary energy intake (EI) minus the energy expended in exercise (EEE) adjusted for kilograms of lean body mass (LBM) (EA = EI – EEE/kg LBM). It is important to differentiate EA from energy balance (EB), a measure of energy status commonly used by clinicians and dieticians to indicate whether an individual is energy replete or in an energy surplus or deficit. EB is the amount of dietary EI minus total energy expenditure (TEE) (EB = EI – TEE). EB accounts for the body’s available energy after all of the necessary physiological processes of the body have taken place. Loucks et al. (2011) have suggested that the calculation of
EA is superior to any calculation of 24hr EB because it involves measurement of only one component of 24hr EE vs. measuring all of the components of 24hr EE (i.e., non-exercise activity thermogenesis and thermic effect of food) (Loucks et al., 2011). Additionally, EA can be measured with respect to any one physiological process that expends energy which is not available for other metabolic processes, i.e. cellular metabolism and thermoregulation. Therefore, EA was used in the Triad model as opposed to EB, because EA focuses specifically on the energy expended during exercise (Nattiv, Loucks et al. 2007). Nonetheless, it remains a challenge for exercising women to estimate their energy requirements. Furthermore, the effect of EA on performance has yet to be explored, nor has the threshold of 30 kcal/kg LBM been examined as a cut-off below which negative effects are translated to markers of performance at the whole-body, running velocity or efficiency, or at the cellular level, i.e., muscle protein turnover, lactate removal, or muscle glycogen content.

Low EA has a suppressive effect on both metabolic and reproductive function in order to preserve energy stores for more vital physiological processes, such as thermoregulation, cellular maintenance and locomotion (Wade, Schneider et al. 1996). Evidence from short-term studies in sedentary, regularly menstruating women (Loucks and Callister 1993; Loucks and Heath 1994; Loucks, Verdun et al. 1998; Loucks and Thuma 2003) and longitudinal experiments in female cynomolgus monkeys (Williams, Caston-Balderrama et al. 2001; Williams, Helmreich et al. 2001) provided the basis for which EA is observed as a relevant factor affecting metabolism and reproduction. As such, the suppressive effects of low EA on fasting T3 concentrations (Loucks and Heath 1994), luteinizing hormone (LH) (Loucks, Verdun et al. 1998; Loucks and Thuma 2003)
and menstrual cyclicity (Williams, Helmreich et al. 2001) are well-elucidated in the literature.

Loucks and colleagues (Loucks and Callister 1993; Loucks and Heath 1994) first examined the effect of manipulations of EA on metabolism, using T3 as the proposed marker of metabolic status, in healthy young regularly menstruating sedentary women. Suppressed T3 was shown to occur in these participants as a result of an energy deficit and was then prevented with appropriate increases in dietary EI (Loucks and Callister 1993). Furthermore, these effects on T3 were independent of exercise intensity and specifically, it was the energy cost of exercise on EA that impacted T3 concentrations. Loucks et al. (1994) revealed in a follow-up study that reductions in T3 (16%) were induced abruptly between 19 and 25 kcal/kg LBM. These researchers then became interested in the effects of these EA manipulations on reproductive function, specifically LH pulsatility. To date, it was unclear whether exercise stress was the mechanism for which disruptions in LH pulsatility occurred and presumably, translate to exercise-associated amenorrhea. Interesting, these results from these experiments disproved the hypothesis that LH pulsatility is disrupted by exercise stress and as such, the investigators proposed that changes in EA induced concomitant changes in LH pulsatility (Loucks, Verdun et al. 1998). Loucks et al. (2003) then investigated the dependence of LH pulsatility on varying EA conditions (balanced, 45 kcal/kg LBM vs. restricted, 10, 20, and 30 kcal/kg LBM) in 29 regularly menstruating, sedentary women. Their results demonstrated that LH pulsatility is disrupted below an EA threshold of 30 kcal/kg LBM, wherein LH pulse frequency decreased and LH pulse amplitude increased.
In prospective experiments in female cynomolgus monkeys (Williams, Caston-Balderrama et al. 2001; Williams, Helmreich et al. 2001), Williams and colleagues evaluated the role of low EA on both the development and reversal of exercise-induced amenorrhea. The researchers gradually increased the daily exercise (12 km/day over 7- to 24-month period) without any changes in food intake in eight adult female monkeys which resulted in the development of amenorrhea. Despite any significant weight loss, the monkeys were observed to have increased menstrual cycle lengths and a 28% decrease in T3 concentrations. In four of the eight amenorrheic monkeys, there were given appropriate energy to reverse the energy deficit (138-181% of energy intake) without any changes in daily exercise. All of these monkeys presented with increases in reproductive hormone concentrations (LH, FSH, estradiol, and progesterone) and eventually, ovulatory cycles were restored. These findings support the premise that metabolic and reproductive adaptations occur as a means to conserve energy, without significant changes in body weight (Williams, Helmreich et al. 2001). Alternatively, increases in EA may appropriately compensate for the underlying energy deficit associated with amenorrhea in exercising monkeys and as such, restoration of regular menstrual function will occur.

2.6 Low Energy Availability in Female Long-Distance Runners

Female long-distance runners and other female athletes may reduce EA for several reasons: 1) intentional, i.e., to improve performance by modifying body size and composition; 2) compulsive, i.e., as the result of a disordered eating behavior or pathological weight control; or 3) inadvertent, i.e., failing to match EI to EEE (Loucks, Kiens et al. 2011). Female distance runners typically adopt restrictive eating behaviors as
a means to maintain or reduce their body weight or fat mass in the effort to achieve optimal distance running performance. As such, female long-distance runners are more likely to reduce EA and are more susceptible per se to inducing an energy deficit by failing to compensate for their energy expending activities. Despite expending more energy, runners may not appropriately increase EI compared to control groups (Mulligan and Butterfield 1990; Wilmore, Wambsgans et al. 1992; Beidleman, Puhl et al. 1995). In a prevalence study in high school female athletes by Hoch et al. (2009), the investigators demonstrated that 6% of athletes experience low EA. In studies in college athletes, investigators have reported higher prevalence estimates of low EA ranging from 12-33% (Reed, De Souza et al. 2013).

Numerous reports on low EI in female long-distance runners exist in the literature (Mulligan and Butterfield 1990; Myerson, Gutin et al. 1991; Wilmore, Wambsgans et al. 1992; Edwards, Lindeman et al. 1993; Beidleman, Puhl et al. 1995; Thompson and Manore 1996) where investigators report EI in female runners ranging from 1,272-2,937 kcal/d. However, when athletes are categorized by menstrual status (amenorrheic vs. eumenorrheic), the amenorrheic athletes demonstrate lower EI compared to their eumenorrheic counterparts (1,272-1,950 kcal/d vs. 1,690-2,937 kcal/d) (Marcus, Cann et al. 1985; Wilmore, Wambsgans et al. 1992; Beidleman, Puhl et al. 1995; Tomten and Hostmark 2006). Findings from Edwards et al. (1993) exhibited that TEE (measured by doubly-labeled water) was greater than EI (measured by diet logs) resulting in a 32% imbalance. Similarly, Beidleman et al. (1995) demonstrated that reported EI was lower than EE in female runners by a larger deficit (-987kcal) vs. controls (-395kcal). Such reductions in EI are often linked to abnormal eating attitude/behavior and disturbed body
image (Myerson, Gutin et al. 1991; Edwards, Lindeman et al. 1993). It is possible that an energy deficit may result from underreporting and thus, findings with respect to self-reported EI and EE are difficult to interpret.

Table 2.1 summarizes the studies assessing the components of EA (EI and EEE) in female long-distance runners specifically. Our review of the studies resulted in five studies wherein the investigators determined EA or provided data from which we could estimate EA in female long-distance runners (Myerson, Gutin et al. 1991; Wilmore, Wambsgans et al. 1992; Beidleman, Puhl et al. 1995; Kopp-Woodroffe, Manore et al. 1999; Tomten and Hostmark 2006). EA values in these studies ranged 19-59 kcal/kg LBM. Alternatively, studies in which EB was determined predominantly exhibited that female long-distance runners presented in a negative energy balance (Mulligan and Butterfield 1990; Beidleman, Puhl et al. 1995). In a review by Loucks (2007), 25 studies of female eumenorrheic runners and 12 studies of female amenorrheic runners were examined and demonstrated a range in EA of 12-29 kcal/kg FFM. An EA threshold of 30 kcal/kg FFM is advanced as a discriminating factor between women with energy conservation and reproductive dysfunction (below 30 kcal/kg FFM) and those women that are energy replete and regularly menstruating (above or equal to 30 kcal/kg FFM). However, this threshold has yet to demonstrate utility in a field setting and future research is necessary to evaluate menstrual cyclicity in female long-distance runners with an EA below 30 kcal/kg FFM.
Table 2.1. Studies assessing the components of energy availability (EA) (energy intake (EI) and exercise energy expenditure (EEE)) in female long-distance runners.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Study Population</th>
<th>Methods</th>
<th>EA (kcal/kg LBM or FFM)</th>
<th>EI (kcal/d)</th>
<th>EEE (kcal/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilmore et al. 1992</td>
<td>Amen runners (n=8) Mean Age: 20.4±1.6 yr Mean BMI: 18.4 kg/m²</td>
<td>EI: 3-day diet logs EEE: 3-day activity diary FFM: Hydrostatic weighing</td>
<td>Amen: 28.5±11.6</td>
<td>Amen: 1781±283</td>
<td>Amen: 476±247</td>
</tr>
<tr>
<td></td>
<td>Eumen runners (n=5) Mean Age: 23.6±5.7 yr Mean BMI: 18.7 kg/m²</td>
<td></td>
<td>Eumen: 27.6±12.5</td>
<td>Eumen: 1690±272</td>
<td>Eumen: 402±222</td>
</tr>
<tr>
<td>Myerson et al. 1991</td>
<td>Amen runners (n=7) Mean Age: 29.9±1.5 yr Mean BMI: 19.4 kg/m²</td>
<td>EI: 24-hr dietary recall, 6-day diet logs EEE: Measurement of energy cost at average reported training pace (multiplied by average km/day) FFM: Hydrostatic weighing</td>
<td>Amen: 27.5</td>
<td>Amen: 1729±236</td>
<td>Amen: 526±48</td>
</tr>
<tr>
<td></td>
<td>Eumen runners (n=10) Mean Age: 29.1±1.2 yr Mean BMI: 19.5 kg/m²</td>
<td></td>
<td>Eumen: 32.5</td>
<td>Eumen: 1933±382</td>
<td>Eumen: 537±38</td>
</tr>
<tr>
<td>De Souza et al. 1998</td>
<td>Sedentary eumen women (n=11) Mean Age: 26.2±1.2 yr Mean BMI: 23.0 kg/m²</td>
<td>EA was compared by cycle</td>
<td>SedOvul: 30.0±1.2</td>
<td>SedOvul:1804.7±64.6</td>
<td>SedOvul: 0</td>
</tr>
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<td></td>
<td>Recreational female runners (n=24) Mean Age: 27.8±1.3 yr Mean BMI: 21.7 kg/m²</td>
<td>EI: 7-day prospective nutritional diaries were completed every month during the study period in the early follicular phase EEE: Training activities were recorded on training cards BW: Body Weight measured in kg</td>
<td>ExOvul: 23.3±1.6</td>
<td>ExOvul:1837.0±79.6</td>
<td>ExOvul: 479.5±53.0</td>
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<td></td>
<td>SedOvul (n=28 cycles) ExOvul (n=24 cycles) ExLPD (n=21 cycles) ExAnov (n=6 cycles)</td>
<td></td>
<td>ExLPD: 26.5±1.8</td>
<td>ExLPD:1992.6±84.8</td>
<td>ExLPD: 494.3±64.1</td>
</tr>
<tr>
<td></td>
<td>Loucks et al. 2007 25 studies of female eumen runners 12 studies of female amen runners</td>
<td>EI, training mileage and body composition data was used to estimate EA</td>
<td>Range of EA: 12 - 29</td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>Methods</td>
<td>Results</td>
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<tr>
<td>Beidleman et al. 1995</td>
<td>Amen runners (n=10)</td>
<td>EB was determined (EI-EE)</td>
<td>Amen: -22.2±9.36</td>
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<tr>
<td></td>
<td>Mean age: 21.5±0.6 yr</td>
<td>EI: 3-day dietary records</td>
<td>Amen: 1950±121</td>
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<td></td>
<td>Mean BMI: 21.5 kg/m²</td>
<td>EE: 3-day period from individual HR/VO₂ curves during rest and exercise,</td>
<td>Amen: 2936±224</td>
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<td></td>
<td></td>
<td>thermic effect of meals</td>
<td>Eumen: 2223±122</td>
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<td></td>
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<td>FFM: Hydrostatic weighing</td>
<td>Eumen: 215±7.5kg/m²</td>
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<td></td>
<td>Eumen runners (n=10)</td>
<td></td>
<td>Eumen: -8.7±28.7</td>
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<td></td>
<td>Mean age: 24.1±1.1 yr</td>
<td></td>
<td>Eumen: 1829±92.9</td>
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<tr>
<td></td>
<td>Mean BMI: 21.6 kg/m²</td>
<td></td>
<td>Eumen: 215±7.5kg/m²</td>
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<tr>
<td>Mulligan and Butterfield 1990</td>
<td>Non-runners (n=5)</td>
<td>EB was determined (EI-EE) Energy Balance (EI-EE)</td>
<td>Non-runners: -42±484</td>
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<tr>
<td></td>
<td>Mean age: 30.6±5.6 yr</td>
<td>EI: Weighed food records</td>
<td>Non-runners: 1744±367</td>
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<td></td>
<td>EE: Minute-by-minute activity diaries</td>
<td>Non-runners: 1786±208</td>
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<td></td>
<td></td>
<td>FFM: Hydrostatic weighing</td>
<td>Non-runners: 22±11±516</td>
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<td>Moderately active runners: V02max =</td>
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<td></td>
<td>&lt;58 ml/kg/min (n=9)</td>
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<td></td>
<td>Mean age: 37.0±4.2 yr</td>
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<td></td>
<td>Very active runners: V02max =</td>
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<tr>
<td></td>
<td>&gt;63 ml/kg/min (n=7)</td>
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<td></td>
<td>Mean age: 29.8±8.0 yr</td>
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<tr>
<td>Kopp-Woodroffe et al. 1999</td>
<td>Collegiate runner (19yr and BMI of 20.4 kg/m²)</td>
<td>EI: Self-reported 7-day consecutive weighed-food records were used (pre- and post-20-week program)</td>
<td>Pre-intervention: EA=20.16</td>
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<td></td>
<td></td>
<td>EE: Self-reported 7-day activity records (pre- and post 20-week program)</td>
<td>Pre-intervention: EI=1729</td>
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<td></td>
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<td>FFM: Hydrostatic weighing</td>
<td>Pre-intervention: EEE=628</td>
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<tr>
<td>Deuster et al. 1986</td>
<td>Elite distance runners (n = 51)</td>
<td>EI: Self-reported 3-day dietary records</td>
<td>Not Reported</td>
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<tr>
<td></td>
<td>Mean age: 29.1±0.8 yr</td>
<td></td>
<td>EI: 2397±104</td>
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<tr>
<td></td>
<td>Mean BMI: 19.1 kg/m²</td>
<td></td>
<td>Not Reported</td>
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<tr>
<td>Kaiserauer et al. 1989</td>
<td>Eumen runners (n=9)</td>
<td>EI: 3d diet records</td>
<td>Not Reported</td>
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<tr>
<td></td>
<td>Mean age: 26±3 yr</td>
<td></td>
<td>RMR: 2490</td>
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<tr>
<td></td>
<td>Mean BMI: 19.5±0.3 kg/m²</td>
<td></td>
<td>RMSC: 1688</td>
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<td></td>
<td>Eumen Controls (n=7)</td>
<td></td>
<td>AR: 1582</td>
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<td></td>
<td>Mean age: 22±1 yr</td>
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<td></td>
<td>Mean BMI: 21.8±1.1 kg/m²</td>
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<td></td>
<td>Amen runners (AR) (n=8)</td>
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<td></td>
<td>Mean age: 27±2</td>
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<tr>
<td></td>
<td>Mean BMI: 18.8±0.6</td>
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<tr>
<td>Study</td>
<td>Group Description</td>
<td>Age (Mean±SD)</td>
<td>BMI (Mean)</td>
<td>EI Description</td>
<td>EI Value (Mean±SD)</td>
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<tr>
<td>Nelson et al. 1986</td>
<td>Amen runners (n=11)</td>
<td>25.2±1.47</td>
<td>20.5</td>
<td>Self-reported 3-day diet diary</td>
<td>1730±152</td>
</tr>
<tr>
<td></td>
<td>Eumen runners (n=17)</td>
<td>29.2±1.23</td>
<td>20.3</td>
<td></td>
<td>Not Reported</td>
</tr>
<tr>
<td>Drinkwater et al. 1984</td>
<td>Amen runners (n=14)</td>
<td>24.9±1.3</td>
<td>19.7</td>
<td>3-day diet logs</td>
<td>1622.7±145.1</td>
</tr>
<tr>
<td></td>
<td>Eumen runners (n=14)</td>
<td>25.5±1.4</td>
<td>21.1</td>
<td></td>
<td>Not Reported</td>
</tr>
<tr>
<td>Marcus et al. 1985</td>
<td>Amen runners (n=11)</td>
<td>20.0 ± 0.4</td>
<td>18.6</td>
<td>3-day diet record, dietary interview, and nutritional assessment</td>
<td>1272±136</td>
</tr>
<tr>
<td></td>
<td>Eumen runners (n=6)</td>
<td>23.8±1.7</td>
<td>20.1</td>
<td></td>
<td>Not Reported</td>
</tr>
<tr>
<td>Tomten and Hostmark 2006</td>
<td>Regularly menstruating runners (R) (n=10)</td>
<td></td>
<td></td>
<td>Food and drink weighed on electronic scales and recorded for 2 week days and 1 weekend-day, food diaries,</td>
<td>R: 58.8 kcal/kg LBM</td>
</tr>
<tr>
<td></td>
<td>Irregularly menstruating runners (IR) (n=10)</td>
<td></td>
<td></td>
<td></td>
<td>IR: 40.0 kcal/kg LBM</td>
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</tbody>
</table>
2.7 Relationship Between Energy Status and Performance in Female Long-Distance Runners

Performance is operationally defined as the execution of a sport in a competitive environment (VanHeest and Mahoney 2007). However, this definition varies in a research setting. Researchers often measure the execution of performance indirectly by evaluating certain physiological factors associated with performance in athletes, such as submaximal or maximal oxygen uptake ($\text{VO}_{2}\text{max}$), blood lactate concentrations during and at maximal exercise, and muscle mass or fiber composition. A multitude of factors, in addition to energetic factors, may affect performance in female long-distance runners to include self-motivation, social support, athlete-coach relationship, and other intrapersonal and interpersonal skills (Iso-Ahola 1995). Psychological stress also can have direct and indirect implications on performance (Filaire, Maso et al. 2001; VanHeest and Mahoney 2007; Chaouachi, Leiper et al. 2012). As such, controlling for the external and internal factors affecting athletic performance is a challenge and therefore, athletes must be evaluated in the specific competitive event wherein all of these factors may predominate. Taken together, it is difficult to assess athletic performance in a research setting that best replicates the true sport environment and to date, there has yet to be an investigation that evaluates the effect of energy status on performance outcomes (i.e., time trial finishing time, running velocity) in female long-distance runners.

The primary goal of competitive athletic participation in female long-distance runners is the achievement of the fastest finishing race time. To achieve successful performance at this task, female long-distance runners strive to achieve a body size and body composition that minimizes resistance of movement against gravity (i.e., higher
muscle/fat ratio) in addition to optimally mobilize energy from several fuel sources, i.e., muscle and liver glycogen and adipose tissue triglycerides (Loucks 2004). In order for female athletes to continuously perform at an elite-level, a proper amount of EA is suggested to be advantageous in training and competition. Although runners often acutely reduce EA, performance may be negatively affected by chronically low EA, particularly in the presence of drastic weight loss and disruption of endocrine function (Gabel 2006). Loss of lean body mass negates training efforts specific to strength and endurance performance and may also translate detrimental effects to immune, endocrine, and musculoskeletal function (Burke, Loucks et al. 2006).

Prior research in men provides evidence of the relationship between both an acute and a chronic energy deficit on health outcomes in addition to physical performance (Nindl, Friedl et al. 1997; Friedl, Moore et al. 2000). In judo athletes, Filaire et al. (2001) determined that 7-day food restriction adversely affects physiological, psychological and performance outcomes. Similarly, acute weight loss via energy restriction impaired anaerobic performance in college wrestlers (Rankin, Ocel et al. 1996). However, when replenished with a 75% carbohydrate diet compared to a 47% carbohydrate diet over five hours after weight loss, performance tended to return to initial levels. In a study by Horswill et al. (1990), decrements in performance (i.e. sprint performance) concomitant with weight loss were also observed in wrestlers consuming a low-carbohydrate diet. Decrements in performance following an acute energy deficit are well-documented; however, the effects of severe weight loss on physical performance in young, lean, and healthy men are not often chronically detrimental (Nindl, Friedl et al. 1997). Nindl et al. (1997) demonstrated significant decreases in fat-free mass, strength and power-related
performance outcomes following an 8-week Army course with an energy deficit (1000 kcal/d). Notably, at 5 weeks post, fat-free mass and physical performance measures returned to initial values. In another study in army rangers, endocrine responses to energy deficiency in a multi-stressor environment (sustained workload, inadequate sleep and thermal strain) were characterized to include decreases in $T_3$, IGF-1, and testosterone (Friedl, Moore et al. 2000). Alternatively, refeeding improved this endocrine profile despite continuation of other stressors. Energy restriction studies (Horvath, Eagen et al. 2000; Zachwieja, Ezell et al. 2001) in women and men infer that a moderate energy deficiency may not be unfavourable for exercise performance. In a study by Zachwieja et al. (2001) in physically active men and women, five mile time trial time improved despite a two week reduction in energy (-750kcal.day$^{-1}$) and carbohydrate (5.2 vs. 3.7g.kg.day$^{-1}$) intake. However, the change in performance was not different compared to the control group. Fugde et al. (2006) confirmed previous evidence that Kenyan runners are in a negative energy balance (higher EEE compared to respective EI) during intense training and further, that despite a reduction in body mass, endurance running performance may be enhanced when consuming a high carbohydrate diet. In contrast, Horvath et al. (2000) showed that a reduction in EI and fat intake is not conducive to optimal endurance performance. Based on these findings in the literature, the effects of energetic factors on performance are inconclusive and future investigation in this research area specifically in female long-distance runners is necessary.
2.8 Metabolic Considerations in Female Runners: Changes in REE, T3 and Ghrelin

REE is a major determinant of total daily energy expenditure and is defined as the energy requirements to maintain physiological function and energy homeostasis. Suppressed REE is notably associated with energy deprivation (Shetty 1984) and is often observed alongside changes in metabolic hormones indicative of energy deficiency (Myburgh, Berman et al. 1999; De Souza, Lee et al. 2007). Specifically, energy deficiency translates to a cascade of energetic adaptations, including a reduction in REE, and simultaneous alterations in fasting circulating hormone concentrations, including reduced T3, to restore homeostasis (De Souza, Hontscharuk et al. 2007; De Souza, Lee et al. 2007). Previous reports in exercising women with FHA demonstrate an association between a suppressed REE and a chronic energy deficiency. Findings from studies in female runners (Myerson, Gutin et al. 1991; Wilmore, Wambsgans et al. 1992; Beidleman, Puhl et al. 1995) suggest that an adaptive response in reproductive function is associated with physiologic mechanisms to conserve energy in response to inadequate EI relative to EEE. De Souza et al. (2007) found decreases in REE controlled for fat-free mass (FFM) and serum T3 in exercising women with amenorrhea compared to those women with ovulatory cycles (sedentary and exercising). Moreover, exercising women with subclinical menstrual disturbances (consistently anovulatory or inconsistent presentations of ovulatory, LPD, or anovulatory cycles) also presented with lower REE controlled for FFM and T3 compared to sedentary, ovulatory women (De Souza, Lee et al. 2007). These findings confirm an association between REE and T3 in women categorized by exercise and menstrual status. Furthermore, a dose-response relationship exists between REE and T3 such that increases in energy conservation (reductions in
REE and T3) occur concomitant with increases in severity of menstrual dysfunction. Similarly Myerson et al. (1991) found significantly lower REE in amenorrheic runners vs. their eumenorrheic and sedentary control counterparts despite similar EEE among groups. As such, amenorrheic female runners may maintain an energy balance and stable body weight by way of a decrease in REE and other accompanying energy conservation responses (i.e., reductions in T3) in spite of consuming significantly lower EI than eumenorrheic runners.

T3 is the most active form of thyroid hormone and plays a significant role in the control of several physiological functions such as growth and development, metabolism, body temperature, and heart rate (Silverthorn 2009). T3 is tightly linked to regulation of metabolism based on its strong relationships with REE, oxygen consumption, and total energy expenditure (Danforth and Burger 1989). Experiments in animals and humans support the direct and indirect effect of energy and macronutrient intake on thyroid hormone status and REE (Wimpfheimer, Saville et al. 1979; Burger, Berger et al. 1980; Rosenbaum, Hirsch et al. 2000). As such, T3 is a key marker of adaptations to energy deficiency and exercise training. The reduction in T3 is inferred as a governing metabolic signal that may initiate energy conservation mechanisms to restore a eumetabolic state in underweight individuals (Danforth and Burger 1989). De Souza et al. (2007) found lower serum T3 concentrations in all exercising groups with menstrual disturbances compared to the sedentary ovulatory group. In women with anorexia nervosa, Onur et al. (2005) demonstrated suppressed plasma T3 concentrations concomitant with decreased REE. Alternatively, weight gain in these anorexic patients resulted in increases in T3 concentrations and REE independent of FFM (Onur, Haas et al. 2005). Studies in
sedentary, regularly menstruating women exposed to 4-5d manipulations of EA demonstrated that low-T3 syndrome was induced within four days following severe EA restriction and notably, only partial dietary compensation for EEE might be necessary to prevent reductions in T3 concentration (Loucks and Callister 1993; Loucks and Heath 1994). The mechanism whereby low T3 is developed may be explained by the energy cost of exercise, not necessarily the stress of exercise, and accordingly, reductions in T3 were prevented in a study by Loucks and Callister (Loucks and Callister 1993) by increasing dietary EI to match EEE. Suppressed T3 concentrations are also consistently observed in the presence of an energy deficit in amenorrheic athletes (Marcus, Cann et al. 1985; Myerson, Gutin et al. 1991; Loucks, Laughlin et al. 1992). Thus, T3 has been implicated as a relevant hormone associated with chronic energy deficiency sufficient to cause reproductive function in exercising women.

Ghrelin is a hormone secreted from a distinct endocrine cell type, also known as X/A-like or ghrelin cells, in the stomach and gastrointestinal tract (Kojima, Hosoda et al. 1999; Date, Kojima et al. 2000). In humans, ghrelin concentration rises before meals and falls within 1–2 h after meals (Cummings, Purnell et al. 2001), as well as increases during periods of fasting (Toshinai, Mondal et al. 2001). Ghrelin has become a well-known metabolic signal indicative of an energy deficit and is also involved in the restoration of energy homeostasis (Nakazato, Murakami et al. 2001). Ghrelin concentrations in normal weight young women have been shown to be responsive to decreases in body weight, fat mass, and REE (Leidy, Gardner et al. 2004). Substantial evidence of elevated fasting ghrelin concentration in anorexic and amenorrheic women is documented (Tanaka, Naruo et al. 2003; Tolle, Kadem et al. 2003; Ackerman, Slusarz et al. 2012). De Souza et al.
(2004) demonstrated that amenorrheic female athletes exhibit at least 85% higher fasting ghrelin concentrations compared to control groups. Interestingly, despite presenting with elevated fasting ghrelin, amenorrheic female athletes notably display low EI and a metabolic profile consistent with chronic energy deficiency. Alongside this increase in ghrelin concentrations, peptide YY (PYY) concentrations were also elevated in exercising women with hypothalamic amenorrhea (Scheid, Williams et al. 2009). PYY also plays an important role in the regulation of energy homeostasis and to this end, chronically elevated fasting PYY may represent a physiological mechanism in support of energy restriction in exercising women with FHA despite elevated fasting ghrelin concentrations. Ghrelin and PYY are suggested as critical signals linked to the development of clinical outcomes associated with reproductive dysfunction as observed in models of chronic energy deficiency, i.e., women with anorexia nervosa and exercising women with FHA.

**2.9 Conclusions**

Female athletes often engage in dietary energy manipulations for intentional, compulsive, or inadvertent reasons subsequent to the development of chronic energy deficiency. Chronic energy deficiency typically occurs in athletes participating in leanness-focused sports, i.e., long-distance running. Chronic energy deficiency is a key factor associated with the Female Athlete Triad, an interrelated syndrome of low EA with or without disordered eating, menstrual dysfunction, and low bone mineral density presenting alone or in combination (Nattiv, Loucks et al. 2007). The prevalence of low EA/chronic energy deficiency in female long-distance runners is not well-established.
Furthermore, there are several ways in which energy status has been defined in the literature and to this end, it is unclear which energetic factor has the greatest influence on Female Athlete Triad related clinical and performance outcomes. The measurement of competitive performance is notably challenging and several factors must be controlled for, including intrapersonal and interpersonal skills, psychological stress, and other environmental factors. Prior research in male wrestlers, judo athletes, and army rangers demonstrated the negative consequences of acute and chronic energy deficiencies on health outcomes and physical performance. However, to our knowledge, no investigators have examined the association between energy status and performance in high-risk female athletic groups, such as long-distance runners. Metabolic hormones, such as T3 and ghrelin, serve as key markers of adaptations to energy deficiency and exercise training. Future investigation is necessary to characterize changes in energetic factors (i.e., REE, T3, and ghrelin) across a competitive season in female long-distance runners and further, determine the effect of energy status on performance outcomes measured under competitive conditions.
2.10 References


Chapter 3

Methods

3.1 Experimental design: This study was a prospective investigation examining energetic, metabolic, and performance characteristics in 18 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. REE was measured using indirect calorimetry and a ventilated hood system. Metabolic hormone concentrations were determined from a fasting blood sample assayed for TT₃ and ghrelin. Menstrual status was confirmed by self-report and menstrual history evaluation. Body composition was determined using DXA. The research staff measured test race time via a competitive 5km time trial on an accurately measured outdoor course. A maximal oxygen uptake (VO₂max) test was completed on a treadmill using indirect calorimetry and a modified Astrand protocol. EA was calculated by measuring EI via three-day nutritional logs, EEE using the Ainsworth Compendium (Ainsworth et al., 2011), 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 athletic participation (i.e., injury, academic ineligibility). The Institutional Research Board approved this study and all participants
signed an informed consent document if ≥18yr or a parental guardian signed the informed consent if the participant was 17yr.

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 following questionnaires: (1) Health, Exercise and Nutrition Survey that included information on demographics, medical, exercise, menstrual, bone health, and nutrition history; (2) Stress and Recovery Survey; and (3) Perceived Stress Survey. Additional pre-season measurements included an REE test, a fasting blood sample, a body composition and bone density DXA scan, and a VO2max test. A competitive 5km time trial was completed to determine test race time and running velocity. Interim body weight and survey completion took place mid-season. 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/m²). Body composition was assessed using dual-energy x-ray absorptiometry (DXA) during the pre- and post-season. Subjects were scanned on a GE Lunar iDXA (enCORE 2008 software version
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 REE:** 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 (°C), humidity (%H₂O), and barometric pressure (mmHg) were measured. Participants were instructed not to exercise/only participate in light training or ingest caffeine within 24 h, refrain from ingesting food and alcohol within 12 h 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 (Harris and Benedict 1919) \[655.0955 + 9.5634 \text{(weight)} + 1.8495 \text{(height)} - 4.6756 \text{(age)}\].

REE measurements were performed between 0830 and 1100 h 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 (VO₂; mL/min) and carbon dioxide production (VCO₂; mL/min) were measured every 20 s. To calculate REE, data for VO₂ and VCO₂ were only used if steady state was attained. Steady state was achieved when the volume of expired air, VO₂, and respiratory quotient values were not varying by more than 10%. REE was calculated using the Weir equation (Weir 1990): \[\text{REE (kcal/day)} = [3.94(\text{VO}_2) + 1.11(\text{VCO}_2)] \times 1.44.\]

A ratio of the actual REE to predicted REE (REE/pREE) was calculated once during each
monitoring period using the Harris–Benedict equation (Harris and Benedict 1919) and then averaged for the entire study period. To provide an estimate of energy status, we expressed REE as a ratio of actual to predicted REE based on the predicted calculated using the Harris–Benedict equation (Harris and Benedict 1919).

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 (Myburgh, Berman et al. 1999; De Souza, Hontscharuk et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011). Reductions in REE have been observed in several studies in exercising women with amenorrhea (De Souza, Hontscharuk et al. 2007; De Souza, West et al. 2008). We compared laboratory-assessed REE with a prediction equation for REE to estimate how much each individual’s measured REE deviated from the pREE. In anorexia nervosa, (Melchior, Rigaud et al. 1989; Polito, Fabbri et al. 2000; Konrad, Carels et al. 2007), most data published compared measured REE to the Harris-Benedict predicted REE (Harris and Benedict 1919). In these studies, investigators reported a suppressed ratio of measured REE to Harris-Benedict predicted REE (Harris and Benedict 1919) of 0.60-0.80 during periods of low body weight and before refeeding in anorexic women (Melchior, Rigaud et al. 1989; Polito, Fabbri et al. 2000; Konrad, Carels et al. 2007). 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 less than 0.9 is often considered energy deficient (De Souza, Hontscharuk et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011). 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≤REE/pREE<0.90), and energy replete (REE/pREE≥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 (De Souza, Hontscharuk et al. 2007). After allowing clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for fifteen minutes at 4°C. A 24-hour urinary sample, used to measure bone resorption, was also collected from the subjects who had fasted for the previous eight hours. The 24-hour urinary sample was collected from the subject following their first morning void of the first day up until the first morning void of the second day. Both the serum and urine samples were aliquoted into 2 mL polyethylene storage tubes and stored in a freezer at -80°C.

3.8 Serum hormone measurements: TT3 and ghrelin concentration were analyzed as previously described (De Souza, Leidy et al. 2004; De Souza, Hontscharuk et al. 2007). TT3 was analyzed using a chemiluminescence-based immuno assay analyzer, while the sensitivity for total ghrelin was measured using radioimmunoassay. 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 EI: Dietary EI (kcal/day) was assessed using three-day diet logs recorded for two week days and one weekend day, as previously described (De Souza, Hontscharuk et al. 2007; Gibbs, Williams et al. 2011). 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 (Goris and Westerterp 1999). Participants were recommended to weigh (ECKO Kitchen Scale, World Kitchen, LLC, Rosemont, IL, USA) or measure (using standard measuring 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 EEE: 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. (2000) 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.

3.11 EA: EA (kcal·kg\(^{-1}\) LBM) was determined as EI minus EEE relative to kg LBM [EA = (EI – EEE)/LBM (kg)] (Loucks and Callister 1993; Loucks and Heath 1994) using dietary EI data assessed from three-day diet logs, EEE 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 **VO\textsubscript{2max}:** Measurement of VO\textsubscript{2max} was performed during the pre- and post-season on a treadmill using indirect calorimetry and the modified Astrand protocol (Astrand and Saltin 1961). Gas exchanged was monitored continuously by a breath-by-breath system (SensorMedics Vmax metabolic cart; Yorba Linda, CA). We considered VO\textsubscript{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 (Saltin and Astrand 1967).

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 abnormal or severe if a volunteer was 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 normal/regular if a volunteer was 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.

3.15 **Training logs:** 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.

3.16 **Statistical analysis:** All data were analyzed using the SPSS for Windows (version 18.0, Chicago, IL) statistical software package. Data was tested for nonnormality, homogeneity of variance, and outliers before statistical hypothesis tests were performed. Since the TT₃ concentrations were not normally distributed, a logarithmic conversion of the TT₃ concentrations was used to normalize the data. 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 and ghrelin, EA, and test race time across the competitive season. Paired t-test and one-way ANOVA analyses were performed to confirm time and group effects, respectively. Primary correlational analyses were performed between distance running performance (test race time in minutes, running velocity, VO₂max) and (i) energetic variables (EA, EI, EEE, REE, and fasting concentrations of TT₃ and ghrelin), (ii) anthropometric/body composition variables (body mass, BMI, percent body fat, fat mass, and LBM), and (iii) exercise volume weekly minutes. Secondary correlational analyses were also performed (i) to determine the association between REE (REE, REE controlled for LBM, and REE/pREE) and fasting concentrations of Log TT₃ and ghrelin to corroborate our operation definition
of energy status and (ii) to determine the association between distance running performance (test race time in minutes, running velocity) and VO$_2$\textsubscript{max} to corroborate our performance markers. Correlational analyses were performed between primary and secondary outcome variables measured at pre- and post-season.
3.17 References


Chapter 4

Manuscript

The effect of energy status on energetic factors and performance across a competitive season in female long-distance runners

4.1 Introduction

The relationship between optimal nutrition and successful athletic performance is well-documented and notably critical in female athletes (Loucks 2004). Female athletes that participate in endurance or aesthetic sports (i.e., distance running, gymnastics, and figure skating) wherein leanness is considered advantageous for performance are often considered at a higher risk for energy deficiency, which is characterized by energetic and metabolic adaptations to inadequate energy intake (EI) relative to energy expenditure (Torstveit and Sundgot-Borgen 2005). Specifically, competitive distance running may represent a high pressure environment for weight control or loss translating to chronic energy deficiency (Sundgot-Borgen and Torstveit 2010). Female long-distance runners and other female athletes may induce a chronic energy deficiency for several reasons: 1) intentional, i.e., to improve performance by modifying body size and composition; 2) compulsive, i.e., as the result of a disordered eating behavior or pathological weight control; or 3) inadvertent, i.e., failing to match EI to EEE (Loucks, Kiens et al. 2011). Chronic energy deficiency is one of the key underpinning factors associated with the Female Athlete Triad (Triad) (low EA with or without disordered eating, menstrual irregularities, and low bone mineral density) in exercising women (Nattiv, Loucks et al.
The prevalence of low EA has been shown to range from 6 to 33% in female athletes (Loucks 2007; Hoch, Pajewski et al. 2009; Reed, De Souza et al. 2013) and as such, the effects of low EA and associated changes in energetic status on performance in female long-distance runners is an important research area to explore. Athletes are recommended to consume adequate energy intake during periods of high-intensity and/or long duration training to maintain body weight and health and optimize training effects (Rodriguez, Di Marco et al. 2009). To our knowledge, the energetic factors affecting performance in female long-distance runners are unclear and previous reports are inconclusive on whether an energy deficit in this specific athletic population is detrimental or advantageous to performance (Loucks 2004; Sundgot-Borgen and Torstveit 2010).

Chronic energy deficiency has a suppressive effect on both metabolic and reproductive function in order to preserve energy stores for more vital physiological processes, such as thermoregulation, cellular maintenance and locomotion (Wade, Schneider et al. 1996). Subsequent adaptations in resting energy expenditure (REE) and metabolic hormones, such as triiodothyronine (TT3), and ghrelin, occur to restore a eumetabolic state translating to a disruption of luteinizing hormone (LH) pulsatility (Loucks, Verdun et al. 1998; Loucks and Thuma 2003) and menstrual cyclicity (Williams, Helmreich et al. 2001) in human and animal experiments. As such, chronic energy deficiency is a primary factor promoting the induction of subclinical (luteal phase defects and anovulation) and clinical menstrual disturbances (functional hypothalamic amenorrhea and oligomenorrhea), which may result in musculoskeletal injury and low bone mineral density (Nattiv, Loucks et al. 2007). Particularly, the risk of a bone stress
injury in female long-distance runners may have a detrimental effect on their future running career and overall quality of life. Chronic energy deficiency may also directly affect performance, particularly in the presence of weight loss and disruption of endocrine function (Gabel 2006). Specifically, loss of lean body mass may negate training efforts specific to endurance performance and can translate negative effects to immune, endocrine, and musculoskeletal function (Burke, Loucks et al. 2006).

Several investigators have provided evidence of an association between an energy deficiency and decrements in performance in active populations (i.e., adolescent wrestlers and US army rangers) (Nindl, Friedl et al. 1997; Roemmich and Sinning 1997; Friedl, Moore et al. 2000). Acute energy restriction has been shown to adversely affect physiological, psychological, and performance outcomes (Horswill, Hickner et al. 1990; Rankin, Ocel et al. 1996; Horvath, Eagen et al. 2000; Filaire, Maso et al. 2001; Zachwieja, Ezell et al. 2001). However, the effect of chronic energy deficiency on performance is unclear. Findings from energy restriction studies (Horvath, Eagen et al. 2000; Zachwieja, Ezell et al. 2001) in women and men are inconclusive such that there is evidence that a moderate energy deficiency may not be unfavourable for exercise performance (Zachwieja, Ezell et al. 2001; Fudge, Westerterp et al. 2006); whereas other investigators (Horvath, Eagen et al. 2000; VanHeest, Rodgers et al. In Review) showed that energy restriction and menstrual dysfunction, markers of chronic energy deficiency, are not conducive to optimal endurance performance in female runners and swimmers. Optimally, during high volume in-season training, female competitive distance runners should match EI to energy expenditure to provide adequate metabolic fuel protective of lean body mass and physiological function. To date, the relationship between chronic...
energy deficiency and long-distance running performance in female long-distance runners has yet to be determined using a prospective study design. Additionally, there is limited data wherein performance (test race time) was directly measured in female long-distance runners categorized by energy status under competitive sport conditions. Overall, the objective of this study is two-fold (1) to characterize the change in energetic factors (REE, fasting concentrations of TT₃ and ghrelin, and EA) across a competitive season in female long-distance runners categorized by energy status and (2) to examine distance running performance during a competitive time trial (test race time) from pre- to post-season in female long-distance runners categorized by energy status. We hypothesize that: (1) female long-distance runners with severe energy deficiency will demonstrate a greater decrease in REE, EA, and fasting TT₃ with a concomitant greater increase in fasting ghrelin across the competitive season compared to runners with replete energy status and (2) female long-distance runners with severe energy deficiency will demonstrate a greater decrease in performance (decrease in test race time) from pre-season to post-season compared to female long-distance runners with replete energy status and moderate energy deficiency.

4.2 Methods

4.2A Experimental design: This study was a prospective investigation examining energetic, metabolic, and performance characteristics in 18 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. REE was measured using indirect calorimetry and a ventilated hood system. Metabolic hormone concentrations were determined from a fasting blood sample assayed for TT<sub>3</sub> and ghrelin. Menstrual status was confirmed by self-report and menstrual history evaluation. Body composition was determined using 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<sub>2</sub>max) test was completed on a treadmill using indirect calorimetry and a modified Astrand protocol. EA was calculated by measuring EI via three-day nutritional logs, EEE using the Ainsworth Compendium (Ainsworth et al., 2011), and lean body mass 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 athletic participation (i.e., injury, academic ineligibility). The Institutional Research Board approved this study and all participants signed an informed consent document if ≥18yr or a parental guardian signed the informed consent if the participant was 17yr.

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 completed the following questionnaires: (1) Health, Exercise and Nutrition Survey that included information on demographics, medical, exercise, menstrual, bone health, and nutrition history; (2) Stress and Recovery Survey; and (3) Perceived Stress Survey.
Additional pre-season measurements included an REE test, a fasting blood sample, a body composition and bone density DXA scan, and a VO2max test. A competitive 5km time trial was completed to determine test race time and running velocity. Interim body weight and survey completion took place mid-season. Participants were also given logs to continuously record their training and menstrual characteristics on a daily basis across the season.

4.2D **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/m²). Body composition was assessed using dual-energy x-ray absorptiometry (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.

4.2E **REE:** 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 (°C), humidity (%H₂O), and barometric pressure (mmHg) were measured. Participants were instructed not to exercise/only participate in light training or ingest caffeine within 24 h, refrain from ingesting food and alcohol within
12 h 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 (Harris and Benedict 1919) \[655.0955+9.5634 \text{(weight)}+1.8495 \text{(height)}-4.6756 \text{(age)}.\] REE measurements were performed between 0830 and 1100 h 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 20 s. 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 (Weir 1990): \[\text{REE (kcal/day)}=[3.94(\text{VO}_2)+1.11 (\text{VCO}_2)]\times1.44.\]

A ratio of the actual REE to predicted REE (REE/pREE) was calculated once during each monitoring period using the Harris–Benedict equation (Harris and Benedict 1919) and then averaged for the entire study period. To provide an estimate of energy status, we expressed REE as a ratio of actual to predicted REE based on the predicted calculated using the Harris–Benedict equation (Harris and Benedict 1919).

**4.2F Energy status:** Energy status was defined using an objective laboratory-based measure, REE, to identify individuals who exhibit energetic adaptation to an energy deficiency (Myburgh, Berman et al. 1999; De Souza, Hontscharuk et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011). Reductions in REE have been observed in several studies in exercising women with amenorrhea (De Souza, Hontscharuk et al.
2007; De Souza, West et al. 2008). We compared laboratory-assessed REE with a prediction equation for REE to estimate how much each individual’s measured REE deviated from the pREE. In anorexia nervosa, (Melchior, Rigaud et al. 1989; Polito, Fabbri et al. 2000; Konrad, Carels et al. 2007), most data published compared measured REE to the Harris-Benedict predicted REE (Harris and Benedict 1919). In these studies, investigators reported a suppressed ratio of measured REE to Harris-Benedict predicted REE (Harris and Benedict 1919) of 0.60-0.80 during periods of low body weight and before refeeding in anorexic women (Melchior, Rigaud et al. 1989; Polito, Fabbri et al. 2000; Konrad, Carels et al. 2007). 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 less than 0.9 is often considered energy deficient (De Souza, Hontscharuk et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011). 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≤REE/pREE<0.90), and energy replete (REE/pREE≥0.90).

4.2G 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 (De Souza, Hontscharuk et al. 2007). After allowing clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for fifteen minutes at 4°C. A 24-hour urinary sample, used to measure bone resorption, was also collected
from the subjects who had fasted for the previous eight hours. The 24-hour urinary sample was collected from the subject following their first morning void of the first day up until the first morning void of the second day. Both the serum and urine samples were aliquoted into 2 mL polyethylene storage tubes and stored in a freezer at -80°C.

**4.2H Serum hormone measurements:** TT3 and ghrelin concentration were analyzed as previously described (De Souza, Leidy et al. 2004; De Souza, Hontscharuk et al. 2007). TT3 was analyzed using a chemiluminescence-based immuno assay analyzer, while the sensitivity for total ghrelin was measured using radioimmunoassay. 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 EI:** Dietary EI (kcal/day) was assessed using three-day diet logs recorded for two week days and one weekend day, as previously described (De Souza, Hontscharuk et al. 2007; Gibbs, Williams et al. 2011). 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 (Goris and Westerterp 1999). Participants were recommended to weigh (ECKO Kitchen Scale, World Kitchen, LLC, Rosemont, IL, USA) or measure (using standard measuring 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 EEE: 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. (2000) 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 EA: EA (kcal·kg⁻¹ LBM) was determined as EI minus EEE relative to kg LBM [EA = (EI – EEE)/LBM (kg)] (Loucks and Callister 1993; Loucks and Heath 1994) using dietary EI data assessed from three-day diet logs, EEE 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.

4.2L 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.

4.2M VO₂max: Measurement of VO₂max was performed during the pre- and post-season on a treadmill using indirect calorimetry and the modified Astrand protocol (Astrand and Saltin 1961). Gas exchanged was monitored continuously by a breath-by-breath system.
(SensorMedics Vmax metabolic cart; Yorba Linda, CA). We considered VO\textsubscript{2}max to be achieved if three of the following four criteria were obtained: (1) attainment of age-predicted maximal heart rate \((208 – (0.7\times\text{age}))\); (2) respiratory exchange ratio \(\geq 1.1\); (3) plateau in oxygen consumption despite an increase in exercise workload; (4) attainment of a rating of perceived exercise score \(\geq 18\) (Saltin and Astrand 1967).

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 abnormal or severe if a volunteer was 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 normal/regular if a volunteer was 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.

4.2O Training logs: 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.

4.2P Statistical analysis: All data were analyzed using the SPSS for Windows (version 18.0, Chicago, IL) statistical software package. Data was tested for nonnormality, 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 and 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. Correlational analyses were performed between distance running performance (test race time in minutes) and our primary variables (EA, REE, and fasting concentrations of TT3 and ghrelin) to determine the association between distance running performance (test race time in minutes) and the above mentioned energetic factors (EA, REE, and fasting concentrations of TT3 and ghrelin) following a competitive season in female long-distance runners.

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. Study participants were 19.5±0.3 yr, weighed 54.6±1.4 kg with 22.9±0.8% body fat, and had a VO2max of 61.2±1.7 mL/kg/min. Participants were engaged in 409±53 min/week of purposeful exercise training in the past 6 months. Pre-season exercise training volume was 264±32 min/wk (Range: 83-510 min/wk). The mean exercise training volume across the competitive season (10-12 wk) was 248±33 min/wk. Pre-season 5km test race time in all participants was 21.4±0.6 minutes, which equals a mean running velocity of 8.9±0.3 mph. Participants presented with an average REE, REE controlled for LBM, and
REE/pREE of 1223±28 kcal/d, 31.0±0.9 kcal/kg LBM, and 0.88±0.02 REE/pREE, respectively. Of the total participants, 11% were amenorrheic, 17% were oligomenorrheic, 56% were eumenorrheic, and 17% were currently using oral contraceptives.

4.3B Anthropometric and body composition characteristics across a competitive season: Measured values of body mass, BMI, percent body fat, fat mass, and LBM across a competitive season in female long-distance runners grouped by energy status are shown in Table 4.2. There were no differences (p<0.05) in body mass, BMI, and LBM among groups at pre- or post-season. The severe ED group had a percent body fat 18% higher (p=0.012) than the energy replete group at pre-season and 18% (p=0.027) and 19% higher (p=0.018) than both the energy replete and moderate ED groups, respectively, at post-season. There was no difference in percent body fat between the moderate ED and energy replete group at pre- (p=0.229) and post-season (p=0.839). The severe ED group had a fat mass 20% (p=0.016) and 24% higher (p=0.006) than the moderate ED and energy replete groups, respectively, at pre-season and 28% (p=0.006) and 25% higher (p=0.011) than the moderate ED and energy replete groups, respectively, at post-season. There was no difference in fat mass between the moderate ED and energy replete group at pre- (p=0.649) and post-season (p=0.763). There were no time effects, group x time interactions, or group effects (p>0.05) for body mass, BMI, and LBM. There was a group x time interaction (p=0.011) and group effect (p=0.035) for percent body fat, however, no time effect was observed (p=0.982). Similarly, there was a group x time interaction (p=0.042) and group effect (p=0.011) for fat mass, however, no time effect was observed (p=0.464).
4.3C **REE characteristics across a competitive season:** Measured REE controlled for LBM and REE/pREE across a competitive season in female long-distance runners grouped by energy status are shown in Figure 4.1. REE controlled for LBM was 14% (p=0.020) and 21% lower (p=0.002) in runners with severe ED than the moderate ED and energy replete runners, respectively, at pre-season. As expected, REE/pREE was lower in the severe ED group than the moderate ED (p=0.002) and energy replete runners (p<0.001), respectively, at pre-season. At post-season, the severe ED group had lower REE controlled for LBM (p=0.027) and REE/pREE (p<0.001) than the energy replete group. The moderate ED group also demonstrated lower REE/pREE than the energy replete group at pre- (p=0.001) and post-season (p=0.006). REE was lower in the severe ED and moderate ED vs. the energy replete group at pre- (1125±34 kcal/d and 1188±24 kcal/d vs. 1355±24 kcal/d, p<0.001 and p=0.001, respectively) and post-season (1188±49 kcal/d and 1170±47 kcal/d vs. 1346±35 kcal/d, p=0.022 and p=0.013, respectively). There was no time effect and group x time interaction within groups (p>0.05) for REE, REE controlled for LBM, and REE/pREE. A group effect was observed for REE (p=0.001), REE controlled for LBM (p=0.015), and REE/pREE (p<0.001).

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 VO2max, are shown in Figure 4.2. At pre-season, the severe ED group had a test race time 16% slower (4.1 seconds) (p=0.005) than the moderate ED group. No differences in test race time were observed between the severe ED (p=0.141) and moderate ED groups (p=0.102) vs. the energy replete group at pre-season. However, at post-season, the severe ED group
had a test race time 16% (3.7 seconds) (p=0.002) and 11% slower (2.6 seconds) (p=0.023) than the moderate ED and energy replete groups, respectively. Pre-season running velocity was faster (p=0.005) in the moderate ED group vs. the severe ED group. At post-season the energy replete (p=0.040) and moderate ED groups (p=0.003) had a faster running velocity than the severe ED group. There were no differences in VO2max values among groups (p=0.249) at pre-season. However, VO2max at post-season was 17% (p=0.009) and 16% lower (p=0.014) in the severe ED group vs. the moderate ED and energy replete groups, respectively. No differences in VO2max were observed between the moderate ED and energy replete group at pre- (p=0.724) and post-season (p=0.844). There were trends in time (p=0.062) and group effects (p=0.052) for VO2max. However, there was no group x time interaction (p=0.367) for VO2max.

4.3F 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. EA did not differ among groups at pre- (p=0.524) or post-season (p=0.814). No time, group x time, and group effects were observed (p>0.05) for EA and EI. There was a group x time interaction (p=0.035) for EEE. However, no time (p=0.309) and group effects (p=0.925) were reported for EEE.

4.3G Metabolic hormones across a competitive season: Fasting Log TT3 and ghrelin concentrations across a competitive season in female long-distance runners grouped by energy status are shown in Figure 4.3. At pre-season, Log TT3 was lower (p=0.043) in the severe ED group vs. the moderate ED group. A trend to statistical significance for differences in pre-season Log TT3 (p=0.067) was observed between the severe ED group and the energy replete group. No differences were observed in pre-season Log TT3
(p=0.741) between the energy replete vs. the moderate ED group. Log TT3 was similar among groups at post-season (p=0.359). No time (p=0.293), time x group (p=0.097), and group effects (p=0.154) were observed for Log TT3. Ghrelin was similar among groups at pre- (p=0.427) and post-season (p=0.374). There was a time effect (p=0.005) for Ghrelin within groups. There were no group x time (p=0.900) and group effects (p=0.320) for Ghrelin.

4.3H Correlational Analyses: Correlational analyses between distance running performance and (i) energetic variables, (ii) anthropometric/body composition variables, and (iii) exercise training in female long-distance runners are presented in Table 4. Pre-season test race time was associated with pre-season EEE (r=-0.669, p=0.003) and pre-season exercise volume (r=-0.763, p<0.001). At pre- and post-season, test race time was positively correlated (p<0.05) with body mass, percent body fat, and fat mass. Pre-season running velocity was associated with pre-season EEE (r=0.693, p=0.002). Post-season running velocity was associated with post-season body mass (r= -0.523, p=0.045). At pre- and post-season, running velocity was negatively correlated with (p<0.05) with percent body fat and fat mass. At pre- and post-season, Log TT3 was positively correlated with REE controlled for LBM (r=0.521, p=0.032 and r=0.525, p=0.030, respectively). At pre- and post-season, VO2max was associated with test race time (r= -0.735, p=0.001 and r= -0.769, p=0.001 respectively) and running velocity (r=0.759, p=0.001 and r=0.751, p=0.002 respectively).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>19.5±0.3</td>
</tr>
<tr>
<td>Age of menarche (yr)</td>
<td>13.7±0.4</td>
</tr>
<tr>
<td>Gynecological age (yr)</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.7±1.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>54.6±1.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.9±0.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
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</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>12.5±0.6</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>39.8±1.1</td>
</tr>
<tr>
<td>Fat-Free Mass (kg)</td>
<td>42.1±1.1</td>
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<tr>
<td>REE (kcal/d)</td>
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</tr>
<tr>
<td>REE/LBM (kcal/kg LBM)</td>
<td>31.0±0.9</td>
</tr>
<tr>
<td>REE/pREE</td>
<td>0.88±0.02</td>
</tr>
<tr>
<td>Amenorrhea (%)</td>
<td>11.1</td>
</tr>
<tr>
<td>Oligomenorrhea (%)</td>
<td>16.7</td>
</tr>
<tr>
<td>Eumenorrhea (%)</td>
<td>55.6</td>
</tr>
<tr>
<td>Oral Contraceptives Use (%)</td>
<td>16.7</td>
</tr>
<tr>
<td>VO₂max (mL/kg/min)</td>
<td>61.2±1.7</td>
</tr>
<tr>
<td>Test Race Time (min:sec)</td>
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</tr>
<tr>
<td>Running Velocity (mph)</td>
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<tr>
<td>History of Exercise (min/wk)</td>
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</tr>
<tr>
<td>Exercise Volume (min/wk)</td>
<td>251.7±33.1</td>
</tr>
<tr>
<td>Total Body BMD (g/cm²)</td>
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<tr>
<td>L1-L4 BMD (g/cm²)</td>
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<tr>
<td>Total Hip BMD (g/cm²)</td>
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</tr>
<tr>
<td>Femoral Neck BMD (g/cm²)</td>
<td>1.09±0.03</td>
</tr>
</tbody>
</table>

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

* = History of exercise (min/wk) in past 6 months
Table 4.2 Anthropometric and body composition characteristics across a competitive season in female long-distance runners (n=18) grouped by energy status.

<table>
<thead>
<tr>
<th></th>
<th>PRE-SEASON</th>
<th>POST-SEASON</th>
<th>Time Effect p-value</th>
<th>Group x Time Interaction p-value</th>
<th>Group Effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass (kg)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Severe ED</td>
<td>57.8±2.5</td>
<td>58.9±3.2</td>
<td>0.106</td>
<td>0.410</td>
<td>0.197</td>
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<tr>
<td>Moderate ED</td>
<td>51.9±2.2</td>
<td>51.9±2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Replete</td>
<td>54.2±2.2</td>
<td>54.8±2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Severe ED</td>
<td>20.6±0.3</td>
<td>20.9±0.5</td>
<td>0.111</td>
<td>0.518</td>
<td>0.315</td>
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<tr>
<td>Moderate ED</td>
<td>19.7±0.7</td>
<td>19.7±0.7</td>
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</tr>
<tr>
<td>Energy Replete</td>
<td>19.4±0.7</td>
<td>19.6±0.7</td>
<td></td>
<td></td>
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<tr>
<td><strong>Percent body fat</strong></td>
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<td></td>
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<tr>
<td>Severe ED</td>
<td>25.3±1.2³</td>
<td>26.1±1.3⁶</td>
<td>0.982</td>
<td>0.011</td>
<td>0.035</td>
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<tr>
<td>Moderate ED</td>
<td>22.7±1.0</td>
<td>21.5±1.2</td>
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<tr>
<td>Energy Replete</td>
<td>20.7±1.3</td>
<td>21.1±1.5</td>
<td></td>
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<tr>
<td><strong>Fat mass (kg)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Severe ED</td>
<td>14.7±1.0⁶</td>
<td>15.4±1.3⁶</td>
<td>0.464</td>
<td>0.042</td>
<td>0.011</td>
</tr>
<tr>
<td>Moderate ED</td>
<td>11.7±0.6</td>
<td>11.1±0.7</td>
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<tr>
<td>Energy Replete</td>
<td>11.2±0.7</td>
<td>11.5±0.7</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>LBM (kg)</strong></td>
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<tr>
<td>Severe ED</td>
<td>40.7±1.9</td>
<td>40.9±2.0</td>
<td>0.070</td>
<td>0.304</td>
<td>0.539</td>
</tr>
<tr>
<td>Moderate ED</td>
<td>37.7±1.8</td>
<td>38.5±2.0</td>
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<tr>
<td>Energy Replete</td>
<td>40.7±2.0</td>
<td>40.9±2.1</td>
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</tr>
</tbody>
</table>

Data expressed as mean±SEM; BMI = Body mass index; LBM = Lean body mass; VO₂max = Maximal Oxygen Uptake.

Group differences evaluated at pre- and post-season using One-way ANOVA analyses.

³ = p<0.05, Severe ED vs. Energy Replete

⁶ = p<0.05, Severe ED vs. Moderate ED and Energy Replete
Figure 4.1A: REE Controlled for LBM. Significant differences existed between the severe ED group and the energy replete group at pre-season, and significant differences existed between the severe ED group and both the moderate ED group and energy replete group at post-season.

Figure 4.1B: REE/REE. Significant differences existed between the severe ED group and both the moderate ED group and energy replete group at pre- and post-season measurements.
Figure 4.2

**Figure 4.2A: Test Race Time:** At pre-season, the severe ED group had a test race time 16% slower (4.1 seconds) (p=0.005) than the moderate ED group. At post-season, the severe ED group had a test race time 16% (3.7 seconds) (p=0.002) and 11% slower (2.6 seconds) (p=0.023) than the moderate ED and energy replete groups, respectively.

**Figure 4.2B: Running Velocity:** At pre-season, the severe ED group had a slower running velocity (p=0.005) than the moderate ED group. At post-season, the severe ED group had a slower running velocity than the moderate ED and energy replete groups, respectively.

**Figure 4.2C: VO2max:** VO2max at post-season was 17% (p=0.009) and 16% lower (p=0.014) in the severe ED group vs. the moderate ED and energy replete groups, and there were no significant differences at pre-season.
**Table 4.3.** Energy availability characteristics across a competitive season in female long-distance runners grouped by energy status.

<table>
<thead>
<tr>
<th></th>
<th>PRE-SEASON</th>
<th>POST-SEASON</th>
<th>Time Effect p-value</th>
<th>Group x Time Effect p-value</th>
<th>Group Effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EA (kcal/kg LBM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe ED</td>
<td>37.5±4.5</td>
<td>41.0±2.5</td>
<td>0.987</td>
<td>0.694</td>
<td>0.614</td>
</tr>
<tr>
<td>Moderate ED</td>
<td>46.3±9.6</td>
<td>45.7±5.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Replete</td>
<td>46.6±5.2</td>
<td>43.6±7.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EI (kcal/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe ED</td>
<td>1900±126</td>
<td>2093±110</td>
<td>0.647</td>
<td>0.710</td>
<td>0.771</td>
</tr>
<tr>
<td>Moderate ED</td>
<td>2128±292</td>
<td>2069±209</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Replete</td>
<td>2165±233</td>
<td>2203±278</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EEE (kcal/d)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe ED</td>
<td>376±70</td>
<td>415±87</td>
<td>0.309</td>
<td><strong>0.035</strong></td>
<td>0.902</td>
</tr>
<tr>
<td>Moderate ED</td>
<td>458±63</td>
<td>332±85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Replete</td>
<td>427±25</td>
<td>438±49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM; EA = Energy availability; EI = Energy intake; EEE = Exercise energy expenditure; LBM = Lean body mass
Figure 4.3A: Log TT3 At pre-season, Log TT3 was lower (p=0.043) in the severe ED group vs. the moderate ED group. A trend to statistical significance for differences in pre-season Log TT3 (p=0.067) was observed between the severe ED group and the energy replete group. No differences were observed in pre-season Log TT3 (p=0.741) between the energy replete vs. the moderate ED group. Log TT3 was similar among groups at post-season (p=0.359). No time (p=0.293), time x group (p=0.097), and group effects (p=0.154) were observed for Log TT3.

Figure 4.3B: Total Ghrelin: Ghrelin was similar among groups at pre- (p=0.427) and post-season (p=0.374). There was a time effect (p=0.005) for Ghrelin within groups. There were no group x time (p=0.900) and group effects (p=0.320) for Ghrelin.
Table 4.4. Correlational analyses between distance running performance and (i) energetic variables, (ii) anthropometric/body composition variables, and (iii) exercise training in female long-distance runners.

<table>
<thead>
<tr>
<th></th>
<th>Pre-Season Test Race Time (min)</th>
<th>Post-Season Test Race Time (min)</th>
<th>Pre-Season Running Velocity (mph)</th>
<th>Post-Season Running Velocity (mph)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energetic Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE (kcal/d)</td>
<td>-0.013</td>
<td>0.113</td>
<td>-0.013</td>
<td>0.147</td>
</tr>
<tr>
<td>REE (kcal/kg LBM)</td>
<td>-0.184</td>
<td>0.193</td>
<td>0.417</td>
<td>0.371</td>
</tr>
<tr>
<td>REE/pREE</td>
<td>-0.257</td>
<td>0.504</td>
<td>0.357</td>
<td>0.102</td>
</tr>
<tr>
<td>Log TT₃</td>
<td>-0.388</td>
<td>0.124</td>
<td>-0.266</td>
<td>0.410</td>
</tr>
<tr>
<td>Ghrelin (pg/mL)</td>
<td>0.240</td>
<td>0.353</td>
<td>0.212</td>
<td>0.466</td>
</tr>
<tr>
<td>EA (kcal/d/kg LBM)</td>
<td>-0.058</td>
<td>0.825</td>
<td>0.054</td>
<td>0.854</td>
</tr>
<tr>
<td>EI (kcal/d)</td>
<td>-0.208</td>
<td>0.408</td>
<td>-0.007</td>
<td>0.981</td>
</tr>
<tr>
<td>EEE (kcal/d)</td>
<td>-0.669</td>
<td>0.003</td>
<td>-0.508</td>
<td>0.064</td>
</tr>
<tr>
<td><strong>Anthropometric/Body Composition Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>0.477</td>
<td>0.046</td>
<td>0.573</td>
<td>0.026</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.302</td>
<td>0.223</td>
<td>0.435</td>
<td>0.105</td>
</tr>
<tr>
<td>Percent Body Fat</td>
<td>0.600</td>
<td>0.009</td>
<td>0.806</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>0.739</td>
<td>&lt;0.001</td>
<td>0.870</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>0.183</td>
<td>0.467</td>
<td>0.113</td>
<td>0.689</td>
</tr>
<tr>
<td><strong>Exercise Training</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise Volume (min/wk)</td>
<td>-0.763</td>
<td>&lt;0.001</td>
<td>-0.535</td>
<td>0.060</td>
</tr>
</tbody>
</table>

Note: The values in bold indicate statistical significance.
4.4 Discussion

In this prospective study, we examined the effects of energy status on distance running performance in 18 female long-distance runners categorized by energy status. As hypothesized, the female runners that presented with a severe ED demonstrated the slowest test race time and running velocity at pre-season, which was significantly slower than the moderate ED group. These performance decrements were also observed in the severe ED group at post-season testing, indicative of an association between energy status and performance across the season. Notably, at post-season, the severe ED group had a test race time 16% (3.7 seconds) and 11% slower (2.6 seconds) than the moderate ED and energy replete groups, respectively. Typically, the goal of training for long-distance runners is to peak at competitive races. When female runners with severe ED fail to improve across the season, this may result in negative outcomes in peak training and performance. Despite approximately three seconds discriminating the severe ED group from the other two groups at pre- and post-season, this time difference is substantial in terms of runner placement in a competitive long-distance/cross-country race. Overall, our findings demonstrate an association between severe energy deficiency and decrements in running performance in female long-distance runners across a competitive season. Notably, REE was identified as a relevant marker of performance at pre- and post-season and thus, REE testing throughout the season may be appropriate for monitoring energy status with implications for tracking the progression of performance toward peak competitive races.

In our study, we demonstrated an association between energy status and performance in female long-distance runners. However, contrary to our hypothesis, a
dose-response relationship was not observed and to this end, decrements in performance were only exhibited in the severe ED runners, i.e., these decrements were only observed in those runners with an REE less than 84% of predicted REE. Contrary to our hypothesis, moderate ED runners, those with an REE between 84%-90% of predicted REE, did not differ in performance outcomes from energy replete runners with REE greater than or equal to 90% of predicted REE. As such, these two groups had comparable test race times and running velocity despite different REE profiles. Similarly, Van Heest et al. (In Review) demonstrated in ovulatory suppressed swimmers that REE:pREE fell consistently below 85% across the season, and in these swimmers, performance was significantly worse than their regularly ovulating counterparts, who exhibited REE:pREE values between 95-104%. There are a number of factors that could explain this lack of a dose-response association. Quite possibly, the threshold below which performance decrements are observed may be lower than an energy deficiency sufficient to disrupt metabolic and reproductive function, defined as an REE less than 90% of predicted REE (De Souza, Hontscharuk et al. 2007; De Souza, Lee et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011). In fact, a moderate energy deficiency may not negatively affect performance or may need to present long-term to result in slower running velocity/test race time and overall decrements in performance. Similar findings have been demonstrated in studies of energy restriction in runners (Zachwieja, Ezell et al. 2001; Onywera, Kiplamai et al. 2004; Fudge, Westerterp et al. 2006), wherein a moderate energy deficit did not affect subsequent performance in these athletes. In a study by Zachwieja et al. (2001) in physically active men and women, five mile time trial time improved despite a two week reduction in energy (-750kcal.day$^{-1}$) and
carbohydrate ($5.2 \text{ vs. } 3.7 \text{g.kg.day}^{-1}$) intake. However, the effect of moderate ED on chronic performance adaptations is unclear and impairments in performance may not necessarily be observed until REE is severely suppressed for a chronic period of time in female runners.

Chronic energy deficiency is marked by inadequate metabolic fuel to sustain all major physiological processes and as such, critical functions i.e., thermoregulation, cellular maintenance, are protected; whereas, less critical functions, reproduction and growth, are suppressed. However, the effect of chronic energy deficiency on performance in athletes is not as well-understood. As previously demonstrated in amenorrheic exercising women, suppressed REE and fasting TT3 are adaptations that occur to restore a eu-metabolic state (energy replete) and may be used as markers of energy deficiency in exercising women (De Souza, Hontscharuk et al. 2007; De Souza, Lee et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011; VanHeest, Rodgers et al. In Review). As expected, REE and fasting TT3 was suppressed in the severe ED group relative to other groups. In a study by Van Heest et al (In Review), ovarian steroid and metabolic hormones (including TT3) were highly correlated with sport performance in ten junior elite female swimmers over the 12-week season. Specifically, TT3 was suppressed in ovarian suppressed swimmers at week 12 compared to cyclic swimmers and to this end, the ovarian suppressed swimmers demonstrated a 9.8% decrease in 400m swim velocity compared to an 8.2% improvement in cyclic swimmers at week 12. Taken together, these findings demonstrate the importance of evaluating REE, fasting TT3, and menstrual status in female athletes at the pre-season to determine the capacity for improvement in performance across the season. This association between
energy/menstrual status and performance may have implications for pre-participation screening of athletes. Early detection would enhance the ability to intervene in those athletes presenting with severe energy deficiency to avoid poor performance outcomes across the season, particularly at peak competitive events, and potential clinical subclinical and clinical sequelae consistent with energy deficiency (Ihle and Loucks 2004; De Souza, Toombs et al. 2010).

We hypothesized that EA would significantly increase across the season, however, we observed no change in EA across the season within or among female runner categorized by energy status. The mean EA values among our groups ranged from 37.5 to 46.6 kcal/kg LBM and 41.0 to 45.7 kcal/kg LBM at pre- and post-season, respectively. These EA values are notably higher than the range of EA in amenorrheic runners observed in the literature (12-29 kcal/kg LBM) (Loucks 2007). In addition, our findings are in contrast with a report in elite female swimmers (VanHeest, Rodgers et al. In Review) wherein EA and energy intake were significantly lower in athletes that did not improve swim velocity vs. those athletes that did improve swim velocity across the season. In a prospective study in elite female soccer players across a season, Reed et al. (2013) showed that EA values declined from pre- to mid-season and increased from mid-season to post-season measurements. Researchers have suggested that low EA consistent with energy deficiency may vary among exercising women (Gibbs, Williams et al. 2013); however, to our knowledge, the effect of low EA on performance outcomes in female runners is unknown. Accurate measurement of EA in free-living individuals is difficult, especially due to the self-reported nature of diet and exercise training logs (Sawaya, Tucker et al. 1996; Conway, Seale et al. 2002; Reed, Bowell et al. 2011; Gibbs, Williams
et al. 2013). Loucks and colleagues have suggested that it is unclear whether EA or energy balance (EB), which considers all components of 24-hr energy expenditure, is the better variable to examine in terms of the effect of energy status on performance (Loucks, Kiens et al. 2011). In our study, objective measurements of REE and fasting TT3 were shown to corroborate severe energy deficiency in association with decrements in performance. To this end, we would suggest that objective measures of REE or certain metabolic hormones, i.e. TT3, may in fact be more appropriate for defining energy status in exercising women than EA or EB.

Decrements in VO2max, an indicator of maximal aerobic capacity, may also be exhibited in athletes with chronic energy deficiency. In our study, VO2max was significantly lower in female runners with severe energy deficiency; whereas moderate ED and energy replete runners had higher VO2max values (9 to 17% greater) than the severe ED runners at pre- and post-season, respectively. In a study by De Souza et al. (2008), similar findings of lower VO2 max values were observed in energy deficient exercising women compared to their energy counterparts. However, numerous investigators (Marcus, Cann et al. 1985; Nelson, Fisher et al. 1986; Kaiserauer, Snyder et al. 1989; De Souza, Maguire et al. 1990; Myerson, Gutin et al. 1991; Wilmore, Wambsgans et al. 1992) found no differences in VO2max between exercising women categorized by menstrual status. Since VO2max is not a direct marker of performance, a strength of our study is our measurement of test race time under competitive conditions in female long-distance runners as a means of confirming an association between energy status and actual performance outcomes across a season.
To date, this is the first study wherein investigators characterized metabolic hormones prospectively across a competitive season in female long-distance runners categorized by energy status. Energy deficient female runners are suggested to maintain energy balance by way of a decrease in REE and other accompanying energy conservation responses, including alterations in metabolic hormones such as TT3 and ghrelin. In fact, suppressed fasting TT3 concentrations have been demonstrated to be highly associated with suppressed REE (Onur, Haas et al. 2005). Fasting TT3 was also lower in the severe ED group consistent with previous reports on energy-deficient and amenorrheic exercising women (De Souza, Hontscharuk et al. 2007; De Souza, Lee et al. 2007). Specifically, pre-season fasting TT3 were significantly lower in the severe ED group compared to the moderate ED group and trended (p=0.067) to a significant difference between the severe ED and the energy replete group. Since TT3 is indicative of energy status, future investigation should explore TT3 as a metabolic biomarker for performance in female runners.

Total ghrelin is also a well-established metabolic marker of energy deficiency in amenorrheic athletes. In a previous study in amenorrheic runners, investigators exhibited at least 85% higher fasting ghrelin concentrations compared to control groups (De Souza, Leidy et al. 2004). Furthermore, this rise in ghrelin concentration has been shown to be responsive to decreases in REE (Leidy, Gardner et al. 2004). Typically, ghrelin is negatively correlated with body fat mass (Jurimae, Cicchella et al. 2007) and is responsive to diet- and exercise-induced changes in body weight (Leidy, Gardner et al. 2004; Leidy, Dougherty et al. 2007). Certain investigators hypothesized that energy deficiency in athletes during training would promote increases in ghrelin to stimulate
appetite resulting in higher energy intake (Maestu, Jurimae et al. 2008). However, few investigators have examined ghrelin in athletes following exercise training across a competitive season (Maestu, Jurimae et al. 2008; Ramson, Jurimae et al. 2008). Maestu et al. (2008) demonstrated that ghrelin is sensitive to initial decrease in percent body fat but plateaus beyond which further increases associated with energy deficiency or weight loss will not be observed. To this end, it is possible that fasting ghrelin is not sensitive to temporarily increased training volume (Jurimae, Maestu et al. 2011). In our study, ghrelin decreased across the season within the groups. Possible explanations for this finding are the down-regulation of ghrelin receptors associated with high volume training (Kraemer, Durand et al. 2004; Ramson, Jurimae et al. 2008) and as such, ghrelin may be a better indicator of de-adaptation/overtraining vs. training adaptations in athletes across a competitive season.

We also observed associations between performance outcomes (test race time, running velocity) and factors related to anthropometry/composition and training. These findings are expected as endurance running represents a sport wherein leanness and low body weight are often considered advantageous for performance. However, it is important to emphasize that anthropometry/body composition parameters have a greater impact on running performance in women participating in half-marathon, marathon, and ultra-marathon events (Knechtle, Knechtle et al. 2011; Schmid, Knechtle et al. 2012); whereas, neuromuscular factors such as running economy and lactate threshold may manifest as better predictors of performance in runners that primarily compete in 3 to 10km events (Kelly, Burnett et al. 2008). In addition, power to weight ratio is a relevant contributor to running velocity (Sundgot-Borgen and Torstveit 2010). Thus, loss of LBM
may negatively influence muscular endurance and strength. In a prospective investigation in lean, healthy men following a long-term energy deficit (Nindl, Friedl et al. 1997), loss of LBM (-12%) was associated with decrements in physical performance measures (i.e., simulated power clean and vertical jump). Notably, LBM did not change in our participants across the season. Future research is necessary to characterize changes in neuromuscular, strength, and power-related performance outcomes in female long-distance runners grouped by energy status as per the operational definition in this study.

A major strength of our study is the prospective evaluation of performance outcomes in female long-distance runners across the competition season. Competitive performance is difficult to measure and our study represents one of the few reports on the effect of energy status on performance in female distance runners. This approach captures the psychological or behavioral factors that impact performance. However, there were notable limitations. The measurement of EA is challenging using self-report logs to assess energy intake and exercise energy expenditure in exercising women under free-living conditions. Previous investigators have demonstrated that study participants are prone to under-report daily food intake on diet logs (Martin, Su et al. 1996; Sawaya, Tucker et al. 1996) and over-report exercise energy expenditure comparison to doubly labeled water measurements (Conway, Seale et al. 2002), resulting in an overall underestimation of EA. Furthermore, EA may be transient in runners considering the changes in dietary and training regimens across a season, and as such, the use of EA as an indicator of energy deficiency in free-living participants is questionable (Gibbs, Williams et al. 2013). There are several methods to assess and define energy status. For our purposes, energy status was defined as using an objective laboratory-based measure,
REE, to identify individuals who exhibit energetic adaptation to an energy deficiency, and expressed as a ratio of actual REE to predicted REE based on the predicted calculated using the Harris–Benedict equation (Harris and Benedict 1919). In prior reports in exercising women, we have defined an REE/pREE score less than 0.90 as an energy deficiency, and in the current study, we categorized our participants according to tertiles of REE/pREE to appropriately capture whether a dose-response relationship exists with respect to energy status. Last, based on the rigorous training schedules of our participants, we limited our measurements to pre- and post-season. Evaluation of energy status and performance outcomes at certain time points throughout the season (i.e., mid-season) would have better characterized changes in energy status, metabolic hormones, and performance outcomes across a competitive season.

Overall, our findings provide evidence of an association between energy status and performance in female long-distance runners across a competitive season. In contrast to our hypothesis, we did not observe a dose-response relationship between REE and performance outcomes; however, a severe ED was associated with decrements in test race time, running velocity, and VO2max in female long-distance runners following the competitive season. As shown in this study, decreases in REE below 84% of predicted REE are indicative of sub-optimal energy status that can be detrimental to performance. As such, REE testing may be an appropriate measure to implement at pre-season and throughout the season in order to monitor energy status and avoid decrements in relevant performance outcomes. Early intervention would be beneficial both short-term for performance outcomes and long-term for health-related outcomes, i.e., menstrual, musculoskeletal, psychological, and cardiovascular health. Monitoring energy status in
female long-distance runners may assist in establishing performance expectations and effective dietary/training regimens for each runner to enhance ability to achieve optimal energy status concomitant with peak performance.
4.5 References


4.6 Figure Captions

Figure 4.1. Resting energy expenditure (REE) controlled for lean body mass (LBM) and measured REE compared to predicted REE (REE/pREE) across a competitive season in female long-distance runners grouped by energy status. REE controlled for LBM (kcal/kg LBM) across a competitive season in female long-distance runners grouped by energy status (Severe Energy Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group (REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel A. REE/pREE across a competitive season in female long-distance runners grouped by energy status (Severe Energy Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group (REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel B. 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.

Figure 4.2. 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 (Severe Energy Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group (REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel A. Running velocity (mph) across a competitive season in female long-distance runners grouped by energy status (Severe Energy Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group
(REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel B. Maximal oxygen uptake (VO2max) (mL/kg/min) across a competitive season in female long-distance runners grouped by energy status (Severe Energy Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group (REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel C. 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.

Figure 4.3. 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 Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group (REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel A. The symbol (*) indicates p<0.05, Severe ED Group vs. the Moderate ED group. Fasting ghrelin concentration (pg/mL) across a competitive season in female long-distance runners grouped by energy status (Severe Energy Deficient group (REE/pREE < 0.84) (red line), Moderate Energy Deficient group (REE/pREE >=0.84 to <0.90) (blue line), and Energy Replete Group (REE/pREE >=0.90) (green line)) is shown in Panel B.
Chapter 5
Summary, Conclusions, and Implications

Female long-distance runners with chronic energy deficiency are likely at a higher risk of the Female Athlete Triad clinical sequelae, including low EA with or without disordered eating, menstrual dysfunction, and low bone mineral density (Nattiv et al., 2007). These female athletes may develop chronic energy deficiency as a result of the failure to match dietary energy intake to energy expenditure (Torstveit and Sundgot-Borgen, 2005). Chronic energy deficiency has a suppressive effect on both metabolic and reproductive function in order to preserve energy stores for thermoregulation, cellular maintenance and locomotion (Wade, 1996). To date, the impact of chronic energy deficiency on performance in female long-distance runners is unclear.

The purpose of our study was to examine energetic factors, to include EA, REE, TT$_3$, and ghrelin concentrations, that impact distance running performance during a competitive season in female distance runners. In the current study, we characterized the changes in energetic factors in female long-distance runners across a competitive season and examined the effects of energy status on distance running performance in 18 female long-distance runners categorized by energy status. Participants (aged 17-25) were assessed pre- and post-competitive running season (10-12 weeks). It was initially hypothesized that increases in EA, REE, and TT$_3$ with concomitant decreases in ghrelin would promote improvements in distance running performance (specifically, decreases in test race time in minutes) in female distance runners following a competitive season.
Overall, our results provided evidence of an association between severe energy deficiency and decrements in distance running performance. REE/pREE and REE were found to be lower in the severe ED group than the moderate ED runners at pre-season, as well as lower than the moderate ED and the energy replete group at post-season. The moderate ED group also demonstrated lower REE/pREE than the energy replete group at pre- and post-season. At pre-season, the severe ED group had a slower test race time and running velocity than the moderate ED group. No differences in test race time and running velocity were observed between the severe ED and moderate ED groups vs. the energy replete group at pre-season. At post-season, the severe ED group had a slower test race time and running velocity than both the moderate ED (3.7 seconds) and energy replete groups (2.6 seconds). At pre-season, Log TT3 was lower in the severe ED group vs. the moderate ED group. A time effect was observed for ghrelin within groups, but no changes in EA and fasting TT3 were observed across the season.

Contrary to our hypothesis, a dose-response relationship was not observed in performance, and decrements in performance were only exhibited in the severe ED runners. The moderate ED runners did not differ in performance outcomes from energy replete runners. As such, these two groups had comparable test race times and running velocity despite different REE profiles. There are a number of factors that could explain this lack of a dose-response association. For one, the conventional threshold below which performance decrements are observed may be in fact lower than an energy deficiency sufficient to disrupt metabolic and reproductive function (REE less than 90% of predicted REE) (De Souza, Hontscharuk et al. 2007; De Souza, Lee et al. 2007; De Souza, West et al. 2008; Gibbs, Williams et al. 2011). As such, a moderate energy deficiency may not
negatively affect performance or may need to present long-term to result in slower running velocity/test race time and overall decrements in performance.

In summary, a severe ED was associated with decrements in test race time, running velocity, and VO2max in female long-distance runners following the competitive season. It was concluded that decreases in REE below 84% of predicted REE are indicative of sub-optimal energy status that can be detrimental to performance. Based on this association between severe energy deficiency and decrements in distance running performance, we suggest monitoring REE and key metabolic hormones, such as TT3, as markers of performance outcomes in order to promote the early detection of poor energy status in female distance runners. One possible method, REE testing, may be favorable in optimizing running performance across a season. Implementation at pre-season and throughout the season in order to monitor energy status and avoid decrements in relevant performance outcomes could prove beneficial for female distance runners with respect to performance outcomes and long-term health-related outcomes. Monitoring energy status in female long-distance runners may assist in establishing performance expectations and effective dietary/training regimens for each runner to enhance ability to achieve optimal energy status concomitant with peak performance at desired times. Future research is necessary to confirm this association in a larger sample of female runners.
5.1 References


ACADEMIC VITA

Mitchell S. McTavish

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Education

The Pennsylvania State University

University Park, PA

- B.S. in Kinesiology – Movement Science Option
- Scholar in Schreyer Honors College
- Dean’s List Recipient

May 2013

Related Experience

Research Lab Assistant

2011 – Present

Noll Laboratory: The Pennsylvania State University
Department: Kinesiology – Exercise Physiology

- Volunteered in Women’s Health and Exercise Lab
- Competent in laboratory testing for the following procedures:
  - Resting metabolic rate
  - Maximal oxygen uptake
  - Underwater weighing for body composition
- Processing blood and urine samples
- Completed NIH and PSU IRB Training Courses

Activities

Health Education and Learning THON

2010 – 2011

- Volunteered as part of Penn State’s Panhellenic Dance Marathon
- Assisted in raising money from various activities as part of the fundraising division

Kinesiology Club

2010 – Present

- Attended monthly meetings and participated in Kinesiology-themed presentations
- Facilitated contact with club officers

Penn State Club Track

2009 – 2010

- Participated in team meetings, practices, events, and fundraising
- Developed team-building skills

Work Experience

Warren General Hospital

June 2012 – August 2012

Warren, PA
• Completed a 100-hour internship with a Urological surgeon
• Observed several surgeries involving the entire urological tract including structures such as the kidneys, ureters, and prostate gland
• Experienced direct hands-on experience with patients
• Volunteered an additional 15 hours with the physician

KCS Energy, Inc. 2005 – Present
Warren, PA
• Tended and maintained oil wells and their surrounding environment

Jackson Valley Country Club 2005 – Present
Warren, PA
• Maintained grounds and golf course facilities
• Completed various duties working in the restaurant

Research Interests
I have broad interests in achieving optimal athletic performance, particularly through proper dietary and exercise training in athletes. My research has focused on female long-distance runners specifically for the purpose of evaluating whether these athletes maintained proper energy status in order to maximize performance. Although I have little experience with the subject matter, I am also interested in the psychological aspects of optimizing performance. All in all, I appreciate the role of both physiological and psychological factors on performance, and would like to further investigate the precise roles of each of these factors on athletic performance.