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THE THERMIC EFFECT OF FOOD IN ENERGY DEFICIENT WOMEN

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ABSTRACT

Women suffering from anorexia nervosa, an extreme case of starvation and energy deficiency, exhibit altered metabolism in response to ingestion of a caloric load. The possibility of this phenomena has yet to be investigated in exercising women with functional hypothalamic amenorrhea (FHA), a condition which is often secondary to moderate energy deficiency. The purpose of this study was to characterize and compare the metabolic profiles of young, exercising women with either amenorrheic or eumenorrheic menstrual statuses. 7 amenorrheic (AMEN) and 9 ovulatory, eumenorrheic control (OV) women, who participated in aerobic or resistance training for at least 3 hr/wk, ingested a 700 kcal liquid mixed macronutrient meal (14% protein, 64% carbohydrate, and 22% fat) and underwent measurements of resting energy expenditure (REE), thermic effect of food (TEF), and respiratory quotient (RQ) by indirect calorimetry over a 6 hour period. Paired t-tests were used to compare REE measurements and TEF area under the curve (AUC). Two-way (group*time) repeated measures ANOVA were conducted to assess differences in absolute TEF (kcal/day), relative TEF (kcal/kgFFM/day), and RQ. Groups did not significantly differ in age, height, body mass, gynecological age, body fat percentage, fat mass, fat free mass, or lean body mass. BMI was different between the groups, where the OV group had a significantly higher BMI than the AMEN group ($p=0.024$). There were no significant differences between groups for ratio of measured REE to Harris-Benedict predicted REE ($p=0.448$) or TEF AUC ($p=0.692$). There were no significant ($p>0.05$) interaction or main effects for absolute (kcal/day) REE or relative (kcal/kgFFM/day) REE. There was no significant interaction or main effect for group with respect to RQ ($p>0.05$), but there was a significant main effect for time ($p=0.006$) with RQ significantly increasing from rest 15 minutes postprandially. These results suggest that metabolic adjustments, such as elevated TEF and a higher postprandial reliance on carbohydrate, typically seen in anorexia nervosa are not present in less severe cases of energy deficiency. However, future research is required to more thoroughly assess energetic status.

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Chapter 1

Introduction

This preamble is needed to explain the link between energy status and reproductive function in exercising women

The Female Athlete Triad (Triad) is a medical syndrome consisting of low energy availability (EA), suboptimal bone health, and menstrual dysfunction, each of which can exist alone or in combination with one another [1]. The estimated prevalence of the Triad varies widely in the literature [2–4]; however, it is clear that this condition is more common in athletes than non-athletes [1,5], and particularly common in athletes who participate in sports emphasizing leanness for aesthetic or performance purposes [2,4,6]. Athletes participating in leanness sports, such as gymnastics and distance running, are at increased risk of energy deficiency resulting from insufficient energy intake relative to the increased demands of exercise energy expenditure [6]. Energy deficiency results via several mechanisms including: purposeful undereating to restrict caloric intake in an effort to achieve a perceived ideal body composition; disordered eating behaviors including high dietary cognitive restraint and drive for thinness [7–9]; or inadvertently failing to match energy intake to energy expenditure [1].

In models of energy deficiency and low EA, compensatory mechanisms adjust metabolism such that overall energy is conserved and ensuring that the energetic needs of essential bodily functions are met. Specifically, the most immediately vital physiological functions of cellular maintenance, thermoregulation, and locomotion are maintained while expenditure is shunted away from the non-essential metabolic costs of growth and reproduction [11]. Metabolic alterations indicative of an energy deficiency include suppressed resting energy expenditure (REE), suppressed concentrations of total triiodothyronine (TT_3) and insulin-like growth factor 1 (IGF-1) [12], and an increase in fuel availability

via increased concentrations of growth hormone (GH)[2,13] and cortisol [14–16]. Suppression of reproductive function is demonstrated by decreased luteinizing hormone (LH) pulse frequency and increased pulse amplitude [17] and decreased estrogen and progesterone production [18,19] resulting in menstrual disturbances such as functional hypothalamic amenorrhea (FHA), defined as the absence of menses for at least 90 days [20].

Exercising women are at risk for developing the conditions of energy deficiency and low EA by failing to adequately increase energy intake to match the increased energy expenditure due to exercise. Total daily energy expenditure (TDEE) is comprised of REE, activity thermogenesis, and the thermic effect of food (TEF) [21]. Resting energy expenditure, accounting for 65-70% of TDEE, is the energy required to maintain essential bodily processes [10] and is suppressed in exercising women with FHA [22]. Activity thermogenesis, 15-25% of TDEE, is further subdivided into exercise associated thermogenesis (EAT) and non-exercise associated thermogenesis (NEAT). Exercise associated thermogenesis is the energy expended during purposeful physical activity and is elevated in exercising women, while NEAT is the energy expended during all other activities outside of sleeping, eating, and intentional exercise [23]. The TEF accounts for 10-15% of daily energy expenditure and is the increase in energy expended during the digestion, absorption, and storage of food [10]. The TEF has yet to be quantified in women with FHA, but is has been elevated in other models of energy deficiency such as anorexia nervosa [24,25], which may contribute to the inability of exercising women with FHA to the meet the energy requirements necessary for resumption and maintenance of menses.

While the TEF has yet to be reported in exercising women with FHA, studies of anorexia nervosa provide evidence that the TEF is elevated during an energy deficit. Most investigators have reported that the TEF was greater in patients prior to weight gain compared to healthy controls and compared to patients who were able to meet their target weight [24–26]. Moukaddem *et. al.* reported higher TEF values in anorexic patients during the refeeding phase of treatment compared to semi-starvation [27], however differences between studies may be attributed to feeding mode and meal composition since the

TEF increases in relation to the caloric load [25] as well as the amount of carbohydrate ingested [24]. As an adaptation to energy deficiency, an elevation in the TEF is counterintuitive because it does not align with typical mechanisms of energy conservation in which caloric expenditure is decreased. A more complete understanding of the energy demands of exercising women, including measurement of the thermic response to a meal of known caloric load and macronutrient composition, is essential for managing energy intake at a sufficient level to maintain reproductive function and overall health.

An additional metabolic component that may be altered during an energy deficiency and thus warrants further investigation in exercising women with FHA is substrate utilization. Respiratory quotient (RQ), measured by indirect calorimetry as the rate of CO_2 production divided by the rate of oxygen consumption (VCO_2/VO_2), varies according to energetic status [24,26,28] and meal composition [29]. The RQ approaches 1.0 after glucose consumption, 0.70 after fat consumption, and 0.83 after a normal mixed macronutrient meal [29]. In anorexia nervosa patients, conflicting results have been reported in the literature such that basal RQ was lower prior to refeeding and weight gain [12,26,27], and higher in patients before refeeding [24,28] and compared to healthy controls [26]. In one study of exercising women, there was no difference in basal RQ between women with FHA and those with eumenorrheic menstrual cycles [30]. These discrepancies remain when measuring postprandial RQ, which may partially be explained by differences in the macronutrient composition of the meal ingested. Further variability may be due to the length and severity of the energy deficiency. Glycogen is stored in finite amounts and, if depleted, an increased reliance on fat or protein as fuel would be necessary, and this shift would be reflected by a lower RQ. An improved understanding of fuel utilization in exercising women with FHA, a milder form of energy deficiency than anorexia nervosa, is important for a more complete understanding of the metabolic alterations known to occur in this population. This will be beneficial for developing diet prescriptions to reverse amenorrhea and resume menses.

1.1 Objective

Due to the limited and inconsistent information regarding metabolism and energy homeostasis in energy deficient women, the overall objective of this study is to assess the metabolic profiles of exercising women with varying menstrual statuses during resting conditions and after the consumption of a 700 kcal liquid meal of mixed macronutrient composition (14% protein, 64% carbohydrate, 22% fat). Metabolic profiles will be defined as assessments of TEF, REE, and RQ in exercising women with FHA (AMEN) and exercising women with ovulatory (OV) menstrual cycles.

1.2 Specific Aim 1

To determine the relationship of REE, as measured by indirect calorimetry, to menstrual status in exercising women with FHA (AMEN) and ovulatory, exercising women (OV).

The following hypotheses will be tested:

H1: Absolute REE (kcal/day) will be lower in AMEN compared to OV.

H2: Relative REE (kcal/day/kg LBM) will be lower in AMEN compared to OV.

H3: The ratio of actual REE to Harris-Benedict predicted REE (REEa/REEp) will be lower in exercising women with FHA compared to exercising women with ovulatory, eumenorrheic menstrual cycles.

1.3 Specific Aim 2

To determine the relationship of the TEF, as measured by indirect calorimetry, to menstrual status in exercising women with FHA (AMEN) and ovulatory, exercising women (OV) in response to consumption of a liquid, mixed composition meal.

The following hypotheses will be tested:

H1: Absolute TEF (L/min) will be greater in AMEN compared to OV at all time points (0, 60, 120, 180, 240, and 300 minutes).

H2: Relative TEF (mL/kg/min) will be greater in AMEN compared to OV at all time points (0, 60, 120, 180, 240, and 300 minutes).

H3: The integrated area under the curve (AUC) for 300 minutes following meal ingestion will be greater in AMEN compared to OV.

1.4 Specific Aim 3

To determine the relationship between fuel utilization, measured as a ratio of expired CO_2 to inspired O_2 (VCO_2/VO_2) and referred to as RQ (measured by indirect calorimetry), and menstrual status in exercising women with FHA (AMEN) and ovulatory, exercising women (OV) in response to consumption of a liquid, mixed composition meal.

The following hypothesis will be tested:

H1: A greater reliance on carbohydrate for fuel, as determined by a higher RQ value, will be observed in AMEN at 0, 60, 120, 180, 240, and 300 minutes after consumption of a liquid, mixed composition meal compared to OV.

1.6 Statistical Analyses

All data will be checked for assumptions of normality and values will be reported as mean $\pm$ standard error of the mean with significance set as $p<0.05$. TEF will be expressed as the elevation above REE at each time point as an absolute term, relative to body mass, and as the integrated increase in energy expenditure above REE (area under the curve) during the 5 hours after meal consumption. Differences between groups will be analyzed using two-sided independent t-tests will be performed in order to

compare REE under basal conditions. A two-way repeated measures analysis of variance (ANOVA) will compare RQ and TEF between groups at all time points (0, 60, 120, 180, 240, and 300 minutes) with a Tukey post-hoc test when significant main effects are found. All analyses will be conducted on SPSS v. 24.

1.7 Experimental Design Overview

This experiment is a cross-sectional study designed to investigate the energetic demands of exercising women with and without menstrual disturbances. Specifically, this study will assess REE and RQ, during basal conditions and TEF and RQ for 5 hours following consumption of a 700 kcal mixed-macronutrient-composition liquid meal (14% protein, 64% carbohydrate, 22% fat). Responses will be compared between exercising women with ovulatory, eumenorrheic menstrual cycles (OV) and those who have had an absence of menses for $\geq$ 90 days (AMEN).

1.8 Participants

Participant eligibility included 1) women; 2) between the ages of 18 and 35yrs; 3) BMI between 16-25 kg/m^2; 4) currently participate in aerobic or resistance training for at least 3hr/wk; 5) gynecological age > 5 years; 6) weight stable (+/- 2kg); 7) not experiencing regular menstrual bleeding within the past 3 months OR have regular menstrual cycles of 26-35 days for the past 6 months; 8) non-smoker; 9) not pregnant, breastfeeding, or lactating; and 10) not taking hormonal contraceptives for the past 6 months.

1.9 Rationale

In the absence of adequate energy intake to match energy expenditure, females face disruption of many bodily systems vital for growth [31] and reproduction [17,32]. Total daily energy expenditure is comprised of REE, TEF, and activity thermogenesis from both exercise and non-exercise activities [21]. The components of TDEE are known to be altered in models of an energy deficiency, thus in order to adequately match energy intake to energy expenditure, a more accurate and complete understanding of each energy component is necessary. If this total expenditure is not regularly countered by sufficient caloric consumption, an energy deficit can develop which may lead to compromised health. A prolonged energy deficiency results in metabolic and hormonal disruptions [16], which impact overall health and well-being leading to menstrual dysfunction [17], low bone mass [14], cardiovascular complications [33,34], and decreased athletic performance [35]. Both TT_3 concentration and REE are suppressed in models of a chronic energy deficiency as an attempt to conserve energy [9,22,36].TT_3 contributes to the synthesis and degradation of carbohydrates, lipids, and proteins and, when suppressed, is matched by similar declines in REE [37]. By contrast, TEF has been observed at abnormally high values in anorexia nervosa patients [24,25,27]. The mechanism for this increase in TEF is interesting, as any increase in energy expenditure could be deleterious to recovery/refeeding efforts in severely energy deficient patients. Those who suffer from conditions such as disordered eating and/or the Triad struggle to meet energy needs for complex reasons and a more complete understanding of these reasons is necessary in order to maximize the effectiveness of prevention and treatment strategies. Examining the precise differences in TEF and substrate utilization between exercising women with amenorrhea and those with ovulatory, eumenorrheic menstrual cycles will allow for a more thorough understanding of the energy deficiency underlying the Triad.

To date, research exists in anorexia nervosa patients examining the TEF and substrate utilization [24,25,27]; however, there are gaps in the literature pertaining to these markers in less extreme cases of energy deficiency, for example, in exercising women or those with general disordered eating behaviors

and FHA. Mild energy deficiencies and disordered eating are still detrimental to health and performance and should be examined thoroughly. Such cases are especially prevalent in exercising populations. In a study of 425 collegiate female athletes of varying sports, up to 30% of subjects reported behaviors consistent with disordered eating, while only 2-3% reported a clinical diagnosis of anorexia or bulimia [2]. The present study directly compares the metabolic profiles of exercising women of differing menstrual statuses and thus allows for a means to identify the metabolically disruptive mechanisms present in more commonly encountered cases of energy deficiency.

Previously amenorrheic patients who achieve energy balance and resume menses experience dramatic increases in bone mineral density (BMD) [38–40], as well as estrogen and progesterone [16]. This suggests the critical nature in restoring normal menstrual cyclicity as it relates to the overall health of women. Conducting this work is a promising step toward the prevention of an energy deficiency as well as the development of a refeeding method which takes into account the changes in TEF and substrate utilization that occur during energy deficiency.

1.10 Expected Findings and Applications

It is anticipated that exercising women with amenorrhea will have higher TEF values compared to women with eumenorrheic, ovulatory menstrual cycles. An elevated TEF in already energy deficient subjects is a factor that is not commonly considered by patients or medical professionals when creating refeeding plans to restore energy balance. This oversight could be consequential, as the TEF accounts for 10-15% of daily energy expenditure [21] depending on total energy content [41] and protein content [42] of consumed meals. The TEF is strongly correlated with lean mass [41] and can be affected by weight gain [43], but the mechanisms for these variations are not well understood. It is expected that the current

study will provide a more complete understanding of these mechanisms, as they are important considerations for subjects during the refeeding process.

It is also expected that RQ values will be higher at all time points following a mixed composition meal in women with amenorrhea as compared to women with ovulatory, eumenorrheic menstrual cycles, indicating higher carbohydrate oxidation rates comparable to the elevated RQ values observed in anorexic patients following a meal [12,24]. Turning carbohydrate to fat is energetically expensive, and this mechanism could also explain the increase in TEF [24]. The present study is expected to provide further insight into this possible mechanism and its implications for optimal meal composition in energy deficient patients. In addition, it is anticipated that REE will be lower in women with amenorrhea compared to women with ovulatory, eumenorrheic menstrual cycles, which would be consistent with adaptations to a chronic energy deficit [22,36,44].

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Chapter 2

Literature Review

2.1 Introduction

The presence of a chronic energy deficiency in women results in the initiation of compensatory mechanisms which can be deleterious to overall health and wellbeing. Specifically, during a prolonged energy deficit, energy expenditure is shunted away from the non-essential metabolic costs of growth and reproduction in order to maintain the physiological functions of cellular maintenance, thermoregulation, and locomotion [1]. These energetic adjustments can contribute to clinical issues such as low bone mineral density [2–4], impaired lipid profiles and endothelial dysfunction [5], hypoestrogenism [6,7], and menstrual disturbances such as functional hypothalamic amenorrhea [8–10]. This list is clearly expansive, and demonstrates the impact of an energy deficiency on several bodily systems and, thus, demonstrates the critical nature of maintaining an energy balance.

Fundamental thermodynamic principles necessitate that to be in energy balance, caloric intake must equal energy expenditure, and therefore, an energy deficit can develop when energy intake is less than energy expenditure. Energy input consists of food intake; while, caloric output includes resting energy expenditure (REE), thermic effect of food (TEF), and activity thermogenesis [11,12]. REE, accounting for 65-70% of daily energy expenditure, is the energy required to maintain essential bodily processes [12]. Activity thermogenesis, 20-30% of total daily energy expenditure (TDEE) [13], is further subdivided into exercise associated thermogenesis (EAT) and non-exercise associated thermogenesis (NEAT); where EAT is the energy expended during purposeful physical activity and NEAT is the energy expended during all other activities outside of sleeping, eating, and intentional exercise [14]. TEF accounts for 10-15% of daily energy expenditure and is the increase in energy expended during the digestion, absorption, and storage of food [15,16].

Because physically active women typically expend large amounts of energy via EAT, it is imperative that they adequately increase energy intake sufficiently to counter this high energetic output. Failure to increase caloric intake to match the high energy expenditure seen in an active population, and achieve energy balance, may be done inadvertently or purposefully. Purposeful avoidance of energy balance is demonstrated by conscious participation in excessive exercise or restriction of food intake in an effort to acquire a perceived ideal body composition. This may explain why energy deficient women often exhibit elevated drive for thinness scores, a marker associated with higher levels of body dissatisfaction and disordered eating [17,18]. By contrast, inadvertent under-eating occurs when caloric intake does not meet energy expenditure in the absence of conscious dietary restriction. This may develop due to logistical issues such as demanding practice or travel schedules.

Regardless of whether the inception of an energy deficit is due to psychological or logistical factors, there is evidence to suggest that the condition is maintained, or even exacerbated, by persisting dysregulation of appetite and digestive energetics. Appetite is a result of the complex interaction of physiological and environmental cues, and a disturbance of hunger sensations may be an important part of the etiology of conditions related to energy deficiency, such as anorexia nervosa or the Female Athlete Triad. Simultaneous or asynchronous changes in neuroendocrine control, gut peptide secretion, substrate utilization, and/or dietary induced thermogenesis may affect appetite perception and digestive efficiency in this population. This review of literature aims to outline existing evidence to support this notion.

2.2 Neurohormonal Controls of Hunger and Satiety

Proper neurohormonal control of hunger and satiety signals is critical in weight management, as these sensations are strong predictors of food intake [19]. The release and disposal of gut peptides, which control satiety signals, are tightly regulated by feedback systems involving both the gastrointestinal tract and the brain. The hypothalamus has been implicated for its role in the pathways classically thought to

coordinate hunger and satiety signals and is, therefore, likely to impact energy balance. The lateral hypothalamic area (LHA) has been defined as the "feeding center" of the brain, as its stimulation induces food intake [20] and lesions to the area precede a reductions in food consumption and weight [21]. Conversely, the ventromedial nuclei (VMN) of the hypothalamus serves as the "satiety center" of the brain, based on animal studies which illustrate that destruction of the VMN results in weight gain and increased appetite [22]. Although the feeding and satiety centers of the brain are not directly connected, this does not mean that they act independently of one another. The LHA and VMN communicate indirectly via two distnict areas in the hypothalamus: the paraventricular nucleus (PVN) and the arcuate nucleus (ARC). Neural circuitry from hunger and feeding centers converge in the PVN and ARC, suggesting a role for these areas in the integration of hunger and satiety signals [23]. This intricate neural anatomy may allow the LHA and VMN to act reciprocally to facilitate an appropriate satiety or hunger response [24].

There are five criteria by which a particular hormone can be classified as a physiological regulator of appetite as outlined by Nori Geary [25]. These criteria include a) secretion associated with change in eating, b) receptors at the site of action, c) having a physiologic dose that can be reproduced through IV infusion, d) removal of the hormone/receptor prevents the eating effect and replacement at physiologic dose normalizes the eating effect, and e) infusion of an antagonist prevents the eating effect. Given that these criteria are met, the hormone can be designated as either orexigenic (appetite stimulating) or anorexigenic (appetite suppressing). Orexigenic hormones are typically produced peripherally, during a fasted state, and target neurons located in the feeding center, where neurotransmitters are consequently released to produce an appetite stimulating effect on the brain [26]. While multiple neurotransmitters may be released, neuropeptide Y (NPY) is arguably the most notable in the present context. Injection of NPY into the hypothalamus causes potent hyperplasia in an animal model [27], and concentrations of NPY increase in the hypothalamus during states of food deprivation [28,29]. In addition to initiating the cascade-like pathway known as hunger signaling, some orexigenic peptides

also act to simultaneously suppress satiety signals in the brain. Specifically, the orexigenic gut peptide ghrelin may indirectly inhibit anorexigenic neurons by binding to receptors in the PVN [30]; thus, demonstrating the indirect communication between the hunger and satiety centers mentioned previously. Anorexigenic gut hormones act in a similar fashion; being produced peripherally in a state of energy balance, stimulating anorexigenic neurons in the satiety center, and indirectly blunting activity of orexigenic neurons through integration centers in the brain [31]. Orexigenic and anorexigenic hormones can also be split into additional subgroups: those that regulate long term satiety and those that regulate short term satiety [32]. Short-term hormones such as ghrelin and PYY mediate hunger signals from meal to meal, i.e. "fullness"; whereas, long-term hormones such as leptin tend to reflect adiposity and energy store levels over a longer period of time [33].

2.3 Physiological Roles of Gut Peptides

Ghrelin

Ghrelin is a hypothalamic peptide and potent orexigenic hormone secreted by the stomach [34]. Ghrelin was first recognized for its role as a growth hormone (GH) secretagogue [35], but subsequent studies have demonstrated that ghrelin is versatile, affecting a number of bodily systems. Ghrelin activity has been recorded in several biological domains including reproductive physiology [36,37], energetics [38,39], and bone formation [40], but the peptide is most commonly studied for its role in the gastrointestinal system. In healthy individuals, ghrelin concentrations peak under fasted conditions [41], and secretion is suppressed following meal consumption [42] in proportion to the ingested caloric load [43]. Ghrelin infusion increases hunger sensations and food intake in both humans [44] and animal models [45]. The relationship between circulating ghrelin levels and body weight, however, is not entirely clear. Ghrelin levels are suppressed in obese patients [46] and increase with subsequent weight loss [47], but infusion of ghrelin in rat models induces adiposity [48]. Although findings are not consistent

throughout the literature, additional metabolic factors are associated with changes in ghrelin levels. Shiiya et al. [49] reported both oral and intravenous administration of glucose decreased plasma ghrelin levels in healthy subjects. In addition, Saad et al. [50] observed a potent decrease in plasma ghrelin upon insulin infusion in 8 healthy subjects, suggesting a reciprocal relationship between the two hormones. These results disagree with those described by Schaller et al who found no relationship between ghrelin secretion and glucose infusion and found a decrease in ghrelin to be significant only at supraphysiological levels of insulin infusion [51]. Ghrelin is often secreted in conjunction with other meal-related hormones, which may explain the confounding results reported in the literature pertaining to ghrelin modulation by insulin and glucose.

In the context of the female reproductive system ghrelin has an important and complex function. Luteinizing hormone pulsatility is suppressed in women in response to elevated ghrelin levels [52,53], and plasma ghrelin concentrations decrease with estrogen-replacement therapy [54]. Luteinizing hormone suppression in response to exogenous ghrelin administration is also observed in estrogen-treated ovariectomized rats [37,55] and rhesus monkeys [56]. However, ghrelin secretion may also be modulated by estrogen itself, suggesting a bidirectional influence of the two hormones. Female rats experience an increase in ghrelin expression and production upon ovariectomy [57,58]. Ovariectomized female rats and male rats are also more sensitive to the orexigenic effects of exogenous ghrelin administration than rats with ovaries [37,55]. The relationship between ghrelin and reproductive hormones is interesting and may help to explain sex differences in weight gain and appetite.

PYY

Peptide YY (PYY) is a gut peptide secreted by L-type endocrine cells of the gastrointestinal tract [59,60]. Two forms of PYY are present in the blood: PYY 1-36 and the truncated form, PYY 3-36 [61]. Structural variations mediate marked differences in binding affinities between the two forms of PYY. Specifically, PYY 1-36 binds to and activates all five Y (Y1-Y5) receptors while PYY 3-36 only has a high affinity for the Y2 receptor [62]. Both forms are biologically active, but the PYY 3-36 form is most

prominently stored and circulated, and is more active in controlling food intake and regulating appetite [61]. Plasma PYY concentrations are low in the fasted state and increase upon meal ingestion [63] in proportion to caloric intake [64] with fat being a strong stimulus for release [65]. PYY is generally considered to be an anorexigenic satiety hormone, but controversy exists regarding this classification [66] because despite consistent demonstrations of decreased food intake upon intravenous administration of PYY [31,67–69], some reports have failed to find a correlation between plasma PYY concentrations and subjective measures of satiety or fullness [70,71]. However, evidence of a positive correlation between PYY and satiety [72] or increased satiety upon exogenous PYY administration [67,73] are not lacking. These disparities may be due to inaccurate self-reporting, as subjective appetite surveys are not always reliable [74]. However, a possible physiological explanation for these inconsistencies is that while PYY certainly has some anorexigenic roles in food intake and appetite control, it is not the sole agent in these regulatory pathways and the physiology behind satiety sensations and signaling is far too complex to be captured through investigation of a single gut peptide. Individual differences among subjects across studies such as age, sex, lean body mass, and energy status may also prevent consistent results.

Indeed, there is evidence that additional factors besides meal ingestion may affect PYY concentrations. A 2016 study by Kiessl et al. [71] found that higher psychological stress was related to decreased PYY release and food intake in both obese and non-obese women, although subjective appetite was unrelated to PYY levels. Both high intensity and continuous cardiovascular training acutely increase PYY and decrease hunger sensations following a bout of exercise [75,76], however resistance training has no effect on circulating PYY concentrations [77]. With respect to body composition, obesity is associated with low levels of plasma PYY [78] with an increase following weight loss [79]. This contradiction suggests a dysfunction of this hormone as either a result or cause of obesity, and this abnormality could potentially reinforce the condition.

Leptin

Leptin is an anorexigenic hormone secreted primarily by white adipose tissue in proportion to percent body fat [80] and is commensurably higher in women than in men [81]. Leptin release is pulsatile and, in healthy individuals, indirectly related to both pituitary-adrenal axis function [82] and ghrelin secretion [83]. Leptin receptors are found both peripherally and centrally [84], and the presence of receptors in the limbic system suggests an interaction between leptin and the food reward system [85]. The anorexigenic effect of leptin is supported by evidence that leptin levels are indirectly related to energy intake in healthy humans [86,87] and exogenous leptin administration results in decreased food intake in rats [88,89]. Exogenous leptin administration also results in subsequent weight loss in human [90] and animal [91] models. Because the amount of adipose tissue is relatively stable in the short-term (i.e. over the course of a day), the strong positive relationship between leptin production and adipose tissue suggests long-term regulatory action by the hormone in the context of nutritional status, weight maintenance, and food intake. However, there is recent evidence to suggest that leptin may also act as an acute, short-term regulator of energy balance. For example, insulin stimulation in humans results in increased leptin secretion [92]. Because insulin is released acutely upon meal ingestion, it is possible that leptin has some effect on individual meal size. Leptin is also produced in small amounts in the stomach [93], suggesting a role for the hormone in digestion and further supporting the possibility of acute action by leptin. Additional research is needed to confirm this theory.

Despite an extensive amount of academic literature on the subject, the specific contribution of leptin to energy balance and the pathology of obesity is still unclear. Although it is thought to be a condition rarely found in nature, humans [94] and animals [95] who are genetically leptin deficient exhibit morbid obesity, yet leptin levels are elevated in obese individuals who are not leptin deficient [91,96]. Mutations in the leptin receptor cause obesity [97], and though the abnormality has been observed in humans [98], it is extremely rare and not likely to be the primary cause of the disease [99]. With respect to leptin as a treatment for obesity, weight loss is still observed in obese subjects treated with exogenous leptin just as in lean subjects [90,91]. Overall, it seems leptin is significant to the

physiology of obesity, but leptin alone is likely not the sole vehicle for the development of such a complex disease.

Outside of weight regulation, leptin has a plethora of associations relevant toother physiological systems. Leptin administration upregulates FSH production in male rats and LH production in female rats [100]. Further, exogenous estrogen induces [101], while testosterone inhibits [102], leptin production in humans. Leptin promotes angiogenesis [103], piquing interest of the hormone in relationship to cardiovascular pathologies commonly seen in those with obesity. Leptin administration increases sympathetic nerve behavior in mice [104], which has prompted investigation of the hormone relative to its relationship to hypertension. Glucocorticoids stimulate leptin gene expression [105] meaning the hormone could also be important to the etiology of inflammatory conditions.

Cholecystokinin (CCK)

CCK is an anorexigenic hormone secreted from the intestine following meal consumption. Specifically, CCK is released upon nutrient stimulation of neuroendocrine secretory cells in the lumen of the small intestine [106]. CCK receptors are present in the GI tract, pancreas, gallbladder, and brain [25]. The hormone was first discovered for its ability to induce gallbladder contractions and subsequent emptying [107], but has more recently been studied for its effects on satiety and food intake. CCK injections in rats reduce meal size and increase meal frequency with no change in total food intake, suggesting only short term appetite suppression by the hormone [108]. The same reduction of meal size upon CCK infusion is also observed in humans [109,110]. Endogenous CCK's role as a true appetite suppressant in the body has been questioned because the majority of CCK studies use pharmacological doses of CCK rather than physiological doses [25] when relating the hormone to food ingestion. Despite this controversy, the hormone is typically classified as a satiety-inducing agent.

2.4 Gut Peptides in Anorexic Models

Ghrelin and PYY

Ghrelin secretion is altered in anorexic patients when compared to healthy individuals, suggesting an important role for it in the etiology of anorexia nervosa. Women with anorexia nervosa have significantly higher fasting ghrelin levels than controls [111–114], in a pattern that is more prominent in the binge-purge subset of the disease than the restrictive subset [115]. Specifically, alterations in ghrelin secretory dynamics observed in anorexic populations include increased burst mass, increased burst amplitude, and higher pulsatile and total ghrelin secretion [113]. Plasma ghrelin levels do not decrease in anorexic patients following meal ingestion as is seen in healthy controls [112]. However, plasma ghrelin concentrations decrease in anorexic patients following dietary and cognitive-behavioral therapy [111] and weight gain [114].

Ghrelin's roles as a GH secretagogue and orexigenic hormone are also abated in the context of anorexia nervosa. Broligo et al. [116] found that exogenous ghrelin infusion resulted in blunted effects on GH secretion in anorexic subjects when compared to healthy, normal weight women. In a similar study, Miljic et. al. found that both GH secretion and appetite were suppressed in anorexic patients compared to healthy, constitutionally thin subjects [117]. This is consistent with a number of other studies documenting decreased appetite in anorexic subjects [118–120]. Further, Misra et al. [121] compared healthy weight adolescent girls to adolescent girls with anorexia nervosa, and found that ghrelin strongly predicted plasma estradiol concentrations, plasma cortisol concentrations, and bone mineral density at the lumbar spine in healthy girls, but not in anorexic subjects. All of these findings suggest that although plasma ghrelin is elevated in anorexic patients, its biological effects are significantly blunted.

It is possible that the increased ghrelin levels present in anorexic patients could, partially, be due to hypoestrogenism [122,123]. Hypoestrogenism and overall suppression of the reproductive axis are well-documented consequences of being in an energy deficient state [8,124–126]. As previously discussed, secretion patterns of ghrelin are, on some level, dependent on estrogen, with decreased

estrogen consistently resulting in an increase in ghrelin secretion. While a lack of estrogen may explain elevated ghrelin levels, it does not account for the lack of appetite observed in anorexic patients. The previously described animal models would suggest that hypoestrogenism, as seen in anorexia nervosa, would result in increased hyperphagia and food intake. Because this does not align with what is actually observed in humans, it is plausible that appetite suppression in women with anorexia nervosa could be due, in part, to increased activity of anorexigenic gut hormones.

Studies examining aberrations in PYY in anorexic women are lacking compared to those of ghrelin, but the existing literature is consistent. Fasting PYY levels are elevated in female anorexia nervosa subjects compared to both healthy age-matched controls [127], and obese and morbidly obese subjects [128]. The increase in PYY typically observed in healthy individuals following meal consumption is also larger, and remains elevated for longer, in anorexic patients when compared to controls [129]. Overnight fasting PYY concentrations are inversely related to markers of insulin resistance [127] and to BMD at the spine [130] in anorexic subjects. Scheid et al. [131] observed direct associations of PYY with occurrences of disordered eating and hypothalamic amenorrhea in exercising women. Although this study did not directly observe anorexic subjects, this finding is relevant as hypothalamic amenorrhea has previously been part of the diagnostic criteria of anorexia [132], and is a result of energy deficiency in general [6]. Nakahara et al found that, in response to a meal, PYY does not completely return to normal concentrations upon recovery from anorexia nervosa, whereas the insulin and glucose responses to a meal are fully restored in this population [129]. However, it is possible that the study duration was not long enough to see a complete restoration of PYY function, as “recovery” was classified as an ability to consume a 2000 kcal mixed meal. On average, this marker occured only 26 days into treatment, while other hormonal anomalies such as hypoestrogenism [133] and elevated ghrelin [111] may take multiple months to completely normalize following a case of chronic energy deficiency.

The simultaneously elevated levels of anorexigenic PYY and orexigenic ghrelin are counterintuitive and not well understood. Nakahara et al. [129] suggests that the elevation of both ghrelin

and PYY in anorexia contribute to the severity of the disease by sending contrasting satiation signals that lead to unstable behaviors in patients, such as inconsistent eating habits and poor self image. Even if true, the relationship is further complicated by the fact that anorexia nervosa patients typically report low appetite despite elevated ghrelin levels. It is possible that anorexic subjects chronically underreport hunger as a result of their extreme fear of weight gain. However, there are also some plausible physiological reasons why hunger sensations would be reduced in this population. Appetite suppression could be due to the increased levels of fasted PYY consistently seen in this population wherein the anorexigenic sensations of PYY "win out" over the orexigenic signals of ghrelin. In this scenario, elevated PYY could be a means of survival used to blunt the discomfort of hunger sensations one would expect in an extremely energy deficient population such as in those with anorexia nervosa, though a physiological mechanism for such a phenomena is lacking and difficult to prove. Alternatively, appetite suppression in anorexia nervosa could be due to a more generalized systemic dysfunction which, in turn, leads to the elevation of both ghrelin and PYY. For example, Lawson et. al. attributes lack of hunger in anorexia to hypercortisolemia resulting in dysregulation of the hypothalamic-pituitary-adrenal axis and ultimately hypoactivating food motivation pathways in the brain [134]. Because both PYY [135] and ghrelin [136] have receptors in the hypothalamus, it is possible that PYY and ghrelin secretion could be altered under such conditions. This could potentially lead to the contradictory serum levels of the hormones seen in anorexia nervosa. All of these explanations are speculative and require further research in order to confirm or deny their validity.

Leptin

Research regarding leptin and anorexia nervosa is relatively consistent. Leptin levels are suppressed in those with anorexia nervosa [137–140] and increase with weight gain [138,141,142]. Soluble leptin receptor, its main binding protein, is also elevated in anorexia [143] suggesting that free leptin levels are even lower than serum concentrations. Leptin levels may also be an important marker for

the psychological severity of anorexia nervosa, as leptin concentrations are negatively related to cognitive restraint measures reported by anorexic patients as measured by the Three Factor Eating Questionnaire [120].

Leptin may, arguably, be more important in the treatment of anorexia than the etiology. Therapeutic use of leptin to restore normal reproductive function in anorexia nervosa may have some potential. As previously stated, amenorrhea is a frequent characteristic of those suffering from anorexia, and although leptin levels do not significantly differ between amenorrheic and eumenorrheic anorexia nervosa subjects, an increase in leptin concentration is associated with resumption of menses in those who are amenorrheic [144]. This suggests that leptin treatment could be a means to treat menstrual dysfunction in anorexia; however, exogenous leptin's effects on body composition must be thoroughly explored as its relationship to body fat is important to consider in a population that is infatuated with body image.

Cholecystokinin (CCK)

It is unclear whether CCK secretion is altered in anorexia nervosa as the literature is relatively inconsistent. Some investigators found fasted CCK to be elevated in subjects with anorexia when compared to healthy controls [145,146]. Other studies found decreased [147] or normal [148] basal CCK concentrations in anorexic subjects. Some investigations have found postprandial CCK is elevated in anorexia [145,146] and elevates more rapidly after meal ingestion [149], but others found no difference in postprandial CCK secretion compared to controls [146,150]. Any abnormalities in CCK are resolved with weight restoration [145].

2.5 Thermic Effect of Food

The Thermic Effect of Food (TEF) is defined as the volume of calories expended in response to the amount of heat generated during the consumption and digestion of a meal [151]. TEF accounts for

about 10-15% of daily energy expenditure in healthy individuals, depending on food choice [11], and has been shown to vary depending on both the energy content [16,152] and macronutrient composition [153–155] of a consumed meal. Relationships among TEF, body weight, and body composition are unclear in the literature. Some studies observed that obese subjects have a lower TEF when compared to normal-weight controls, but that this measure remains unchanged after weight loss [156]. Conversely, some studies show that TEF does change after weight gain and weight loss, with weight gain being associated with an increase [157] and weight loss with a decrease in TEF [158]. TEF is independent of body composition according to some findings [16], while others have found it to be directly related to body fat [159] and to lean body mass [151]. Other factors outside of body composition also affect TEF. Thorough mastication [160] and regular meal patterns [161] are associated with a higher TEF, but insulin resistance [162] and aging [163] are associated with a blunted TEF.

TEF in Models of Energy Deficiency

Research on TEF in anorexia nervosa is limited compared to that of healthy and obese populations. Existing studies consistently suggest that TEF is heightened during the refeeding stage of treatment; however, it's state during other phases of the disease are uncertain. Rigaud and colleagues observed TEF in hospitalized anorexia nervosa patients prior to the onset of a renutrition program designed for weight gain [164]. TEF was higher in anorexic patients than in healthy controls and directly correlated to caloric load. A study by Maoukaddem et al. [164] compared TEF in in anorexic patients in a semistarvation state (before a renutrition program) and again during a refeeding state (6-8 days into the renutrition program). Interestingly, TEF was significantly higher during refeeding than in the semistarvation state. A separate study by Russell similarly measured TEF of 18 hospitalized anorexia nervosa patients before and after weight gain [165]. TEF was elevated in patients preceding refeeding treatment as compared after refeeding. Pre-refeeding values of TEF were also elevated compared to healthy controls, while post-refeeding values were comparable to the control group. An earlier study by

Vaisman and colleagues measured changes in TEF over the course of treatment in hospitalized adolescent anorexic patients, and observed slightly different results than those in studies previously discussed [166]. TEF was reduced compared to controls upon hospital admission, elevated during renutrition, and then comparable to normal levels upon completion of refeeding and hospital discharge.

An elevation in TEF in anorexia nervosa patients may be due to increased occurrence of lipogenesis [165], increased lean body mass upon refeeding [164], and/or increased anxiety level due to fear of weight gain [164]. There is some discrepancy in the state of TEF before starting a refeeding program, but this could be explained by variations in the timeline of data collections, time before beginning the program, and diet composition before the program. However, more research would need to be done to confirm this.

2.6 Respiratory Quotient

Respiratory quotient (RQ), measured by indirect calorimetry as the rate of CO_2 production divided by the rate of oxygen consumption (VCO_2/VO_2), varies according to energetic status [165–167] and meal composition [168]. RQ approaches 1.0 after glucose consumption, 0.70 after fat consumption, and 0.83 after a normal mixed macronutrient meal [168]. In anorexia nervosa patients, conflicting results have been reported in the literature such that basal RQ was lower prior to refeeding and weight gain [164,166,169], and higher in patients before refeeding [165,167] and compared to healthy controls [166]. In one study of exercising women, there was no difference in basal RQ between women with functional hypothalamic amenorrhea (i.e. energy deficient) and those with eumenorrheic menstrual cycles [170]. These discrepancies remain when measuring postprandial RQ, which may partially be explained by differences in the macronutrient composition of the meal ingested. Further variability may be due to the length and severity of the energy deficiency. Glycogen is stored in finite amounts and, if depleted, an

increased reliance on fat or protein as fuel would be necessary, and this shift would be reflected by a lower RQ.

2.7 Conclusion

A thorough understanding of the etiology underlying the development of an energy deficit is important for patients and practitioners seeking means for adequate energy restoration. Although most energy deficiency literature has been in the context of anorexia nervosa, these studies are also useful for etiological assimilation of less severe cases of energy deficiency such as in the Female Athlete Triad. Disruptions in gut hormones and, subsequently, satiety signals may impact eating behaviors resulting in insufficient caloric intake to meet energy needs. In addition, abnormal digestive energetics, demonstrated by consistently elevated TEF in energy deficient subjects, may be both an effect and magnifier of the condition. When developing treatments for energy deficiency, these metabolic factors must be taken in consideration alongside the well-known psychological irregularities these patients face.

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Chapter 3

Methods

3.1 Experimental Design:

A cross-sectional repeated measures design was used to determine the effect of a 700 kcal mixed composition liquid meal (Ensure) on the thermic effect of food (TEF), fuel utilization, subjective appetite, and metabolic hormone responses in young, exercising, amenorrheic (AMEN; n=7) and eumenorrheic (OV; n=9) women. Subjects underwent two experimental conditions over the course of 1-3 months in random order. During test Visit A participants underwent a 6 hour TEF test based on indirect calorimetry measures using a ventilated hood system to monitor REE and fuel utilization following consumption of a liquid mixed-composition meal. Test Visit B was a 6 hour blood draw test following consumption of an identical meal. Menstrual status was determined by self-reported menstrual history evaluation and confirmed by a prospective evaluation of reproductive hormone concentrations measured from daily urine samples.

3.2 Study Participants:

Participant eligibility criteria included: 1) women; 2) between the ages of 18 and 35 years; 3) BMI between 16-25 kg/m^2; 3) currently participating in aerobic or resistance training for at least 3 hr/wk; 4) gynecological age >5 years; 5) weight stable for the past 6 months; 6) not experiencing regular menstrual bleeding within the past 3 or more months OR have regular menstrual cycles of 26-35 days for the past 6 months. Exclusion criteria included smoking, pregnant, breastfeeding or lactating, taking hormonal contraceptives in the previous 6 months, current clinical diagnosis of an eating disorder, use of

medications that would alter reproductive or metabolic hormones, hemoglobin below 11.5mg/dL or hematocrit below 35%. The Institutional Review Board approved this study and all participants signed an informed consent document.

3.3 Study Time Period:

This study involved up to four visits, two screening and two test conditions, to the laboratory over the course of 1-3 months. The two screening sessions lasted approximately two hours each and the test conditions lasted 6-7 hours each. Screening Visit A consisted of an informed consent meeting followed by the completion of surveys, and collection of height (to the nearest 1.0 cm) and weight (to the nearest 0.05 kg) measurements. Participants were asked to fill out 1) a health, exercise and nutrition survey, 2) the three factor eating survey, 3) a survey to assess mental health, 4) a survey to assess attitudes toward eating, 5) questionnaires to assess psychological stress, and 6) a questionnaire to assess emotional eating. Subjects were also instructed how to complete a 3 day food log (two weekdays, one weekend day), 7 day exercise log, and a menstrual/urine collection calendar. Screening Visit B included a fasting blood draw, physical exam, dietician interview, and dual-energy x-ray absorptiometry (DXA) scan by a technician certified by the International Society of Clinical Densitometry (ISCD). Participants then underwent two experimental conditions over the course of 1-3 months (Test Visit A: 6hr TEF test, following consumption of a 700 kcal liquid mixed-composition meal; Test Visit B: 6hr blood draw test following an identical 700 kcal liquid mixed-composition meal) in random order, with at least a one-day washout period between visits. Test visits were conducted during days 2-8 of the follicular phase of the for OV groups and during the first six days of each 28 day monitoring period for the AMEN participants.

3.4 Screening Visits A and B

On a single occasion during the screening period of the study, blood samples were collected between 0730 and 1000hr, stored and processed as previously described [1]. Total body mass was measured to the nearest 0.05 kg on at least 1 occasion during the study with participants wearing shorts and a t-shirt. Height was measured to the nearest 1.0 cm. Body mass index (BMI) was calculated as the average body mass divided by height squared (kg/m^2) and DXA was utilized to determine body composition via a total body DXA. The division of soft tissue into fat (g) and lean tissue (g) was based on an attenuation ratio of high-energy and low-energy photons or R-value and fat free mass (FFM) was used to adjust resting energy expenditure (REE) measurements. Subjects were scanned on a GE Lunar iDXA (General Electric Lunar Corporation, Madison, WI; enCORE 2008 software version 12.10.113) and all women were required to provide a urine sample prior to DXA for confirmation of a negative pregnancy test.

3.5 Dietary EI:

Dietary energy intake (EI) (kcal/day) was assessed using three-day diet logs recorded for two weekdays and one weekend day, as previously described [1,2]. These three-day nutritional logs for 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 [3]. 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).

3.6 Dietician Interview and Standardized Control Meals

During one of the screening visits, participants were asked to meet with a dietician to assess general eating habits and determine participant's food preferences. This information was used to design appropriate standardized control meals for the participant to consume during the 24-hour period prior to the each test visit. These meals included breakfast, lunch, a snack, and dinner. Participants were asked to consume the first meal at the General Clinical Research Center and the remaining meals were sent home with them. Subjects were instructed to eat all of the food provided, and only the food that was provided.

3.7 Test Condition A – TEF and RQ

Resting energy expenditure (REE) was measured during Test Visit A and determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA). Room temperature (°C), humidity (%H_20), and barometric pressure (mmHg) were measured. Prior to testing, participants were instructed not to exercise or ingest caffeine within 24hr, refrain from ingesting food and alcohol within 12hr, and arrive at the lab within 90 minutes of 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 [4] [655.0955+9.5634 (weight)+1.8495 (height)-4.6756 (age)]. REE measurements were performed between 0645 and 0830hr in a lit room at a comfortable temperature setting (20-24 °C). After the volunteers laid 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 45-min measurement period. Oxygen consumption (VO_2; mL/min) and carbon dioxide production (VCO_2; mL/min) were measured every 30 seconds. 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 (RQ) values were not varying more than 10% for a minimum of 10 minutes. Measured REE was calculated using the Weir equation [5]: REE (kcal/day)=[3.94(VO_2)+1.11)

VCO_2)]*1.44. A ratio of the measured REE to predicted REE (mREE/pREE) was calculated once using the Harris-Benedict equation [4] and used for the entire study period.

During Test Visit A, following the 45 minute REE measurement, subjects consumed a standardized mixed-composition liquid meal of 700 kcals (14% protein, 64% carbohydrate, and 22% fat) within a 15 minute period. Directly after meal consumption, the subject laid quietly under the ventilated hood for 45 minutes for measurement of TEF and RQ followed by a 15 minute rest period. During the 15 minute rest period, the subjects were asked to remain lying supine, but the hood was removed. If necessary, the subject was permitted to use the washroom at the beginning of this 15 minute break. A cycle of 30 minutes under the ventilated hood and a 30 minute break with the hood removed (with subject remaining at bed rest) followed for a total TEF measurement period of 5 hours. The data from the final 20 minutes of each of the 30 minute sessions under the hood were used for statistical analyses. TEF was defined as the increase in energy expenditure above baseline REE for 6 hours after a standardized mixed composition liquid meal and was calculated by assessing incremental area under the curve. RQ was defined as the ratio of the amount of carbon dioxide produced to the amount oxygen consumed or $[VCO_2]/[VO_2]$ at a given time point.

3.8 Test Condition B – Blood Sampling

Subjects were asked to arrive at the lab between 0700-0730 for Test Visit B, which was designed to coincide with the end of the REE test in Test Visit A, ensuring that meal consumption and all study timepoints occurred at the same time of day for both test conditions. Volunteers laid supine and an intravenous peripheral line was inserted for serial blood sampling. Blood samples were drawn 30 minutes and 15 minutes before consumption of the standardized 700kcal liquid meal. Follow-up blood draws were collected every 15 minutes for the first hour, every 30 minutes during the second hour, and then

hourly for the last 3 hours. After allowing clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for fifteen minutes at 4° Celsius. Serum samples were aliquoted into 2 mL polyethylene storage tubes and stored in a freezer at -80° C.

3.9 Statistical Analysis:

All data was checked for assumptions of normality and values are reported as mean ± standard error of the mean with significance set as $p<0.05$. TEF is expressed as the elevation above REE at each time point as an absolute term, relative to fat free mass, and as the integrated increase in energy expenditure above REE (area under the curve (AUC)) during the 5 hours after meal consumption. TEF was calculated as the area under the response curve adjusted for REE by subtracting REE from the energy expenditure measured. Differences between groups were analyzed using two-sided independent t-tests to REE and TEF AUC. Two-way repeated measures analyses of variance (ANOVAs) were performed in order to compare RQ and TEF between groups at all time points (0, 60, 120, 180, 240, and 300 minutes) with a Tukey post-hoc test when significant main effects are found. All analyses were conducted on SPSS v. 24.

3.10 References

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Chapter 4

Manuscript

4.1 Introduction

The Female Athlete Triad (Triad) is a medical syndrome consisting of low energy availability (EA), suboptimal bone health, and menstrual dysfunction [1] which is known to affect exercising women [2–4]; particularly those who participate in sports where leanness is emphasized for aesthetic or performance purposes [2,4,5]. Athletes at risk for the Triad often develop a prolonged energy deficit due to insufficient energy intake relative to the increased demands of exercise energy expenditure [5]. This energy deficit can result via several mechanisms including: purposeful undereating to restrict caloric intake in an effort to achieve a perceived ideal body composition; disordered eating behaviors including high dietary cognitive restraint and drive for thinness [6–8]; or inadvertently failing to match energy intake to energy expenditure [1]. In response to the resultant chronic energy deficiency compensatory mechanisms, which can be deleterious to overall health and wellbeing, are initiated. Specifically, during a prolonged energy deficit, energy is shunted away from the non-essential metabolic costs of growth and reproduction in order to maintain the essential physiological functions of cellular maintenance, thermoregulation, and locomotion [9]. In women, these energetic adjustments can contribute to clinical issues such as low bone mineral density [10–12], impaired lipid profiles and endothelial dysfunction [13], and hypoestrogenism [14,15] resulting in menstrual disturbances such as functional hypothalamic amenorrhea (FHA) [16–18].

The energy needs of the body can be split into energy intake and total daily energy expenditure (TDEE). Energy input consists of food intake; while, energy expenditure includes resting energy

expenditure (REE), thermic effect of food (TEF), and activity thermogenesis [19,20]. Resting energy expenditure, accounting for 65-70% of TDEE, is the energy required to maintain essential bodily processes [20] and is suppressed in exercising women with FHA [21]. Activity thermogenesis, 15-25% of TDEE, is further subdivided into exercise associated thermogenesis (EAT) and non-exercise associated thermogenesis (NEAT). Exercise associated thermogenesis is the energy expended during purposeful physical activity and is elevated in exercising women, while NEAT is the energy expended during all other activities outside of sleeping, eating, and intentional exercise [22]. The TEF accounts for 10-15% of daily energy expenditure and is the increase in energy expended during the digestion, absorption, and storage of food [20]. The TEF has yet to be quantified in women with FHA, but it has been elevated in other models of energy deficiency such as anorexia nervosa [23,24]. This elevation may contribute to the inability of exercising women with FHA to the meet the energy requirements necessary for resumption and maintenance of menses. As an adaptation to energy deficiency, an elevation in the TEF is counterintuitive because it does not align with typical mechanisms of energy conservation in which caloric expenditure is decreased. The mechanism for this increase in TEF is of interest as any increase in energy expenditure may be deleterious to recovery/refeeding efforts in severely energy deficient patients.

An additional metabolic component that may be altered during an energy deficiency, and thus warrants further investigation in exercising women with FHA, is substrate utilization. Respiratory quotient (RQ), measured by indirect calorimetry as the rate of carbon dioxide production divided by the rate of oxygen consumption (VCO_2/VO_2), varies according to energetic status [23,25,26] and meal composition [27]. The RQ approaches 1.0 after glucose consumption, 0.70 after fat consumption, and 0.83 after a normal mixed macronutrient meal [27]. In anorexia nervosa patients, conflicting results have been reported in the literature such that basal RQ was lower prior to refeeding and weight gain [25,28,29], and higher in patients before refeeding [26,30] and compared to healthy controls [25]. An improved understanding of fuel utilization in exercising women with FHA, a milder form of energy deficiency than

anorexia nervosa, is important for a more complete understanding of the metabolic alterations known to occur in this population.

To date, research exists in anorexia nervosa patients examining the TEF and substrate utilization [23,24,29]; however, there are gaps in the literature pertaining to these markers in more commonly encountered but less extreme cases of energy deficiency, for example, in exercising women or those with menstrual disturbances. The purpose of the present study is to directly compare the metabolic profiles (REE, TEF, RQ) of exercising women of differing menstrual statuses in order to improve the understanding of metabolic adaptations that may contribute to or result from an energy deficit, and thus allows for a means to identify the metabolically disruptive mechanisms present in more commonly encountered cases of energy deficiency.

4.2 Methods

4.2A Experimental Design:

A cross-sectional repeated measures design was used to determine the effect of a 700 kcal mixed composition liquid meal (Ensure) on the thermic effect of food (TEF), fuel utilization, subjective appetite, and metabolic hormone responses in young, exercising, amenorrheic (AMEN; n=7) and eumenorrheic (OV; n=9) women. Subjects underwent two experimental conditions over the course of 1-3 months in random order. During test Visit A participants underwent a 6 hour TEF test based on indirect calorimetry measures using a ventilated hood system to monitor REE and fuel utilization following consumption of a liquid mixed-composition meal. Test Visit B was a 6 hour blood draw test following consumption of an identical meal. Menstrual status was determined by self-reported menstrual history and confirmed by a prospective evaluation of reproductive hormone concentrations measured from daily urine samples.

4.2B Study Participants:

Participant eligibility criteria included: 1) women; 2) between the ages of 18 and 35 years; 3) BMI between 16-25 kg/m^2; 3) currently participating in aerobic or resistance training for at least 3 hr/wk; 4) gynecological age >5 years; 5) weight stable for the past 6 months; 6) not experiencing regular menstrual bleeding within the past 3 or more months OR have regular menstrual cycles of 26-35 days for the past 6 months. Exclusion criteria included smoking, pregnant, breastfeeding or lactating, taking hormonal contraceptives in the previous 6 months, current clinical diagnosis of an eating disorder, use of medications that would alter reproductive or metabolic hormones, hemoglobin below 11.5mg/dL or hematocrit below 35%. The Institutional Review Board approved this study and all participants signed an informed consent document.

4.2C Study Time Period:

This study involved up to four visits, two screening and two test conditions, to the laboratory over the course of 1-3 months. The two screening sessions lasted approximately two hours each and the test conditions lasted 6-7 hours each. Screening Visit A consisted of an informed consent meeting followed by the completion of surveys, and collection of height (to the nearest 1.0 cm) and weight (to the nearest 0.05 kg) measurements. Participants were asked to fill out 1) a health, exercise and nutrition survey, 2) the three factor eating survey, 3) a survey to assess mental health, 4) a survey to assess attitudes toward eating, 5) questionnaires to assess psychological stress, and 6) a questionnaire to assess emotional eating. Subjects were also instructed how to complete a 3 day food log (two weekdays, one weekend day), 7 day exercise log, and a menstrual/urine collection calendar. Screening Visit B included a fasting blood draw, physical exam, dietician interview, and dual-energy x-ray absorptiometry (DXA) scan by a technician certified by the International Society of Clinical Densitometry (ISCD). Participants then underwent two experimental conditions over the course of 1-3 months (Test Visit A: 6hr TEF test, following

consumption of a 700 kcal liquid mixed-composition meal; Test Visit B: 6hr blood draw test following an identical 700 kcal liquid mixed-composition meal) in random order, with at least a one-day washout period between visits. Test visits were conducted during days 2-8 of the follicular phase of the for OV groups and during the first six days of each 28 day monitoring period for the AMEN participants.

4.2D Screening Visits A and B

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4.2E Dietary EI:

Dietary energy intake (EI) (kcal/day) was assessed using three-day diet logs recorded for two weekdays and one weekend day, as previously described [7,8]. These three-day nutritional logs for 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 [31]. 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).

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4.2G Test Condition A – TEF and RQ

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temperature setting (20-24 °C). After the volunteers laid 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 45-min measurement period. Oxygen consumption (VO_2; mL/min) and carbon dioxide production (VCO_2; mL/min) were measured every 30 seconds. 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 RQ values were not varying more than 10% for a minimum of 10 minutes. Measured REE was calculated using the Weir equation [33]: REE (kcal/day)=[3.94(VO_2)+1.11) VCO_2)]*1.44. A ratio of the measured REE to predicted REE (mREE/pREE) was calculated once using the Harris-Benedict equation [32] and used for the entire study period.

During Test Visit A, following the 45 minute REE measurement, subjects consumed a standardized mixed-composition liquid meal of 700 kcals (14% protein, 64% carbohydrate, and 22% fat) within a 15 minute period. Directly after meal consumption, the subject laid quietly under the ventilated hood for 45 minutes for measurement of TEF and RQ followed by a 15 minute rest period. During the 15 minute rest period, the subjects were asked to remain lying supine, but the hood was removed. If necessary, the subject was permitted to use the washroom at the beginning of this 15 minute break. A cycle of 30 minutes under the ventilated hood and a 30 minute break with the hood removed (with subject remaining at bed rest) followed for a total TEF measurement period of 5 hours. The data from the final 20 minutes of each of the 30 minute sessions under the hood were used for statistical analyses. TEF was defined as the increase in energy expenditure above baseline REE for 5 hours after a standardized mixed composition liquid meal and was calculated by assessing incremental area under the curve. RQ was defined as the ratio of the amount of carbon dioxide produced to the amount oxygen consumed or [VCO_2]/[VO_2] at a given time point.

4.2H Test Condition B – Blood Sampling

Subjects were asked to arrive at the lab between 0700-0730 for Test Visit B, which was designed to coincide with the end of the REE test in Test Visit A, ensuring that meal consumption and all study time points occurred at the same time of day for both test conditions. Volunteers laid supine and an intravenous peripheral line was inserted for serial blood sampling. Blood samples were drawn 30 minutes and 15 minutes before consumption of the standardized 700kcal liquid meal. Follow-up blood draws were collected every 15 minutes for the first hour, every 30 minutes during the second hour, and then hourly for the last 3 hours. After allowing clotting for 30 minutes at room temperature, the samples were centrifuged at 3000 rpm for 15 minutes at 4° C. Serum samples were aliquoted into 2 mL polyethylene storage tubes and stored in a freezer at -80° C.

4.2I Statistical Analysis:

All data was checked for assumptions of normality and values are reported as mean ± standard error of the mean (SE) with significance set as $p<0.05$. TEF is expressed as the elevation above REE at each time point as an absolute term, relative to fat free mass, and as the integrated increase in energy expenditure above REE (area under the curve (AUC)) during the 5 hours after meal consumption. TEF was calculated as the area under the response curve adjusted for REE by subtracting REE from the energy expenditure measured. Differences between groups were analyzed using two-sided independent t-tests to compare REE, and TEF AUC. Two-way repeated measures analyses of variance (ANOVAs) were performed in order to compare RQ and TEF between groups at all time points (0, 60, 120, 180, 240, and 300 minutes) with a Tukey post-hoc test when significant main effects are found. All analyses were conducted on SPSS v. 24.

4.3 Results

4.3A Demographics

Descriptive and anthropometric characteristics of OV and AMEN groups are presented in Table 4.1. Overall, subjects were 23.0 ±1.17 years of age with an average height of 168.16±1.78 cm. Subjects weighed 60.83 ±1.57 kilograms with 26.1±1.29% body fat. There were no significant differences between groups with respect to age, height, body mass, gynecological age, body fat percentage, fat mass, fat free mass, or lean body mass. BMI was significantly different between the groups, where the OV group had a significantly higher BMI than the AMEN group (p=0.024).

Table 4.1. Descriptive and anthropometric characteristics of study participants

	OV (n=9)	AMEN (n=7)	P-value
Age (yrs)	23.33±1.66	22.57±1.74	0.759
Height (cm)	167.85±2.72	168.54±2.33	0.856
Body Mass (kg)	62.90±2.21	58.17±1.92	0.141
BMI (kg/m^2)	**22.30±0.45**	**20.48± .57**	**0.024**
Gynecological Age (yrs)	10.22±1.54	9.29±2.08	0.937
Body Fat (%)	27.89±1.76	23.80±1.64	0.120
Fat Mass (kg)	17.46±1.25	13.90±1.00	0.052
Fat Free Mass (kg)	45.31±2.00	44.61±1.86	0.806
Lean Body Mass (kg)	42.71±1.86	42.23±1.79	0.859

Data are expressed as mean±SE and bold indicates significance (p<0.05); BMI= Body mass index

4.3B Resting Energy Expenditure

Table 4.2 displays REE data for AMEN and OV groups. There were no significant differences between groups for absolute REE (kcal/day) (p=0.281), or when adjusted for fat free mass (kcal/kgFFM/day) (p=0.271). The ratio of measured to predicted REE was 0.78±.02 and 0.81±0.02 for AMEN and OV, respectively, and was not statistically different between groups (p=0.448).

Table 4.2. REE characteristics of ovulatory and amenorrheic subjects

	OV (n=9)	AMEN (n=7)	P-value
REE average (kcal/kgFFM/d)	26.19±0.71	25.04±0.68	0.271
REE average (kcal/day)	1179.43 ± 40.60	1113.08±41.97	0.281
Ratio of measured to predicted REE	0.81±0.02	0.78±0.02	0.448

Data are expressed as mean±SE and bold indicates significance ($p<0.05$); REE=Resting energy expenditure

4.3C TEF

Table 4.3 displays relative TEF and absolute TEF for all time points. For absolute TEF (kcal /day), there was no significant interaction effect (group*time) (p=0.723) or main effects for time (p=0.100) or group (p=0.651). In OV, average absolute TEF began at 227.14±21.19 kcal/day and peaked at a value of 284.52±63.37 kcal/day during minutes 150-180. In AMEN, average absolute TEF was initially 191.3±33.66 kcal/day and peaked during the same time period with a value of 282.17±9.83 kcal/day. Average absolute TEF was the lowest from 270-300 min in both groups, with a value of 123.60±70.47 for OV and 172.54±78.50 for AMEN.

For relative TEF (kcal/kgFFM/day), there was no significant interaction effect (group*time) (p=0.619), or main effects for time (p=0.147), or group (p=0.802). Average relative TEF began at 5.29±0.56 and 4.26±0.63 kcal/kg FFM/day for OV and AMEN, respectively. Average relative TEF in OV peaked at 7.56±0.69 kcal/kg FFM/day at 150-180 min with a nadir during the 270-300 min interval at a corresponding value of 4.28±0.60 kcal/kg FFM/day. The AMEN group followed a similar pattern with a peak relative TEF of 6.36±1.13 kcal/kg FFM during 150-180 min, and the lowest measures occurring during the 270-300 min interval and reported as 4.30±1.84 kcal/kg FFM.

Table 4.3. Relative and Absolute TEF for ovulatory and amenorrheic subjects

	Relative TEF (kcal/kg FFM/day)		Absolute TEF (kcal/day)	
Time	**OV (n=8)**	**AMEN (n=7)**	**OV (n=8)**	**AMEN (n=7)**
(1) 15-60 min	5.29±0.56	4.26±0.63	227.14±21.19	191.35±33.66
(2) 90–120 min	5.67±1.10	5.49±1.22	213.36±57.19	246.58±44.00
(3) 150-180 min	7.56±0.69	6.36±1.13	284.52±63.37	282.17±9.83
(4) 210-240 min	4.94±1.22	6.17±1.72	174.27±71.06	257.88±60.39
(5) 270-300 min	4.28±0.60	4.30±1.84	123.60±70.47	191.35±33.66

Data are expressed as mean±SE and bold indicates significance (p<0.05); TEF=Thermic effect of food; OV=Ovulatory; AMEN=Amenorrheic; FFM=Fat free mass

TEF measurements expressed as AUC (sum for all individual pieces of the area under the curve minus REE) is presented in Figure 4.1. AUC for the 5-hour TEF test was 50.65±4.49 kcal for OV and 47.14±5.56 kcal for AMEN, which was not significantly different between groups (p=0.692).

Figure 4.1. TEF Area Under the Curve

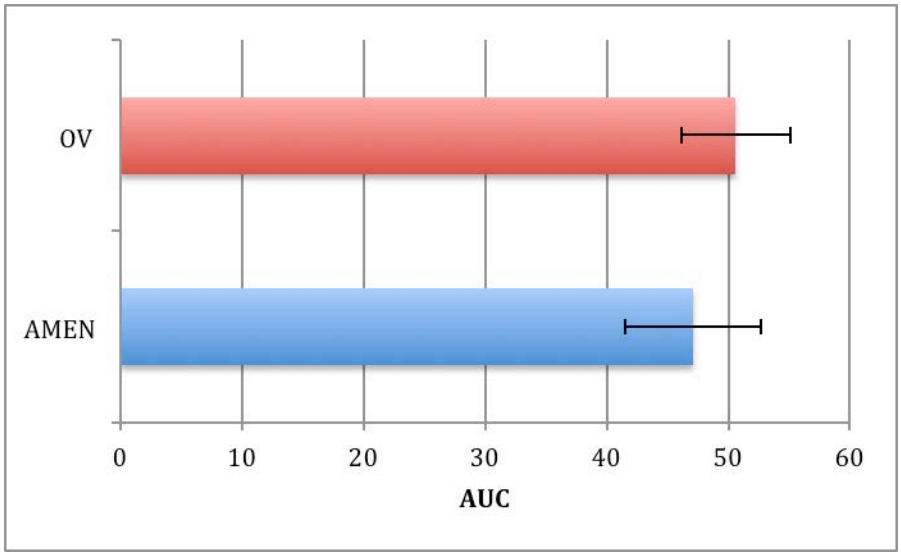

Fig 4.1. TEF Area Under the Curve. There were no significant differences between groups in TEF when expressed as AUC over a 5-hour period following meal ingestion and after correcting for REE.

4.3D Respiratory Quotient

Respiratory quotient (VCO_2/VO_2) during rest and at all postprandial time points is presented in Table 4.4. There was no significant interaction effect (group*time) (p=0.257) or main effect for group (p=0.782) for RQ. However, there was a significant main effect for time (p=0.006). RQ was significantly elevated from rest at 15-60 min and remained elevated through 240 minutes post-prandial before returning to resting values during the 270-300 min interval. RQ values increased from rest after consumption of the mixed composition meal peaking at 0.87±0.02 in OV during the 150-180 min interval and peaking at 0.85 ±0.02 in AMEN during the 210-240 min interval, before decreasing towards resting levels for the remainder of the measurements.

Table 4.4 Average respiratory quotient during rest and at all postprandial time points

	OV (n=9)	**AMEN (n=7)**
(0) rest	0.796±0.007	0.822±0.031
(1) 15-60 min	0.829±0.013	0.847±0.022
(2) 90-120 min	0.853±0.007	0.853±0.012
(3) 150-180 min	0.873±0.015	0.839±0.014
(4) 210-240 min	0.840±0.007	0.846±0.019
(5) 270-300 min	0.828±0.016	0.836±0.011

Data are expressed as mean±SE and bold indicates significance (p<0.05); OV=Ovulatory; AMEN=Amenorrheic

4.4 Discussion

The purpose of this study was to characterize and compare the metabolic profiles of young, exercising women with either amenorrheic or eumenorrheic menstrual statuses. When assessing REE, contrary to our hypotheses, there was no difference between AMEN and OV groups regardless of whether results were expressed in absolute or relative terms. These findings do not align with those demonstrated repeatedly in the literature, where women with functional hypothalamic amenorrhea (FHA) display suppressed REE in comparison to ovulatory women [21,34] as well as a decreased ratio of measured to predicted REE (mREE/pREE) [8,17,35]. De Souza, et al. [8] operationally defined energy deficiency in

exercising females as a mREE/pREE ratio of <0.90, based on classical clinical definitions of energy deficiency in anorexic models, an association of this ratio with hormonal indicators of energy conservation, and a stark contrast of this marker between those with high vs. normal drive for thinness scores. Although there was no significant difference in the mREE/pREE ratio between groups, both the AMEN (0.78±0.02) and OV (0.81±0.02) groups in the present study presented with mean mREE/pREE scores that indicate the presence of a potential energy deficiency according to the established <0.90 criterion.

These results are surprising because amenorrhea is often secondary to energy deficiency in exercising women [16,18], and it would be expected that the mREE/pREE ratio of those with ovulatory menstrual cycles would be ≥0.90, or at least be significantly higher than those in the AMEN group. A potential contribution to the discrepancy in these results compared to previous literature is the fact that energy status exists on a scale, and it is feasible that the subjects in the AMEN group were not severely energy deficient, or were nearing energy balance and/or, concurrently, those in the OV group were only on the borderline of being energy replete or were nearing a deficient state. This would mean that even though the two groups had differing menstrual statuses, their energy statuses were still quite similar, with both favoring the deficient side of the spectrum. Similarly, while energy status can be altered within days or weeks, it may take months for menstrual status to either be lost or recovered in response to changes in energy [36]. As a consequence, the OV group may not be an appropriate control group in which to compare AMEN subjects in order to observe metabolic changes due to energy deficiency since menstrual status may not be the most accurate assessment of current energetic status. Alternatively, the lack of menstrual cycles in the AMEN group may be caused by factors other than energy deficiency, alone. Using a cynomolgus monkey model, Williams et al. [37] found that moderate energy imbalance in combination with exposure to seemingly minor psychological stressors (i.e. change in external living environment) may act to cause significantly more disruptions to menstrual function among individuals in a population than energy deficiency without presence of additional stressors. Lastly, it is important to consider that

perhaps our two groups did, in fact, have different energy statuses from one another, but that energy deficiency is a complex condition consisting of a number of interacting factors. The REE parameter itself may not be enough to classify one as energy deficient or replete, as it is just one part of a larger and more intricate picture of energy deficiency adaptations. Analysis of TT_3 in future studies using this data set could help to clarify the presence and severity of energy deficiency in subjects in both AMEN and OV groups. Downregulation of TT_3 occurs upon meeting a threshold of energy deficiency that is dependent on lean body mass [38], and suppression of this metabolic hormone is often accompanied by reproductive dysfunction [17,39]. However, the possibility of similar energy statuses between the two groups in this study still remains important during analysis of our other primary outcomes.

An additional aim of our study was to characterize the TEF in AMEN and OV subjects in response to ingestion of a 700kcal mixed macronutrient meal. Contrary to our hypotheses, no significant differences in TEF were found between AMEN and OV groups when expressed in relative and absolute terms as well as AUC. While TEF has yet to be reported in exercising women with FHA, our results are in contrast to an elevation of TEF that has been observed several times in the anorexia nervosa literature [23,24,29]. The current findings also fail to align with those of Vaisman et al. [25], who observed a suppression of relative TEF in anorexic subjects prior to undergoing a renutrition program. However, methodical variations among studies such as meal size, method of consumption of caloric load (infusion vs. oral), meal composition, timing of meal consumption with respect to measurement of TEF, status of subjects with respect to energy recovery/treatment, and terms in which TEF is expressed make it difficult compare results between studies. Still, our results are particularly interesting to compare to Rigaud et al. [24] who observed TEF in response to infusion of a caloric load to be higher in anorexic subjects than in controls and reported TEF in terms of AUC. While AUC values in the current study were similar to those observed in healthy women with normal eating behaviors, TEF-AUC for AMEN in our study were still much lower than the anorexic subjects Rigaud and colleagues observed [24]. This suggests that the deviation of our TEF results from those previously published may be accounted for by the fact that

anorexia nervosa is an extreme case of starvation that encompasses a large energy deficit, and differences in TEF may only be statistically observable when comparing such an extreme model of energy deficiency to a healthy individual. None of the AMEN subjects were diagnosed with anorexia nervosa at the time of the current study, and were likely to be much less energy deficient than someone suffering from such a condition. Besides deviations in TEF being statistically non-significant in moderate cases of energy deficiency when compared to controls, noticeable aberrations in TEF (i.e. changes that lead the measure to be significantly distinguishable from that of an energy replete individual) in response to an energy deficiency may be an adaptation that is initiated only when the deficit has progressed to a certain threshold. This threshold may be one that AMEN subjects had not yet encountered, but one that is commonly met by those with anorexia nervosa. Alternatively, and as previously discussed, it is also possible that our AMEN and OV groups did not sufficiently differ in energy status in order to allow for observable differences between groups. Also, changes in TEF may have occurred, but were not detected due to the, aforementioned, potential lack of difference in energy status between groups. This alone could explain the discrepancy in our results from existing literature, but requires additional data to confirm.

This study also examined the relationship between RQ and menstrual status. The two groups did not differ in RQ at any time point throughout the duration of the study. These results refute our hypothesis, which was largely based on anorexia nervosa literature as RQ in response to a mixed meal has yet to be characterized in exercising women with FHA. In anorexia nervosa patients, conflicting results have been reported in the literature such that basal RQ was lower prior to refeeding and weight gain [25,28,29], and higher in patients before refeeding [23,26] and compared to healthy controls [25]. As was the case with TEF, it is difficult to compare the present study to those with similar outcomes due to differences with respect to study methodologies including macronutrient composition of the meal ingested. The hypothesis in the present study was based on speculations by Russel et al. [23], who observed an elevated fasted and postprandial RQ in anorexic subjects and attributed this elevation to increased rates of lipogenesis. This notion is based on the reasoning that the high RQ is reflective the

stoichiometric equation for palmitic acid synthesis (4½ glucose + 4 O_2 palmitate → 11 CO_2 [40]), a main component of human adipose tissue. This is reasonable as elevated postprandial RQ values concurrent with increased rates of fatty acid synthesis have been observed in calorie restricted mice [41]. This process is energetically expensive and could also explain simultaneous elevation of TEF with RQ often seen in anorexic subjects. However, this is contrary to what was observed in the present study as there was no difference between AMEN and OV groups, and though the possibility of energy deficiency in both groups still exists, any elevation in RQ for energy deficient subjects would still be far less than what is typically seen in the anorexic subjects that Russell was referring to. RQ values for all subjects in our study were significantly elevated from baseline, starting at the 15-60 min interval, but the peak RQ values for AMEN (.873 ± .015) and OV (.853 ± .012) were much lower than any values observed in anorexic subjects [23,28] or in mouse models of caloric restriction [41], which approached or exceeded unity. Therefore in the case that both groups were energy deficient, any increase in lipogenesis, reflected by elevated RQ, would not be to the degree seen in other models of energy deficiency. In fact, the basal and peak RQ values in the current study are comparable or in some cases lower to what has been observed in healthy controls in the postprandial state [23], suggesting that the observed increases in RQ in AMEN and OV groups after meal ingestion are not atypical or indicative of the presence of any abnormal metabolic patterns. Therefore, it is also viable that our groups did differ moderately in energy status but that substrate utilization is a process that is only significantly altered or initiated under conditions of an extreme state of energy deficiency such as anorexia. This would mean there would be no detectible differences between our AMEN and OV groups for RQ, and our results may be reflective of this.

4.5 Conclusion

This study did not find any significant differences between amenorrheic and eumenorrheic exercising females in REE, TEF, or RQ. These results do not align with typical observations of these markers in

more extreme cases of energy deficiency such as in anorexia nervosa or, in the case of REE, other studies examining amenorrheic and eumenorrheic athletes. Similarly low mREE/pREE ratios in OV and AMEN groups suggest that subject group assignments may have been inappropriate for the purpose of comparing metabolic outcomes in women of differing energy status, and this requires further analysis. The inclusion of a non-exercising control group may also be an option. It is also possible that energetic adaptations prompting alterations in TEF and RQ are only initiated or statistically distinguishable from controls at extreme levels of energy deficiency, and our results could be an affirmation of this. Future studies should consider a more holistic definition of energy deficiency, a larger sample size, better alignment of meal size and timing with those in the literature, and more hormonal markers in order to test hypotheses.

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EXPERIENCE:

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Women's Health and Exercise Laboratory, State College, PA
Undergraduate Research Assistant: Develop skills such as how to conduct and interpret resting metabolic rate testing and VO2max testing, process urine and blood samples, monitor and assess physical activity with logs and heart rate monitors, perform dietary analysis of diet logs, analyze health history and psychological survey data, interpret dual x-ray absorptiometry scans, and understand the management of study databases. Research under Dr. Mary Jane De Souza.

February 2016 – May 2018

SELECT Exercise Physiology, State College PA
Nominated Member: Read and critically analyze peer reviewed exercise physiology literature, attend lectures by researches, prepare and participate in debates of exercise physiology related topics, preparing to attend the 2016 American College of Sports Medicine Mid-Atlantic conference. One of 10 members nominated by Dr. Mary Jane De Souza.

July 2016 – May 2018

College of Health and Human Development, State College, PA
Peer Mentor: Serve as a student representative at various College of Health and Human Development functions, act as a resource for potential students and incoming freshman. Additionally, attends "Spend a Summer Day," a campus open house for Prospective First Year Students to learn about admissions, academics, and student life at Penn State.

August 2016 – August 2017

Penn State Recreation, State College, PA

Assistant Trainer: Work in all 3 fitness centers on campus, design goal-specific fitness programs for gym members, spot gym members on various exercises, maintain a clean and safe environment.

June 2016 – August 2017 **Penn State Sports Camps,** State College, PA
Duty Counselor: Supervise high school campers at various Penn State sports camps during night hours, answer campers questions, and take injured students to athletic trainer in the case of an emergency.

August 2016 – May 2017 **Penn State Fitness and Bodybuilding Club**, State College, PA
Vice President: Work with other executive board members to plan and execute meetings, schedule guest speakers, recruit new members of all fitness levels, and help members to stay updated on the current issues of the sport of bodybuilding.

June 2016 – August 2016 **Penn State Chemistry Department**, State College PA
Teaching Assistant for General Chemical Principles I: Worked under the supervision of Dr. Linlin Jensen during the summer session. Attended lectures, held office hours, and proctored practice exams. Encouraged students to engage in critical thinking over the course of the semester.

January 2016 – May 2016 **Penn State Center for Fitness and Wellness**, State College, PA
Intern: Administers and analyzes fitness tests for students including blood pressure and heart rate during exercise, handgrip test, body fat percentage, VO2 max, flexibility, curl ups, and push-ups.

Dec. 2014 – Feb 2015 **Penn State Varsity Track & Field Team**, State College, PA
Broadcaster: Announced names, lane assignments, and special announcements at indoor track & field meets, announced at four meets with an audience of 300+.

ACTIVITIES:

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- PSU Fitness & Bodybuilding Club Vice President
- Penn State Weightlifting Club Member
- Blends of Traditional Heritage (B.O.T.H) member
- American College of Sports Medicine Member
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- Organized Penn State Food Pantry Drive for Dr. Terrell Jones