PSYCHOMETRIC INDICATORS OF EATING BEHAVIOR AND RISK FACTORS
ASSOCIATED WITH PHYSIOLOGICAL ADAPTATIONS TO THE
FEMALE ATHLETE TRIAD IN EXERCISING WOMEN

A Dissertation in
Kinesiology

by

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ABSTRACT

The Female Athlete Triad (Triad) is a syndrome of interrelated conditions (low energy availability (EA), menstrual disturbances (MD), and low bone mineral density (BMD)) that exist along a continuum from healthy to pathological clinical sequelae. The objectives of this dissertation were: (1) to examine the association between psychometric indicators of disordered eating behavior (high drive for thinness (DT) and dietary cognitive restraint (DR)) and physiological adaptations to chronic energy deficiency/low EA and MD in exercising women, (2) to examine the changes in psychometric indicators of disordered eating behavior (DR, DT, body dissatisfaction (BD), and bulimia scores) during a randomized controlled trial (RCT) of increased energy intake in women with exercise-associated menstrual disturbances (EAMD) and exercising controls with EAMD and ovulatory cycles, and (3) to determine the risk for low BMD in association with individual and combined Triad risk factors. In Study One, we demonstrated that exercising women with high DT exhibited signs of chronic energy deficiency (suppressed resting energy expenditure (REE) controlled for lean body mass and ratio of measured REE compared to predicted REE) and a greater frequency of severe MD (oligo/amenorrhea) compared to women with normal DT. In Study Two, we demonstrated that exercising women with high DR had a lower EA and a greater frequency of subclinical/clinical MD than women with normal DR. However, there was no difference in frequency of low EA between groups. In Study Three, we demonstrated that refeeding did not exacerbate DR or have adverse effects on DT, BD, and bulimia scores in women with EAMD during a 6 month RCT. In Study Four, we showed a cumulative effect of Triad risk factors on BMD in a large sample of exercising women. In conclusion, we demonstrated: (i) associations between high DT/DR and physiological adaptations to Triad disorders (MD and chronic energy deficiency/low EA) in exercising women, (ii) an RCT of increased energy intake combined with psychological and
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CHAPTER 1

INTRODUCTION

The Female Athlete Triad, or the Triad, is a syndrome of interrelated conditions—low energy availability (EA), menstrual dysfunction, and low bone mineral density (BMD)—which exist along a continuum from healthy to increasingly pathological clinical sequelae in premenopausal athletic and exercising women (90). The Triad is frequently observed in exercising women participating in sport or activity emphasizing leanness and aesthetic appearance (i.e., long-distance running, gymnastics, and figure skating) (120). These female athletes often strive for low body weight and percent body fat via manipulations of energy intake and/or exercise energy expenditure, typically involving energy restriction and/or participation in strenuous exercise training (113,116). Inadequate energy intake relative to exercise energy expenditure, also known as low EA, is a key factor in the development of Triad clinical outcomes (90). Low EA is proposed to induce mechanisms of energy conservation, commonly referred to as an energy deficiency, as a means to repartition fuel to vital bodily processes, i.e., thermoregulation and cellular maintenance (124). Reproductive function (75,76,127) and bone turnover (57) are chronically compromised and clinical consequences may include: (i) menstrual disturbances, both subclinical (luteal phase defects (LPD), anovulation) and clinical (functional hypothalamic amenorrhea (FHA), oligomenorrhea) (31,32); (ii) musculoskeletal injuries and pathological bone loss (33,35,36,102); and (iii) cardiovascular complications (endothelial dysfunction, altered lipid profile) (55,94).
Links among low EA/chronic energy deficiency, disruptions in luteinizing hormone (LH) pulsatility (75) and menstrual cyclicity (20,127) have been well-documented in human and animal experiments. Chronic energy deficiency promotes alterations in energetic components (i.e., suppressed resting energy expenditure (REE)) and metabolic hormones (i.e., supressed total triiodothyronine (TT₃), leptin, insulin, glucose, and insulin-like growth factor-1 (IGF-1) and elevated ghrelin, growth hormone (GH), and cortisol) (30,69,72,73) in order to restore a eumetabolic state and prevent further energy restriction and/or weight loss. It is hypothesized that one or more of these metabolic hormones may be the signal(s) in a cascade of responses resulting in the disruption of gonadotropin releasing hormone pulsatility. Accordingly, energetic and metabolic adjustments may result in decreases in LH pulse frequency and increases in LH pulse amplitude, which suppress the production of ovarian steroid hormones (estrogen and progesterone). As a result, the induction of subclinical (LPD or anovulation) or clinical menstrual disturbances (FHA or oligomenorrhea) may occur in energy-deficient exercising women.

Menstrual disturbances may promote bone loss due to chronically low exposure to estrogen, an anti-resorptive hormone with protective effects on bone, or estrogen-independent mechanisms associated with an energy deficiency (90). Reductions in circulating estrogen concentrations alter bone turnover (77,104) resulting in increases in bone resorption and long-term complications such as low BMD (35,36,78,88), impaired bone microarchitecture (1,37), and musculoskeletal injuries or disorders (i.e., stress fractures, osteoporosis). Chronic energy deficiency represents another mechanism, independent of hypoestrogenism, promoting bone loss (33,57,131,132) and it is the
metabolic hormonal environment associated with an energy deficiency, i.e., reduced IGF-1, TT₃, and leptin concentrations (46,47,125), which suppresses bone formation.

Exercising women may present with an energy deficiency for several reasons: i) intentional, i.e., modifying body size and composition to achieve appearance or performance goals; ii) compulsive, i.e., demonstrating disordered eating and/or pathological weight control behavior; or iii) inadvertent, i.e., failing to match energy intake to energy expenditure (74). Those women with disordered eating attitudes and behaviors are notably susceptible to clinical sequelae associated with the Triad. Disordered eating exists along a continuum of abnormal attitudes and behaviors, ranging from subclinical to clinical eating disorders (i.e., anorexia nervosa and bulimia nervosa) (116). In such a context, self-motivated dieting is a well-recognized factor associated with more severe and persistent disordered eating linked to Triad-related consequences (114). There is notable pressure on exercising women to manage or lose weight as a result of: (i) sociocultural pressures for a thin physique and physical attractiveness, (ii) emphasis on the importance of appearance in sport and fitness, and (iii) perceived performance advantages (116). For female athletes, there is notable pressure to comply with the sport environment, such as rigid training and eating patterns, compliance with restrictive and/or weight-loss diets and unrealistic expectations for body composition. To this end, the prevalence of eating disorders is high in elite female athletes (up to 70%) (116). Conversely, several investigators have reported that recreationally active women may also be at risk for disordered eating/eating disorders (63,111). As such, the risk for disordered eating extends beyond elite female athletes and women with clinical eating disorders. There is considerable evidence linking exercise training to weight and shape preoccupation in exercising women (25-27,44,68), and habitual
exercise training coupled with restrictive eating may lead to subclinical or if severe, clinical disordered eating behavior in an effort to change physical appearance and/or lose weight.

There is substantial cross-sectional evidence of disordered eating attitudes and behavior among exercising women (42,113,114,129). Even subclinical presentations are prevalent in exercising women (28,45,108,123) and are characterized by abnormal eating and weight control patterns, body image concerns, and desire for thinness. Such traits resemble clinical disordered eating, but lack extreme psychopathology. Subclinical disordered eating behavior in exercising women has been associated with elevated scores on the drive for thinness (DT) and body dissatisfaction (BD) subscales on the Eating Disorder Inventory-2 (EDI-2) (13,22,42,44), and higher dietary cognitive restraint (DR) score on the Three Factor Eating Questionnaire (TFEQ) (82,123). Furthermore, subclinical disordered eating behavior often manifests as restrictive eating, which may result in reductions in energy intake and contribute to the development of chronic energy deficiency (28,45). High DT has been proposed as a key factor in the development of the Triad and its clinical conditions (22,28,45,93,119). High DT often promotes stringent dieting and/or exercise behavior associated with a reduction in energy intake that may lead to a disruption in energy homeostasis and a higher frequency of clinical menstrual disturbances (FHA and oligomenorrhea) (45). High DR is defined by a conscious restriction of energy intake in order to achieve or maintain a desired body weight (112). Such eating behavior is frequently observed in women who routinely monitor their food intake and practice potentially harmful methods of weight control. Several investigators have linked high DR with menstrual dysfunction, such as FHA, oligomenorrhea, and anovulation (6,15,81,110,123), and lower BMD (8,15,82,122,123) in premenopausal exercising women.
To date, few investigators have examined the associations between specific psychometric indicators of eating behaviors (i.e., DT, BD, DR) and physiological adaptations to Triad clinical sequelae (i.e., chronic energy deficiency or low EA, and menstrual disturbances) in exercising women (8,28,45,123). Furthermore, the effect of increased energy intake on indicators of disordered eating behavior (i.e., DT, DR, and BD) in exercising women has yet to be explored. Such findings will guide the development of preventive and treatments strategies for Triad disorders in exercising women with exercise-associated menstrual disturbances (EAMD). Non-pharmacological interventions focused on increasing energy intake may be an optimal approach in promoting normalization of energy status and restoration of normal menstrual function in women with EAMD. However, consideration of eating attitudes and behavior is warranted, as changes in DT, BD, and DR may be associated with increases in energy intake, weight gain, and metabolic factors in women with EAMD. Last, there are no studies wherein investigators have evaluated the cumulative risk for low BMD associated with Triad risk factors in a large sample of exercising women. Thus, one of our objectives in this dissertation was to better understand the association between non-invasive and easy-to-obtain indicators of the Triad and risk for low BMD in exercising women. These findings will represent a first step in developing a screening protocol or user-friendly algorithm with the goal of identifying at-risk exercising women that may need further clinical assessment to confirm risk for or incidence of low BMD. Chapter 1 outlines the content of the proposed dissertation with the primary purpose to evaluate psychometric indicators of eating behavior and risk factors associated with physiological adaptations to the Triad (low EA/chronic energy deficiency, menstrual disturbances, and low BMD) in exercising women. In Study One,
surrogate markers of energy deficiency and the distribution of menstrual disturbances were compared in exercising women categorized by DT score. In Study Two, EA and distribution of menstrual disturbances were compared in exercising women categorized by DR score. In Study Three, the effect of a randomized controlled trial (RCT) of increased energy intake on psychometric indicators of disordered eating behavior (DR, DT, BD, and bulimia scores) was examined in women with EAMD. In Study Four, the association between Triad risk factors (individual and in combination) and low BMD was examined to determine the cumulative effect of Triad risk factors on BMD in exercising women.
DISSERTATION PROPOSAL OUTLINE

STUDY ONE: A high drive for thinness is associated with energy deficiency and severe menstrual disturbances: Confirmation in a large population of exercising women

Background

Disordered eating behavior has been well-documented in premenopausal women engaging in recreational and competitive-level exercise (114). Previous reports in female athletes suggest that subclinical or clinical disordered eating may promote chronic energy deficiency potentially leading to the development of clinical consequences associated with the Triad (90,114). The Triad is a syndrome described by low EA (with or without disordered eating), functional FHA, and low BMD (90) alone or in combination, in exercising women. A tool often used to discriminate between disordered vs. healthy eating behavior and attitudes in college-aged women is the EDI-2 (43). The DT subscale of the EDI-2 is used to predict the presence of a subclinical (if severely high, clinical) variant of disordered eating that is often observed in exercising women. Such demonstration of DT is characterized by a preoccupation with body weight and body shape, fear of gaining weight, and high DR (106).

A large body of evidence supports strong correlations among subclinical disordered eating, DT, and amenorrhea in exercising women (28,90,119). Exercise-associated menstrual disturbances (EAMD) are proposed to begin with intense pressures to achieve an unrealistic standard of thinness and also, to enhance weight control (14,114). A high DT has also been proposed as an underpinning factor in the development of the Triad (28,114). Since a high DT promotes diet and/or exercise behavior focused on the achievement of a lean physique, we reasoned that a high DT may be associated with behavioral changes (i.e., dietary energy restriction and/or high exercise volume) likely resulting in the development of an energy deficiency (28).

Physiological evidence of an energy deficiency in exercising women can be demonstrated by a disruption in energetic and endocrine homeostasis, which triggers a reduction in REE, and
alterations in fasting hormone concentrations, including reduced TT₃ and elevated ghrelin concentrations (24,28). These energetic adaptations act as energy-conserving mechanisms translating effects to metabolism (28,29) and reproduction. As such, menstrual disturbances are often observed in energy-deficient exercising women, and therefore, we hypothesize that a greater frequency of clinical menstrual disturbances (amenorrhea and oligomenorrhea) will be observed in exercising women with high DT (28,93,123).

**Brief Rationale**

Substantial evidence exists in support of an association between disordered eating behavior (subclinical or clinical) and the development of conditions associated with the Triad, such as EAMD, low BMD, and musculoskeletal injuries (12,22,45,91,92,102,118,119). Disordered eating, both subclinical and clinical presentations, is well-documented in premenopausal female athletes (14,59,113,119) and exercising women (45,119,120,123). As such, disordered eating behavior represents a key factor in the etiology of the Triad (90). Recently, there has been evidence linking subclinical disordered eating behavior (i.e., high DT and DR) to Triad-related clinical sequelae (28,108,123). Conservative criteria defining disordered eating as clinical eating disorders may not necessarily yield accurate data in female athletes (13) and exercising women (28), and notably, active women identified as “at risk” for clinical disordered eating also often present with EAMD, low BMD, and bone injuries.

We have previously demonstrated that a high DT score may serve as an indicator of underlying energy deficiency based on the observation of a significant negative relationship between DT scores and REE when expressed as the ratio of measured REE to the Harris-Benedict predicted REE (REE/pREE) (28) (**Figure 1**). The exercising women with high DT in our previous study exhibited significantly suppressed REE (controlling for fat-free mass) and a lower ratio of REE/pREE. The ratio of REE/pREE is often utilized in published reports of energy-deficient women
with anorexia nervosa wherein a ratio of 0.60-0.80 was observed (66,83,96). In our previous study, we reported a ratio of \( \text{REE}/p\text{REE} \) of 0.86±0.03 which met our operational definition of an energy deficiency (\( \text{REE}/p\text{REE}<0.90 \)) (28,33).

**Figure 1.** In a previous study from our lab (28), De Souza and colleagues demonstrated an association between a high drive for thinness (DT) score and surrogate markers of an energy deficiency (resting energy expenditure controlled for fat-free mass (REE/FFM), ratio of actual REE to predicted REE, total triiodothyronine (TT3), and ghrelin). Reproduced with permission from De Souza et al., Appetite 2007; 48(3):359-67

To date, there are no studies wherein ovarian steroid profiles of exercising women with high DT are compared to exercising women with normal DT. Due to the inaccuracy of self-reported menstrual status and/or retrospective menstrual history questionnaires (31,32), the analysis of hormonal profiles between groups is undoubtedly important to yield objective characterization of menstrual status by urinary measures of LH, estrone glucuronide (E1G) and pregnanediol glucuronide (PdG) metabolites in this population of exercising women and to determine if
differences observed in menstrual status (ovulatory vs. amenorrheic) and energy status classifications (deficient vs. replete) coincide such that energy-deficient women with high DT have a higher frequency of severe/clinical menstrual disturbances (amenorrhea or oligomenorrhea). This study will represent the first wherein investigators characterize the distribution of menstrual disturbances using hormonal analyses measuring daily urinary E1G and PdG metabolites in exercising women with high DT vs. those women with normal DT. Thus, our findings are expected to provide greater insight into the association between DT score and menstrual function in exercising women. Overall, the rationale for this study is to compare surrogate markers of energy deficiency and the distribution of severe/clinical menstrual disturbances (amenorrhea or oligomenorrhea) in exercising women categorized by DT score. Our objective is to determine whether the DT subscale may provide valuable information to coaches, athletic trainers, and health practitioners attempting to identify an indication of energy and menstrual status in large groups of exercising women where direct assessment is not feasible.

Specific Aims and Hypotheses

Aim 1: To confirm a high DT score as an indicator of energy deficiency (suppressed REE (controlled for lean body mass, LBM) and a ratio of REE/pREE less than 0.90) in a larger population of exercising women compared to our previous publication

Primary Hypothesis

Hypothesis 1i: The exercising women with high DT will demonstrate signs of energy deficiency (suppressed REE controlled for LBM and a ratio of REE/pREE less than 0.90) and thus confirm our previous findings in a larger cohort of exercising women.

Secondary Hypothesis

Hypothesis 1ii: The ratio of REE/pREE will be significantly associated with fasting TT3 concentrations in our exercising women and the energy-deficient exercising women
(REE/pREE<0.90) will have significantly lower fasting TT₃ concentrations vs. those women who are energy replete (REE/pREE>0.90).

**Aim 2: To compare the distribution of severe menstrual disturbances in exercising women categorized by DT score**

Hypothesis 2: The exercising women with high DT will demonstrate a greater frequency of severe menstrual disturbances (amenorrhea or oligomenorrhea); whereas the exercising women with normal DT will demonstrate a greater frequency of eumenorrheic, ovulatory cycles, as corroborated using measures of LH and daily urinary E1G and PdG metabolites.

**Experimental Design/Overview of Research Methodology**

We used a cross-sectional design: (i) to confirm a high DT score as an indicator of energy deficiency in a larger population of exercising women compared to our previous publication (28) and (ii) to compare the distribution of menstrual disturbances in exercising women categorized by DT score. Participants that met inclusion criteria underwent screening to determine study eligibility. An exercise history review was completed and was corroborated with a maximal oxygen uptake (VO₂peak) test to determine exercise status. All participants were considered “exercising” if they were participating in 2 or more hours·week⁻¹ of purposeful exercise (105). Participants completed psychometric measurements of eating attitudes and behaviors (EDI-2, TFEQ). Scores on the DT subscale from the EDI-2 were used to categorize women as high (DT score≥7) or normal DT (DT score<7). Exercising women with high or normal DT were monitored for at least one complete menstrual cycle if regularly menstruating or at least one 28-day monitoring period if not regularly menstruating. Energy status was determined by measurement of REE (kcal·day⁻¹) using indirect calorimetry and a ventilated hood system, as per methods previously published in detail (28,33) and corroborated by serum measurement of TT₃ (ng·dl⁻¹). Predicted REE (pREE; kcal·day⁻¹) was calculated using the Harris-Benedict equation (50). A ratio of the REE/pREE was calculated. Body
composition was measured using dual-energy x-ray absorptiometry (DXA). Dietary energy intake (kcal·day$^{-1}$) was assessed on two occasions during the study using three-day nutritional logs (two weekdays and one weekend day). The reproductive evaluation included a description of menstrual history, confirmation of the presence of an LH peak, and quantification of daily urinary ovarian steroid metabolites, E1G and PdG. The daily urinary samples permitted the characterization of menstrual status (ovulatory, anovulatory, oligomenorrheic, or amenorrheic). The experimental design and study protocol are presented in

**Rationale for Experimental Design/Research Methodology**

**Group Categorization:** A DT score cut-off ($\geq 7$), which corresponds to the 75th percentile for college-aged women (43), represented our operational definition of high DT to best discriminate the exercising women with high DT from those with normal DT. This cut-off was consistent with a previous publication from our lab (28). Other corroborative data included data from Ramacciotti et al. (100) who defined a high DT score as 7 or greater. Torstveit and Sundgot-Borgen (119) suggested the use of a DT score of 15 or greater for classifying athletes “at-risk” for the Female Athlete Triad. However, a DT score that high is likely associated with clinical psychopathology, and our focus was on identifying indications of subclinical disordered eating. Thus, in order to capture subclinical disordered eating, we chose a cut-off on the DT subscale of 7. Additionally, the presence of a fake profile was determined when the DT and BD scores were less than or equal to 3 and the Bulimia score was less than or equal to 1 with a concomitant high score (greater than or equal to 9) on the Perfectionism subscale. The decision to utilize this strategy intended to account for response bias (93). Women with “fake” profiles were then grouped with the high DT women, and their scores were adjusted to reflect the mean DT score of the high DT group (28). A normal DT score was defined as a score less than 7. About 75% of college-aged women have scores less than 7 (43).
Operational Definition of Energy Deficiency: An REE/pREE cut-off (<0.90) represented our operational definition of energy deficiency to best discriminate the exercising women who may present with an energy deficiency from those who are energy replete. We have previously published data using operationally defined energy deficiency as a ratio of REE/pREE less than 0.90 (28,29,33). This definition was based on reports of REE in women with anorexia nervosa (66,83,96), wherein the Harris-Benedict equation (50) was utilized to predict REE. Using a clinical model of starvation, such as anorexia nervosa, during periods of low body weight and prior to refeeding (66,83,96), a reduced ratio of measured REE to that predicted by the Harris-Benedict equation (50) of 0.60-0.80 is often reported.

Menstrual Status Categorization: Eumenorrheic women collected daily urine samples for at least one menstrual cycle, oligomenorrheic women for no more than 90 days, and amenorrheic women for at least one 28-day monitoring period. Menstrual cycle length was defined as the number of days from the first day of menses up to and including the day of the LH surge (31,32). Daily first morning void urine samples were assayed for LH, E1G, and PdG to assess ovulatory status. Ovulatory status was determined by day of the urinary LH surge, identified as an LH peak on the day of or day after the mid-cycle E1G peak (31,32). Specific hormonal criteria for detecting ovulation included a LH surge concentration above 25 mIU·mL⁻¹, the E1G peak concentration above 35 ng·mL⁻¹, and the peak PdG concentration above 5 µg·mL⁻¹ (32,33,62,107).

Menstrual status was deemed abnormal or severe/clinical if a participant was amenorrheic (reported no menses for the past 3 months or longer), oligomenorrheic (reported irregular menses at intervals of 36-90 days) or normal/regular if a participant was eumenorrheic (reported regular menses at intervals of 26-35 days). Self-reported menstrual status was then confirmed prospectively by classifying menstrual cycles by length of the inter-menstrual interval, length of follicular and luteal phases, the presence of menses, and by ovulatory status (ovulatory or anovulatory) as described in previous publications by our lab (31,32).
To determine estrogen and progesterone exposure, E1G and PdG urinary metabolites were compared among groups of menstrual cycles using the trapezoidal integrated area under the curve (AUC) and mean levels of E1G and PdG during the follicular and luteal phases and across the entire cycle. Values from each repeated cycle or monitoring period were averaged. Composite graphs of menstrual cycles depicted by daily E1G and PdG concentrations were determined by taking the mean values from each repeated cycle or monitoring period. For graphing purposes, the E1G and PdG data for eumenorrheic, ovulatory women were aligned by the day of the LH peak defined as Day 0. The amenorrheic, oligomenorrheic, and anovulatory participants’ E1G and PdG data were aligned by chronological day of urinary collections. Both the high DT and normal DT group included oligomenorrheic, amenorrheic, anovulatory and eumenorrheic, ovulatory women and cycles were aligned as per our previous publication (123).

Dataset

Study One included data from a cross-sectional study ("The Active Women’s Study") designed to assess cardiovascular status in exercising women and data from the baseline period of a prospective study ("The REFUEL Study") designed to assess the effects of a 12-month intervention of increased energy intake on indices of bone health and menstrual status in women with EAMD, including FHA (absence of menses for >90 days) and oligomenorrhea (long, inconsistent menstrual cycles of 36-90 days) vs. exercising women with ovulatory cycles.

Description of Participants

Eligibility criteria for this study was: (1) aged 18-35yr; (2) good health as determined by a medical exam; (3) body mass index (BMI) 16-25 kg·m⁻², (4) no chronic illness, including hyperprolactinemia and thyroid disease; (5) stable menstrual status over preceding three months; (5) currently participating in two or more hours·week⁻¹ of purposeful exercise corroborated by a VO₂
max\geq40 \text{ ml·kg}^{-1}·\text{min}^{-1}; \text{ (6) non-smoker; (7) not currently dieting and weight stable for the preceding six months; (8) not taking any hormonal therapy for at least six months; (9) no history or current clinical diagnosis of eating or psychiatric disorders; (10) not pregnant, lactating or planning a pregnancy; (11) no medication use that would alter metabolic or reproductive hormone concentrations; and (12) no other contraindications that would preclude participation in the study.}

**Study Groups Description for Specific Aims 1 and 2**
1. Premenopausal exercising women with high DT (High DT group)
2. Premenopausal exercising women with normal DT (Normal DT group)

**Statistical Analyses**

All statistical analyses were conducted using SPSS version 19.0 (SPSS, Inc., Chicago, IL). All hypothesis tests were two-sided and P<0.05 was considered significant. Data screening was conducted prior to analysis, involving outlier detection and evaluation of assumptions of normality. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percentages for categorical data. Psychometric survey data was recoded and scored to determine subscale or total scores.

To test hypothesis 1: An Independent Student’s $t$-test was completed to compare energetic characteristics between exercising women with high DT and exercising women with normal DT. Primary outcome variables included: REE (kcal·d$^{-1}$), REE controlled for LBM (kcal·kg LBM$^{-1}$), ratio of REE/pREE, and fasting TT$_3$ (ng·dL$^{-1}$). Pearson correlation coefficient analyses were completed to determine associations between DT score and REE parameters. A chi-square test was performed as a cross-tabulation between DT score and energy status (REE/pREE$<0.90$ or REE/pREE$\geq0.90$) to determine the association between a high DT score and our operational definition of an energy deficiency (REE/pREE$<0.90$).
To test hypothesis 2: An Independent Student’s \( t \)-test was completed to compare daily ovarian steroid excretion data between exercising women with high DT and exercising women with normal DT. Primary outcome variables included: E1G Cycle AUC (ng-day-ml\(^{-1}\)), E1G days 2-5 AUC (ng-day-ml\(^{-1}\)), E1G days 2-12 AUC (ng-day-ml\(^{-1}\)), and PdG Cycle AUC (ug-day-ml\(^{-1}\)). Additionally, a chi-square test was performed as a cross-tabulation between DT score and menstrual status (ovulatory, anovulatory, oligomenorrheic, or amenorrheic) to compare the distribution of menstrual disturbances (anovulatory, oligomenorrheic, or amenorrheic) in exercising women when categorized as high vs. normal DT.

**Sample Size Calculations and Justification**

All sample size calculations were performed using G Power 3.1.2 (Universitat Kiel, Germany, 2009). We based our sample size calculations on preliminary data (means and SDs) obtained from the literature. Sample size calculations were performed to determine the number of participants per group required: (i) to detect significant differences (using an Independent Student’s \( t \)-test) in energy status (REE/pREE) and (ii) to compare proportions (using a chi-square test) in menstrual status (eumenorrheic, oligomenorrheic, or amenorrheic) between exercising women with high DT compared to exercising women with normal DT. We chose a previous publication by our lab (28) in sedentary women with normal DT (n=9) and exercising women with (High DT, n=9) or without (Normal DT, n=34) high DT as a basis for these sample size calculations. This publication represents the only evidence in the literature wherein energetic and menstrual variables, similar to the proposed primary outcome variables, are examined in a group of exercising women categorized using a DT score cut-off of 7.

**Sample Size Calculation for Hypothesis 1:** The primary outcome variable used to calculate sample size for energy status was REE/pREE. In our previous publication (28), we showed an REE/pREE of 0.86±0.06 and 0.94±0.02 in exercising women with high DT and those with normal
DT, respectively. Effect size, Cohen’s d, was calculated to have a large effect (1.79) since there was a small percent difference in REE/pREE between groups, 9.0%. Using an effect size d of 1.79, an alpha error probability of 0.05, and a power of 0.80, the required sample size computed to determine differences in REE/pREE between groups was 7 participants in each group for a sample size of 14.

**Sample Size Calculation for Hypothesis 2:** To date, this is the first study to compare the ovarian steroid profiles of daily E1G (ng/ml) and PdG (µg/ml) metabolites and the distribution of menstrual status (eumenorrheic, oligomenorrheic, or amenorrheic) between exercising women with high DT compared to those women with normal DT. Previously in a smaller cohort, De Souza et al. (28) demonstrated that there was a greater proportion of severe/clinical menstrual disturbances (amenorrhea or oligomenorrhea) in exercising women with high DT (n=9) compared to exercising (n=34) and sedentary women with normal DT (n=9) ($\chi^2 = 16.1, p<0.001$).

**Sample Size Justification:** Thus, our sample size calculations indicate that 43 women provided sufficient power (1-$\beta = 0.80$) to detect significant relationships at a large effect size (0.50).

**Expected Findings**

We expected that high DT would be associated with surrogate markers of an energy deficiency (suppressed REE (controlled for LBM) and REE/pREE<0.90) in exercising women, and thus confirm the findings from our previous publication in a larger cohort of exercising women (28). We also anticipated observing a greater frequency of severe/clinical menstrual disturbances (amenorrhea and oligomenorrhea) in the high DT group compared to the normal DT group, as corroborated by ovarian steroid profiles of daily urinary E1G and PdG metabolites. As such, we predict the high DT group would demonstrate suppressed E1G and PdG concentrations vs. the normal DT group, wherein an ovarian steroid profile indicative of ovulation and luteinization would likely be exhibited.

Similar to our previous publication (28), we predicted that the ratio of REE/pREE would be significantly associated with TT$_3$, such that the lower the ratio, the lower the circulating TT$_3$.
concentration. This finding would confirm an association between lower TT₃ and adaptive changes in energy expenditure in exercising women with an REE/pREE<0.90. Since lower circulating TT₃ concentrations would contribute to energy conservation as demonstrated in undernourished or energy-deficient individuals (95), findings from this study were expected to be consistent with previous evidence of an association between TT₃ concentrations and REE.
Study Two: The effect of high dietary restraint on energy availability and menstrual status in exercising women

Background

Disordered eating behavior is notably prevalent in exercising women (28,45) and is often associated with restrictive eating and/or aberrant exercise behavior. As such, inadequate energy intake (EI) relative to exercise energy expenditure (EEE) often contributes to the development of lower EA, a key factor underlying clinical outcomes which are unfavorable to reproductive health in exercising women (74). Furthermore, there is evidence for estrogen dependent (33) and independent mechanisms for bone loss (57) and per se, lower EA may promote potentially irreversible bone loss in women with or without exercise-associated amenorrhea. These detrimental health conditions are commonly associated with the Triad (90), a syndrome first recognized in female athletes, wherein one or more of the following conditions may be present: low EA (with or without disordered eating), menstrual disturbances, and/or low bone mineral density (BMD).

Reductions in EA promote energy conservation mechanisms, reserving fuel for only the most critical physiological processes, i.e., thermoregulation and cellular maintenance (124). Reproductive function is not considered critical for survival and may manifest as subclinical (LPD or anovulation) or clinical menstrual disturbances (FHA or oligomenorrhea) (30). Links between low EA and disruptions in LH pulsatility (75) and menstrual cyclicity (127) have been exhibited in human and animal experiments. However, the association between disordered eating behavior (i.e., a high DR) and EA has not been clearly elucidated in exercising women and lower EA may represent a factor associated with the induction of EAMD in women with high DR.

DR is the conscious restriction of EI in an effort to achieve or maintain a certain body weight (112). Several investigators have linked high DR with menstrual disturbances, such as amenorrhea, oligomenorrhea, and anovulation (6,123) in exercising and sedentary women. Despite differences in
methods for determining menstrual status and wide ranges in prevalence of menstrual disturbances in women with high DR (34-78%) (6,81,110,128), the association between higher DR scores and menstrual dysfunction is well-documented. Lower EI associated with high DR is also often observed in women (64,85,103), but few investigators have explored the link between DR and EA in exercising women (103). A high DR is suggested to promote lower EA by way of persistent monitoring of EI and subsequent chronic energy. As such, high DR is hypothesized to contribute to the development of lower EA through the reduction of EI and/or increase in EEE. Therefore, exercising women with high DR may be at a higher risk for the development of menstrual disturbances and potentially other Triad-related clinical outcomes.

**Brief Rationale**

To date, the association between DR and EA has yet to be examined in exercising women categorized by DR score. Since EA is a key component of the mechanism underlying alterations in LH pulsatility and menstrual cyclicity concomitant with exercise training, an objective of this study was to clarify whether a link between DR and EA exists, such that women with high DR demonstrate lower EA and as a result, a greater frequency of EAMD, both subclinical (LPD or anovulation) and clinical perturbations (amenorrhea or oligomenorrhea). In brief, high DR may represent the underpinning of an eating behavioral phenotype associated with lower EA and menstrual disturbances in exercising women.

In a previous study from our lab (123), we demonstrated that the frequency of clinical menstrual disturbances (amenorrhea and oligomenorrhea) was greater in exercising women with high DR compared to women with normal DR. Among the women in the high DR group, 50% of participants presented with clinical menstrual disturbances (amenorrhea or oligomenorrhea) vs. 26% of participants in the normal DR group. The women with high DR also demonstrated significantly lower PdG AUC values compared to the normal DR group. Additionally, Vescovi et al. (123)
exhibited a progressively greater frequency of clinical menstrual disturbances (amenorrhea and oligomenorrhea) and concomitant reductions in E1G and PdG concentrations with increasing DR scores (Figure 2). One of the objectives of the current study was to confirm previous findings by Vescovi et al. (123) wherein an association was demonstrated between high DR and menstrual dysfunction in exercising women. Notably, menstrual status was objectively characterized by urinary measures of LH, E1G, and PdG metabolites, which represents an optimal strategy to determine menstrual status compared to self-report and/or retrospective measures of menstrual history wherein there is potential inaccuracy (32).

Considering Vescovi et al. (123) did not determine EA in their study, one of our specific aims was to determine the associations between DR score and EA to better understand the etiology of EAMD in women with high DR. Previous researchers have suggested that individuals with high DR exert cognitive control over EI (53) and are less likely to respond to the physiological cues of hunger initiated by an exercise-induced energy deficit. Accordingly, those women with high DR may be less likely to compensate for energy expended during exercise, and therefore, may demonstrate reductions in EA. In a study by Lluch et al. (71) in women with high DR, an exercise-induced energy deficit did not have a significant effect on EI consumed at a subsequent meal or throughout the remainder of the day. To this end, women with high DR may avoid compensating for the energy expended via exercise. Thus, high DR may be a successful strategy for inducing lower EA in combination with exercise and as a result, these exercising women with high DR may be more susceptible to developing subclinical or clinical menstrual disturbances. Therefore, the DR subscale may represent a psychometric indicator of lower EA and menstrual disturbances in exercising women. **Overall, our objective was to determine whether the DR subscale provides valuable information on EA and menstrual status in exercising women. Furthermore, it is important to determine the association between DR score and EA to better understand the etiology of menstrual disturbances in exercising women with high DR.**
Figure 2. Composite graphs of daily estrone-1-glucuronide (E1G, top panel) and pregnanediol glucuronide (PdG, bottom panel) concentrations across the dietary restraint (DR) score quartiles from a cross-sectional study by Vescovi et al. (123). Values are mean±SEM. Reproduced with permission from Vescovi et al., Physiol Behav. 2008; 95(1-2):48-55

Specific Aims and Hypotheses

Aim 1: To compare EA and menstrual status characteristics in exercising women categorized by DR score

Primary Hypothesis

Hypothesis 1i: Exercising women with high DR will demonstrate lower EA (lower EI and higher EEE) compared to women with normal DR.

Hypothesis 1ii: Exercising women with high DR will exhibit a greater frequency of menstrual disturbances (subclinical or clinical menstrual disturbances) compared to women with normal DR.
Secondary Hypothesis

Hypothesis 1iii: DR score will be negatively associated with EA (kcal·kg⁻¹ LBM) and EI (kcal·d⁻¹); whereas DR score will be positively associated with EEE (kcal·d⁻¹) in exercising women.

Aim 2: To compare DR score and EA across the continuum of exercise-associated menstrual disturbances (ovulatory vs. subclinical menstrual disturbances vs. clinical menstrual disturbances) classified using measures of LH and daily urinary E1G and PdG metabolites

Hypothesis 2: DR score will increase whereas EA (kcal·kg⁻¹ LBM) will decrease as menstrual cycle disturbances progress in severity from subclinical menstrual disturbances (LPD or anovulation) to clinical menstrual disturbances (amenorrhea or oligomenorrhea).

Experimental Design/Overview of Research Methodology

This is a cross-sectional study comparing exercising women aged 18-35 years with high DR (n=30) and normal DR (n=56) with respect to psychological, anthropometric, EA, and menstrual characteristics. Participants that met inclusion criteria underwent screening to determine study eligibility. An exercise history review was completed and was corroborated with a VO₂peak test to determine exercise status. In order to be considered an “exercising” woman, participants needed to partake in at least 2 hours of purposeful exercise each week (2). Participants were retrospectively categorized according to their DR scores obtained from the Three Factor Eating Questionnaire (TFEQ). Subjects with a score of ≥13 were classified as having a high DR. Participants who were eumenorrheic (normally menstruating) were monitored for the length of at least one complete menstrual cycle; while women who were amenorrheic or oligomenorrheic (experiencing menses at irregular intervals) were monitored for a least a 28-day monitoring period. EA (kcal·kg⁻¹ LBM) was defined as EI minus EEE relative to kg LBM [EA = (EI – EEE)/LBM (kg)] (72). EI (kcal·d⁻¹) was determined using three-day diet logs and EEE (kcal·d⁻¹) was obtained using seven-day exercise logs,
Polar Heart Rate monitors and/or Ainsworth compendium (3). Body composition (percent body fat, fat mass (kg), LBM (kg)) was assessed using DXA. Classification by menstrual status involved evaluation of self-reported menstrual history, confirmation of the presence of a LH peak, and quantification of daily urinary ovarian steroid metabolites, E1G and PdG (32).

Rationale for Experimental Design/Research Methodology

**Group Categorization:** DR score was obtained from the TFEQ (112), which was completed once during the study. A high DR score was defined as ≥13 based on the Eating Inventory (112) and also represents the 75th percentile in populations of premenopausal (122,123) and postmenopausal women (10,51). The participants were categorized into two groups based on their DR scores: (1) women with high DR and (2) women with normal DR.

**Menstrual Status Categorization:** Eumenorrheic women collected daily urine samples for at least one menstrual cycle, oligomenorrheic women for no more than 90 days, and amenorrheic women for at least one 28-day monitoring period. Menstrual cycle length was defined as the number of days from the first day of menses up to and including the day of the LH surge (31,32). Daily first morning void urine samples were assayed for LH, E1G, and PdG to assess ovulatory status. Ovulatory status was determined by day of the urinary LH surge, identified as an LH peak on the day of or day after the mid-cycle E1G peak (31,32). Specific hormonal criteria for detecting ovulation included a LH surge concentration above 25 mIU·mL⁻¹, the E1G peak concentration above 35 ng·mL⁻¹, and the peak PdG concentration above 5 µg·mL⁻¹ during the luteal phase (32,33,62,107).

Menstrual status was deemed abnormal or clinically severe if a participant was amenorrheic (reported no menses for the past 3 months), oligomenorrheic (reported irregular menses at intervals of 36-90 days) or normal if a participant was eumenorrheic (reported regular menses at intervals of 26-35 days). Self-reported menstrual status was then confirmed prospectively by classifying menstrual cycles by length of the inter-menstrual interval, length of follicular and luteal phases, the
presence of menses, and by ovulatory status (ovulatory or anovulatory) as described in previous publications in our lab (31,32). These determinations were made from the measurement of daily urinary E1G and PdG concentrations by taking the mean values from each repeated cycle or monitoring period similar to a previous publication (32). For graphing purposes, the E1G and PdG data for eumenorrheic, ovulatory women were aligned by the day of the LH peak defined as Day 0. The amenorrheic, oligomenorrheic, and anovulatory participants’ E1G and PdG data were aligned by chronological day of urinary collections. Both DR groups included amenorrheic, oligomenorrheic, anovulatory, LPD, and ovulatory women and cycles were aligned as per our previous publication (123).

Dataset

Study Two included data from a cross-sectional study (“The Active Women’s Study”) designed to assess cardiovascular status in exercising women and data from the baseline period of a prospective study (“The REFUEL Study”) designed to assess the effects of a 12-month intervention of increased energy intake on indices of bone health and menstrual status in women with EAMD, including amenorrhea (absence of menses for >90 days) and oligomenorrhea (long, inconsistent menstrual cycles of 36-90 days), vs. exercising women with ovulatory cycles.

Study Groups Description for Specific Aims 1 and 2

1. Premenopausal exercising women with high DR (High DR group)
2. Premenopausal exercising women with normal DR (Normal DR group)

Description of Participants

Eligibility criteria for this study was: (1) aged 18-35yr; (2) good health as determined by a medical exam; (3) BMI 16-25 kg·m⁻², (4) no chronic illness, including hyperprolactinemia and
thyroid disease; (5) stable menstrual status over preceding three months; (5) currently participating in two or more hours·week$^{-1}$ of purposeful exercise corroborated by a VO$_2$ max$\geq$40 ml·kg$^{-1}$·min$^{-1}$; (6) non-smoker; (7) not currently dieting and weight stable for the preceding six months; (8) not taking any hormonal therapy for at least six months; (9) no history or current clinical diagnosis of eating or psychiatric disorders; (10) not pregnant or lactating or planning a pregnancy; (11) no medication use that would alter metabolic or reproductive hormone concentrations; and (12) no other contraindications that would preclude participation in the study.

Statistical Analyses

All statistical analyses were conducted using SPSS version 19.0 (SPSS, Inc., Chicago, IL). All hypothesis tests were two-sided and P<0.05 was considered significant. Data screening was conducted prior to analysis, involving outlier detection and evaluation of assumptions of normality. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percentages for categorical data. Psychometric survey data was recoded and scored to determine subscale or total scores.

To test hypotheses i, iii, and iv: Independent Student’s $t$-tests were performed to compare EA and menstrual characteristics between groups. The independent variable was DR group (high vs. normal). Primary outcome variables included: (1) EA (kcal·kg$^{-1}$ LBM) and EA parameters (EI (kcal·d$^{-1}$), EEE (kcal·d$^{-1}$), and LBM (kg)) and (2) ovarian steroid excretion characteristics (E1G AUC (ng·day·mL$^{-1}$) and mean (ng·mL$^{-1}$), PdG AUC (ug·day·mL$^{-1}$) and mean (ug·mL$^{-1}$)). Pearson correlation analyses were performed between independent and primary outcome variables to examine the associations between DR score, EA parameters (EA, EI, and EEE), and ovarian steroid excretion characteristics (E1G AUC and mean, PdG AUC and mean).
To test hypothesis 1ii: Chi-squares tests were performed to determine the association between DR group and menstrual status (ovulatory, LPD, anovulation, oligomenorrheic, or amenorrheic) and ovulatory status (ovulatory vs. anovulatory).

To test hypothesis 2: One-way ANOVA with post-hoc comparisons (LSD) were performed to compare DR score and EA (kcal·kg⁻¹ LBM) between menstrual status groupings (ovulatory vs. subclinical menstrual disturbances vs. clinical menstrual disturbances).

**Sample Size Calculations and Justification**

All sample size calculations were performed using G Power 3.1.2 (Universitat Kiel, Germany, 2009). We based our sample size calculations on preliminary data (means and SDs) obtained from the literature. Sample size calculations were performed to determine the number of participants per group required: (i) to compare EA in exercising women when categorized by DR score and (ii) to compare the distribution of menstrual disturbances in exercising women when categorized by DR score. As such, power calculations were completed for key outcome variables (EA, distribution of clinical menstrual disturbances).

**Sample size calculation to test hypothesis 1:** To date, this is the first study to compare EA in exercising women categorized by DR score. The primary outcome variable used to calculate sample size was EA. For a sample size calculation of EA, Reed et al. (103) observed significant differences in EA (28.8±11.5 kcal·kg⁻¹ FFM vs. 42.1±9.2 kcal·kg⁻¹ FFM) between amenorrheic exercising women with high DR (mean DR score=12.0±4.5) and ovulatory exercising women with normal DR (mean DR score: 6.8±4.5), respectively. Effect size, Cohen’s d, was calculated to have a large effect (1.33), since there was a 46.0% difference in EA between groups. Using an effect size d of 1.33, an alpha error probability of 0.05, and a power of 0.80, the required sample size to determine EA was 10 participants in each group for a sample size of 20.
Sample size calculation to test hypothesis 2: Using reported data from Vescovi et al. (123), the following percentages of women who either presented with a clinical menstrual disturbance (amenorrhea or oligomenorrhea) or did not present with a clinical menstrual disturbance in a sample of exercising women categorized by DR score were: 23% (19/84) with high DR and a clinical menstrual disturbance, 23% with high DR but without a clinical menstrual disturbance (19/84), 14% (12/84) with normal DR and with a clinical menstrual disturbance, and 40% (34/84) with normal DR but without a clinical menstrual disturbance. Using the above percentages (23%, 23%, 14%, 40%) for the alternative hypothesis (pH1) and 0.25 for each of the accompanying cells for the null hypothesis (pH0) for the total sum of the expected probabilities to equal 1.0, an effect size \( w \) of 0.38 was computed using the G*Power statistical program. An effect size of 0.38, an alpha error probability of 0.05, a power of 0.80, and 1 degree of freedom, as a 2 x 2 chi square analysis will be performed, requires a total sample size of 55 participants to test hypothesis 2 using a goodness-of-fit test for contingency tables.

Sample Size Justification: Thus, our sample size calculations indicated that 55 women provided sufficient power (\( 1 - \beta = 0.80 \)) to detect significant relationships at a moderate to large effect size (0.38-0.50).

Expected Findings

We expected that high DR would be associated with lower EA, specifically lower EI relative to EEE, in exercising women. We also anticipated observing a greater frequency of EAMD (both subclinical, i.e., LPD or anovulation, and clinical perturbations, i.e., amenorrhea or oligomenorrhea) in women with high DR compared to those women with normal DR, as corroborated by daily urinary E1G and PdG metabolites. Furthermore, we expected that the ovarian steroid profile observed in the high DR group would be indicative of suppressed E1G and PdG concentrations. Alternatively, we predicted a greater frequency of ovulatory cycles in the normal DR group wherein the ovarian steroid
profile would be indicative of ovulation and luteinization. Overall, we expected to demonstrate: (1) lower EA in exercising women with high DR vs. women with normal DR, (2) associations between DR score and EA status, and (3) a greater frequency of menstrual disturbances in exercising women with high DR vs. women with normal DR.
STUDY THREE: REFUEL: The effect of increased energy intake on psychometric indicators of eating behavior in women with exercise-associated menstrual disturbances

Background

Disordered eating and abnormal dieting behavior have well-known negative implications for clinical sequelae in exercising women, including disturbances of energetic and menstrual function, bone loss, and fractures (22,45,119). Prior research findings in exercising women exhibit strong interrelationships among disordered eating behavior, chronic energy deficiency, and FHA (22,45,119), all hallmark conditions associated with the Triad. The Triad is comprised of three components existing along a continuum of severity, alone or in combination, to include low EA (with or without disordered eating), menstrual dysfunction, and low BMD (90). The etiology of Triad-related clinical outcomes in exercising women frequently begins with conscious energy restriction and/or increases in energy expenditure motivated by sport-specific or sociocultural pressure to achieve often unrealistic standards of body size and physical attractiveness (116). Consequently, significant reductions in energy intake relative to energy expenditure may contribute to the development of a chronic energy deficiency (28,45,108,123). Notably, a chronic energy deficiency is the primary factor associated with menstrual disturbances and bone loss in premenopausal exercising women.

Exercising women with disordered eating consistently demonstrate significantly elevated scores on the DT and BD subscales on the EDI-2 (13,42,44), and a high DR score on the TFEQ (81,123). The EDI-2 (43) and TFEQ (112) are multidimensional psychometric tools used for discriminating disordered vs. healthy behavior and attitudes toward food and body image in college-aged women. High DT has been proposed as one of several factors contributing to the development of the Triad and its clinical sequelae (13,22,28,45,93,119,120). This paradigm is based on evidence of disruption of energy homeostasis translating to metabolic and reproductive dysfunction in women.
with high DT (28,45). High DR is characterized by a conscious restriction of energy intake in order to achieve or maintain a desired body weight (112). Exercising women with high DR habitually monitor their energy intake and practice certain methods of pathological weight control (8,91,123). Several investigators have linked high DR to menstrual disturbances, such as FHA, oligomenorrhea, and anovulation (6,7,81,123), in premenopausal exercising and sedentary women. BD is also a symptom associated with disordered eating (22,42,119,120) such that higher BD may represent a psychological characteristic that is linked to weight loss or cycling, energy restriction, and restrained eating, all factors that may induce chronic energy deficiency and associated menstrual disturbances in exercising women.

**Brief Rationale**

The first aim of treatment in amenorrheic exercising women to restore menstrual function is to modify diet and exercise behavior to increase EA by increasing energy intake, reducing energy expenditure, or a combination (38,67,127). Evidence of the efficacy of this non-pharmacologic treatment strategy is documented in reports in women with anorexia nervosa (19,66), retrospective analysis of female athletes with menstrual disturbances (4), case studies of amenorrheic exercising women (38,67), and experiments in female cynomolgus monkeys (126,127). Findings from these studies suggest that the reversal of EAMD may be explained by adequate increases in energy intake that result in the amelioration of an energy deficiency. Kopp-Woodroffe et al. (67) demonstrated an association between 20 weeks of increased energy intake (combined with decreased exercise energy expenditure) and resumption of menses was exhibited in amenorrheic athletes. Dueck et al. (38) exhibited improvements in energy balance in a college-aged amenorrheic runner with concomitant increases in LH pulsatility following 15 weeks of increased energy intake and reduced exercise volume. Interestingly, the role of psychological status and eating behavior in the treatment of EAMD has yet to be addressed. DT, DR, and/or BD may serve as psychological and eating behavioral
characteristics associated with metabolic alterations indicative of the recovery from energy
deficiency (66) and EAMD (subclinical and clinical perturbations) (90). However, there is no
investigation in women with EAMD wherein changes in psychometric indicators of eating behavior
(such as DR, DT, and BD) during a randomized controlled trial (RCT) of increased energy intake
have been characterized.

Findings in women with eating disorders demonstrate improvements in psychological
indicators of disordered eating behavior (obtained from the EDI-2) following refeeding and
behavioral change intervention (19,66,115). Konrad et al. (66) showed an improvement in DT score
in women with anorexia nervosa following treatment without compromising weight gain (19,66).
Similar findings have been presented in women with bulimia nervosa (115) wherein improvements
in DT, bulimia, and BD scores were exhibited following cognitive behavioral therapy and nutritional
counseling. However, to date, it is unclear whether EAMD women without pathologically severe
eating disorders would exhibit similar changes across an increased energy intake intervention with
psychological/nutritional monitoring or if refeeding would have negative implications for the
psychological eating behavior profile, particularly DT, DR, and BD scores, compared to control
participants with EAMD and ovulatory cycles.

Specific Aim and Hypotheses

Aim: To determine the effect of a 6-month RCT of increased energy intake on psychometric
indicators of eating behavior (DT, DR, and BD) in exercising women with EAMD
(EAMD+Cal) compared to exercising controls with EAMD (EAMD Control) and OV (OV
Control).

Hypothesis 1i: EAMD+Cal women will demonstrate a greater decrease in DR score compared to
EAMD and OV controls
Hypothesis 1ii: EAMD+Cal women will demonstrate no change in DT and BD score compared to EAMD and OV controls

**Experimental Design/Overview of Research Methodology**

We used a prospective repeated measures design to determine the effect of a 6-month RCT of increased energy intake on psychometric indicators of eating behavior (DT, DR, and BD) in the EAMD+Cal group vs. EAMD and OV control groups. This study included data from an RCT that was designed to assess the effects of 12 months of increased energy intake (20-30% above baseline energy requirements) on indices of bone health and menstrual status in women with EAMD, including FHA (absence of menses for ≥90 days) and oligomenorrhea (long, inconsistent menstrual cycles of 36-90 days), vs. exercising control participants with EAMD and OV. The study was conducted at two sites, the University of Toronto (UT) and the Pennsylvania State University (PSU) over 6 years. Participants (OV and EAMD women) were recruited on a rolling basis over these 6 years and observed for 12 months. The experimental design of this study is presented in Figure 3. All participants were “exercising” and participated in two or more hours·week⁻¹ of purposeful exercise. All participated who completed 6 months of the RCT were eligible for our analysis.

The screening period was conducted over 2-3 visits. During the screening period, participants were informed of the purpose, procedures, and potential benefits/risks of study participation prior to signing an Informed Consent approved by the Biomedical Institutional Review Board at the PSU or the Human Ethics Boards at the UT. Once consent was obtained, height (cm) and weight (kg) were measured, and participants completed questionnaires to assess demographic, medical history, exercise history, eating attitudes and behaviors (43,112), bone health, psychological health, and stress/mood characteristics (18,23,34,117). The EDI-2 and TFEQ, were administered to participants. A physical exam was performed by an on-site clinician to determine health status and to rule out any physical signs or symptoms of polycystic ovarian syndrome (PCOS) (i.e., acne,
hirsutism) or disordered eating. A fasting blood sample was obtained and analyzed for complete
blood count, CHEM-24 and an endocrine panel, which included follicular stimulating hormone,
luteinizing hormone (LH), estradiol, prolactin, thyroid stimulating hormone, thyroxine, total and free
testosterone, and dihydroepiandrosterone sulfate. Results of the blood test were used to rule out
endocrine and metabolic disease. A research psychologist with trained expertise in clinical eating
disorders completed a semi-structured interview with each participant to rule out current or history of

Figure 3. Experimental Design of Study Three.
clinical eating disorders and other psychiatric disorders. Participants met with a registered dietician upon completion of a three-day diet log (two week days and one weekend day) to determine the participants’ eating patterns, relationships with food, and food preferences (allergies, specific likes and dislikes). Calcium intake was assessed by the Calcium Inventory (86). Those participants with dietary habits that did not comply with study protocol were excluded. All participants were prescribed calcium (1000-1300 mg·d⁻¹) (Caltrate 600 + D, Wyeth Consumer Health care Products) and vitamin D3 (400 IU) (Wyeth Consumer Health Care Products) in an amount equal to, in combination with their normal diet, necessary to meet adequate intake (AI) levels, as listed above. The dietician evaluated whether the participant was a good candidate for a study in which they may have to increase energy intake. The dietician also provided the participant with specific instruction on how to record a three-day diet log and use available measurement tools to accurately measure their food and beverage intake during the study. A DXA scan was performed by a registered technician to assess body composition (percent body fat, fat mass (kg), LBM (kg), FFM (kg)). After finishing screening, the participant completed baseline assessments.

During baseline, participants collected daily urine samples for 28-day monitoring period if in the EAMD group or for an entire menstrual cycle in the OV group. Participants recorded signs and symptoms of menses on menstrual calendars. At baseline, participants arrived at the Women’s Health and Exercise Laboratory between 600-830hr (fasted and having refrained from exercise and caffeine for prior 24hr and alcohol for prior 12hr) and completed the following: (1) body weight and body composition measurement (via DXA), (2) REE testing via indirect calorimetry and a ventilated hood system (28,33,45), and (3) blood sample measurement for the determination of TT₃ (28,45). Participants also completed a peak oxygen uptake (VO₂peak) test on a separate occasion to evaluate physical fitness as per previous publications from our lab (28,45).

Upon completion of baseline procedures, participants with EAMD were randomly assigned to either a treatment group or a control group. Research personnel conducted randomization as per a
block randomization (block size of 8), balance was forced among treatment assignments after every 8th participant was enrolled. The EAMD participants randomly assigned to the treatment group (EAMD+Cal) were provided an energy prescription (increased energy intake 20-30% above baseline energy requirements) and asked to maintain their usual exercise training regimen for the intervention phase of the study. Baseline energy requirements for this study were operationally defined as the sum of REE and purposeful exercise energy expenditure. Participants in the EAMD Control group were asked to maintain their baseline physical activity levels and energy intake. Participants in the EAMD+Cal group were requested to increase their energy intake through the use of nutritional/sports energy supplements or with foods they typically eat. Energy bars (primarily PowerBars, Clif Bars) that contained approximately 250-300 calories were provided by the research staff and advanced as a strategy to increase energy intake throughout the day.

The EAMD+Cal participants met with a registered dietician bi-weekly throughout the entire increased energy intake intervention to monitor compliance to energy prescription (i.e., review participants’ diet logs and provide strategies to achieve target energy intake) and changes in nutritional and eating behavior characteristics. The participants in the EAMD and OV Control groups met with the registered dietician at monthly intervals throughout the study to monitor compliance to control group requirements and ensure maintenance of usual nutritional and eating behavior characteristics. The EAMD+Cal participants met with a clinical psychologist bi-weekly throughout the entire increased energy intervention to monitor general psychological health status, behavior changes in psychological and eating behavior characteristics, and provide assistance in implementing the energy prescription and other lifestyle changes to ensure compliance to the intervention. The participants in the EAMD Control group met with the registered dietician and clinical psychologist at monthly intervals throughout the study. The OV Control group began a calcium and vitamin D run-in period on Day 1 of their next menses. The EAMD participants began the calcium and vitamin D run-in period on day 1 of week 1 of baseline. All groups received oral
calcium and vitamin D supplements to ensure that they consumed the adequate intake of 1000-1300 mg/per day of calcium and 400 IU of vitamin D (usual dietary intake was considered in achieving this goal, and supplemented when necessary). Calcium and vitamin D were used as control measures similar to other studies of bone health (5,17,41,79).

Psychometric assessment of eating behavior (EDI-2, TFEQ), body weight, body composition (via DXA), energy status (REE test), metabolic hormones indicative of energy status (via fasting blood sample measurement, i.e., TT₃), and energy intake (three-day diet logs) were repeated at weeks 5, 9, and 21. OV Control women collected daily urine samples for an entire menstrual cycle on four occasions throughout the intervention, whereas EAMD+Cal and Control groups collected daily urine samples for the entire intervention. Menstrual bleeding/symptoms and exercise training was monitored throughout the study using menstrual calendars and seven-day purposeful exercise logs. The study protocol is presented in Table 1.

Rationale for Experimental Design/Research Methodology

Classification of Baseline Menstrual Status: Initial classification of menstrual status prior to intervention was based on self-reported menstrual history, results of physical exam, urinary E1G, PdG, and LH profiles, and other endocrine measures described above. Participants recorded menses and/or other menstrual symptoms (i.e., cramps, spotting, discharge, etc.) daily on menstrual calendars. Eumenorrheic women collected daily urine samples for one menstrual cycle, oligomenorrheic women for no more than 90 days, and women with FHA for one 28-day monitoring period. Menstrual cycle length was defined as the number of days from the first day of menses up to and including the day of the LH surge as described below (31,32). Daily first morning void urine samples were assayed for LH, E1G, and PdG to assess ovulatory status.
Table 1. Refuel Study Protocol.

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Ovulatory status was determined by the day of the urinary LH surge, identified as an LH peak on the day of or after the mid-cycle E1G peak (31,32). Specific hormonal criteria for detecting ovulation included a LH surge concentration above 25 mIU·mL⁻¹, the E1G peak concentration above 35 ng·mL⁻¹ and the peak PdG concentration above 5 µg·mL⁻¹ during the luteal phase (32,33,62,107).

Menstrual status was defined as EAMD if a participant was FHA (reported no menses for the past 3 months) or oligomenorrheic (reported irregular menses at intervals of 36-90 days). Menstrual status was defined as OV if a participant was eumenorrheic (reported regular menses at intervals of 26-35 days). Self-reported menstrual status was then confirmed prospectively by classifying menstrual cycles by length of the intermenstrual interval, length of follicular and luteal phases, the presence of menses, and by ovulatory status (ovulatory or anovulatory) as described in previous publications in our lab (31,32). These determinations were made from the measurement of daily urinary E1G and PdG concentrations by taking the mean values from each repeated cycle or monitoring period (32). For graphing purposes, the E1G and PdG data for eumenorrheic, ovulatory women were aligned by the day of the LH peak defined as Day 0. The FHA, oligomenorrheic, and anovulatory participants’ E1G and PdG data were aligned by chronological day of urinary collections.

**Dataset**

Study Three included longitudinal data (at baseline, IWk9, and IWk21) from a prospective study (REFUEL study) designed to assess the effects of a 12-month intervention of increased energy intake on indices of bone health and menstrual status in women with EAMD, including amenorrhea (absence of menses for >90 days) and oligomenorrhea (long, inconsistent menstrual cycles of 36-90 days).
Study Groups Description for Specific Aims

(1) Premenopausal exercising women with EAMD who increased energy intake (EAMD+Cal)

(2) Premenopausal exercising women with EAMD control group (EAMD Control)

(3) Premenopausal exercising women with OV control group (OV Control)

Description of Participants

Participants were young adult women primarily from the general population of college-aged women (18-35 yr) at UT and PSU. Recruitment was accomplished through the university campus and community newspapers, television, radio, and bulletin fliers. Eligibility criteria for this study is: (1) aged 18-35yr; (2) good health as determined by a medical exam; (3) body mass index (BMI) 16-25 kg·m⁻²; (4) no chronic illness, including hyperprolactinemia and thyroid disease; (5) stable menstrual status over preceding three months; (6) currently participating in two or more hours week⁻¹ of purposeful exercise; (7) non-smoker; (8) not taking any hormonal therapy for at least six months; (9) no current clinical diagnosis of eating or psychiatric disorders; (10) not pregnant or lactating or planning a pregnancy; (11) no medication use that would alter metabolic or reproductive hormone concentrations; and (12) no other contraindications that would preclude participation in the study.

Statistical Analyses

All statistical analyses were conducted using SPSS version 19.0 (SPSS, Inc., Chicago, IL). All hypothesis tests were two-sided and p<0.05 was considered significant. Data screening was conducted prior to analysis, involving outlier detection and evaluation of assumptions of normality. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percentages for categorical data. Psychometric survey data
was recoded and scored to determine subscale or total scores. Baseline measurements were examined using one-way analysis of variance analysis (ANOVA). A one-way ANOVA with repeated measures with one within factor (time: baseline, IWk9, and IWk21) and one between factor (group: EAMD+Cal vs. EAMD Control vs. OV Control) was performed to compare psychometric indicators of eating behavior (DT, DR, and BD scores), body weight, body composition, energetic and metabolic hormone variables over time. One-way ANOVA analyses were performed to confirm group effects. Paired t-Tests were performed to confirm time effects.

**Sample Size Calculations and Justification**

All sample size calculations were performed using G Power 3.1.2 (Universitat Kiel, Germany, 2009). We based our sample size calculations on preliminary data (means and SDs) obtained from the literature (Konrad et al., 2007; Bratland-Sanda et al., 2010; Sundgot-Borgen et al., 2002; Bryant et al., 2012). Sample size calculations were performed to determine the number of participants per group required to detect differences in DT, BD, and DR scores across three time points (Baseline, IWk9, IWk21) using a one-way ANOVA with repeated measures for time and group effects and group x time interaction.

**Sample size calculations to test hypotheses:** To date, no prospective investigation has been completed wherein the researchers examined the effects of increased energy intake on psychological characteristics indicative of eating behavior (DT, DR, BD) following the recovery of menses in women with EAMD (amenorrhea or oligomenorrhea). In a study by Bratland-Sanda et al. (2010), women with clinical eating disorders receiving inpatient treatment (individual group psychotherapy, psychoeducation, art therapy lessons, and low volume physical activity- 2 days per week, 60 min per session) for 12-20 weeks were categorized in excessive exercise and non-excessive exercise groups. Bratland-Sanda et al. (2010) demonstrated DT score decreased from 15.8±4.3 to 12.5±5.9 in the nonexcessive exercisers (n=24). Based on 21% (3.3) decrease in DT
score, standard deviation of 5.1, a large effect size (Cohen’s d) of 0.65 was calculated. Using an effect size of 0.65, an alpha error probability of 0.05, a power of 95%, and 3 groups (EAMD treatment group (EAMD+Cal), EAMD Control group, OV Control group), the required sample size using a one-way ANOVA with repeated measures for an interaction within and between groups was a total of 9 participants.

Using data from Konrad et al. (2007), DT score decreased from 12.4±5.23 to 8.4±4.96 in 10 women with anorexia nervosa following refeeding (7 to 9 week partial hospitalization eating disorder program). Based on 32% (4) decrease in DT score, standard deviation of 5.1, a large effect size (Cohen’s d) of 0.78 was determined. Using an effect size of 0.78, an alpha error probability of 0.05, a power of 95%, and 3 groups (EAMD+Cal, EAMD Control, OV Control), the required sample size using a one-way ANOVA with repeated measured for an interaction between groups was 9 participants per group or a total sample size of 27 participants. Using data from Sundgot-Borgen et al. (2002), BD score decreased from 17 to 8 in 17 women with bulimia nervosa receiving nutritional counselling (16 wk of outpatient sessions). Based on 53% (9) decrease in BD score, standard deviation of 5, a large effect size (Cohen’s d) of 1.8 was determined. Using an effect size of 1.8, an alpha error probability of 0.05, a power of 95%, and 3 groups (EAMD+Cal, EAMD Control, OV Control), the required sample size using a one-way ANOVA with repeated measured for an interaction between groups was a total of 21 participants. Using data from Bryant et al. (2012), DR score increased from 8 to 9.6 in 58 overweight and obese women and men following 12 weeks of supervised exercise. Based on 20% (1.6) increase in DR score, standard deviation of 4, an effect size (Cohen’s d) of 0.40 was determined. Using an effect size of 0.40, an alpha error probability of 0.05, a power of 95%, and 3 groups (EAMD+Cal, EAMD Control, OV Control), the required sample size using a one-way ANOVA with repeated measured for an interaction between groups was a total sample size of 27 participants.
Sample Size Justification: Thus, our sample size calculations indicated that 27 women provided sufficient power ($1-\beta = 0.80$) to detect significant relationships at a large effect size ($0.50$).

Expected Findings

We expected that increased energy intake with psychological/nutritional monitoring would result in favorable changes in psychological indicators of eating behavior (i.e., reductions in DT, BD, and DR scores) for our EAMD+Cal women. Specifically, the EAMD+Cal women were expected to demonstrate a greater decrease in DR score, with no change in DT and BD scores, across 6 months of the increased energy intake intervention compared to EAMD and OV controls. These expected findings were based on the assumed compliance of the EAMD+Cal group with the intervention and potentially, small effects of the behavioral monitoring (nutritional and psychological/eating behavior monitoring via semi-structured interviews). We did not anticipate that DT and BD scores would change within or among groups across 6 months of the increased energy intake intervention vs. control. It was hypothesized that an increased energy intake intervention may require additional cognitive/psychological support for those EAMD women with subclinical/clinical disordered eating behavior to promote symptom control concomitant with weight gain and recovery of energy status and menstrual function.
STUDY FOUR: Low bone density risk is higher in exercising women with multiple Female Athlete Triad risk factors

Background

The osteogenic impact of exercise on bone health in exercising women is well-documented (39,40,48,52,130) and weight-bearing exercise, in particular, may act as a preventive strategy for low BMD (61,65). However, in the presence of inadequate EI relative to EEE, also known as low EA, certain exercising women may be more susceptible to bone loss associated with low BMD (35,36,78,88) and impaired bone microarchitecture (1,37). As such, exercising women are often at risk for a disruption in menstrual cycles resulting in hypoestrogenism and musculoskeletal complications including osteoporosis and fractures (89). Low EA may negatively impact longitudinal bone growth and maturation in adolescent female athletes (11,21), and may promote bone loss in premenopausal women with or without exercise-associated amenorrhea (57,101). All of these clinical outcomes are associated with an interrelated syndrome known as the Triad (90).

The Triad is a well-documented paradigm in exercising women and is characterized across a continuum of healthy to subclinical and clinical conditions of menstrual disturbances, low BMD, and low EA (with or without disordered eating) (90). The Triad is most often observed in exercising women participating in leanness focused sport/activity (120) characterized by stringent weight control, such as long-distance running, gymnastics, and figure skating. Low EA/chronic energy deficiency may predominate in leanness sport/activity and often results in menstrual disturbances. Furthermore, elevated DR is an eating behavior associated with menstrual disturbances and lower BMD in exercising and sedentary women (6,8,15,81,82,110,122,123) and may represent an important factor to consider in the etiology of Triad-related bone loss. The relationship between hypoestrogenism and low BMD in exercising
women is well-established (22,33,35,36). Reductions in circulating estrogen concentrations promote proliferation of osteoblasts and osteoclasts and subsequently up-regulate bone turnover (77,104). Elevations in bone turnover are marked by a greater production of osteoclasts relative to osteoblasts and therefore, the result is a net resorption of bone. Low EA acts to promote bone loss via an energy deficiency (33,57,101,131,132) and disruptions in metabolic hormones, including reduced IGF-1 and leptin (46,125), which suppress bone formation. Additionally, peak bone mineral accrual in girls is strongly associated with menarche (80) and since adolescence is such a critical time period for bone acquisition, positive benefits of exercise participation on bone may be negated in those exercising girls with late age at onset of menarche (LAOM) and menstrual dysfunction (16,35,60,87).

**Brief Rationale**

Low BMD and musculoskeletal injury is a detrimental clinical outcome associated with the Triad in exercising women. In prior investigations in female athletes, the prevalence of low BMD using the International Society of Clinical Densitometry definition (Z-score≤-2) was low (mean: 9.2%) (8,12,54,92,97,102,109). However, prevalence estimates of low BMD increase when using a less conservative criterion point (ACSM Triad Position Stand) (Z-score<-1) (mean: 20.1%) (8,12,54,92,97,102). Since weight-bearing exercise is typically an osteogenic activity, it is important to consider that exercising women presenting with a BMD Z-score<-1 at any clinically relevant skeletal site may warrant medical attention and as noted above, this may be an optimal criterion for exercising women (90). To this end, a better understanding of the association between Triad-related risk factors and low BMD is warranted in a large population of exercising women and as such, these findings will guide the development of preventive and treatment strategies for low BMD associated with the Triad.
There has been emerging interest in the evaluation of risk factors associated with the Triad that may identify low BMD in exercising women (8,54,102). To date, few investigators have evaluated whether certain combinations of risk factors are associated with a higher percent and risk of low BMD in exercising women. Additionally, it is unknown whether there is a cumulative effect of Triad risk factors on low BMD, such that there is a dose-response relationship between number of Triad-related risk factors and BMD in exercising women. Thus, a comprehensive examination of risk factors to identify low BMD in exercising women is necessary to inform coaches, athletic trainers, and health practitioners of the individual and combinations of Triad risk factors which when presenting together translate to an increased risk of low BMD. Such findings would inform more intensive efforts of developing a screening process identifying risk of low BMD in exercising women wherein those women meeting the criteria for certain risk factors associated with the Triad (or number of risk factors) would be recommended for direct assessment of BMD. Practical criteria indicative of risk for low BMD are advised (i.e., easy to obtain and non-invasive self-reported or field measures of Triad-related characteristics) since an objective measure of BMD (i.e, DXA scan or other imaging assessment) is expensive and often not feasible in a clinical or field setting. Such data may also improve preventive strategies for low BMD associated with the Triad in exercising women.

Specific Aims and Hypotheses

**Aim 1:** To evaluate the association between Triad risk factors (individually and in combination) and low BMD in exercising women

Hypothesis 1i: Exercising women with menstrual dysfunction (current oligo/amenorrhea (OA) and LAOM) and low BMI in combination will demonstrate the highest percent and risk for low BMD (Z-Score<-1 and ≤-2).
Hypothesis 1ii: Exercising women with varying combinations of Triad risk factors (current OA, LAOM, low BMI, elevated DR, and participation in lean sport/activity) will demonstrate a greater likelihood of low BMD (Z-Score<-1 and ≤-2) in comparison to women without varying combinations of Triad risk factors (i.e. individual or no present risk factors).

Aim 2: To determine whether an increase in percent of low BMD in exercising women occurs alongside an increase in number of Triad risk factors

Hypothesis 2i: The percent of low BMD (Z-Score<-1 and ≤-2) in exercising women will increase in association with an increase in number of Triad risk factors, such as current OA, LAOM, low BMI, elevated DR, and participation in lean sport/activity.

Hypothesis 2ii: Current OA, LAOM, low BMI, elevated DR, and lean sport/activity participation will represent significant predictors of risk for low BMD (Z-Score<-1 and ≤-2).

Experimental Design/Overview of Research Methodology

This retrospective study was designed: (i) to examine the association between Triad risk factors (individually and in combination) and low BMD among a large population (n=437) of exercising women and (ii) to determine whether an increase in percent of low BMD in exercising women occurs in association with an increase in number of Triad risk factors. The study consisted of baseline data from 4 prospective multi-center studies completed in the United States and Canada (Pennsylvania State University (PSU), University of Toronto (UT), University of California at Los Angeles (UCLA), and San Diego State University (SDSU) (Table 2).

Data collection from UCLA, collected between the fall of 1996 and spring of 2001, consisted of female Division I collegiate track and field and cross-country athletes (n=17). Participants were followed, prospectively, for five years. Data collection for the SDSU study occurred during the 2003-2004 academic year. Female participants were between ages 13-18 yr
with initial onset of menarche or, if not menstruating, were between ages 15-18 yr (n=272). Participants were evaluated through their respective 3-4 month sport season. Data collection from PSU and the UT, between 2005 and 2011, consisted of data from a cross-sectional study designed to assess cardiovascular status in exercising women (n=34) and data from the baseline period of a prospective study designed to assess the effects of a 12-month intervention of increased EI on indices of bone health and menstrual status in women with exercise-associated menstrual disturbances vs. exercising women with regularly ovulatory menstrual cycles (n=114).

Table 2. Description of four prospective studies from which cross-sectional merged data was obtained for retrospective analyses.

<table>
<thead>
<tr>
<th>Study Location</th>
<th>Study Dates</th>
<th>Study Design</th>
<th>Study Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCLA</td>
<td>Fall of 1996 and Spring of 2001</td>
<td>Prospective 5yr study</td>
<td>Female Division I collegiate track and field and cross-country athletes (n=17)</td>
</tr>
<tr>
<td>SDSU</td>
<td>2003-2004 academic yr</td>
<td>3-4 month sport season</td>
<td>Female participants aged 13-18 yr with initial onset of menarche or, if not menstruating, aged 15-18 yr (n=272)</td>
</tr>
<tr>
<td>UT</td>
<td>2005 to 2011</td>
<td>Prospective study (3 menstrual cycles)</td>
<td>Young healthy adult women primarily from the general population of college-aged women (18-35 yr)</td>
</tr>
<tr>
<td>PSU &amp; UT</td>
<td>2005 to 2011</td>
<td>12-month intervention</td>
<td>Young healthy adult women primarily from the general population of college-aged women (18-35 yr)</td>
</tr>
</tbody>
</table>

Participants were young, healthy adult women primarily from the general population of college-aged women (18-35 yr). These participants engaged in at least two or more hours·week\(^{-1}\) of purposeful exercise and included competitive and recreational-level exercising women. For each study, one or more questionnaires were completed to obtain demographic and general background information including eating attitudes and behaviors, menstrual function, sports participation, exercise, and injury history, and medication use. Height and weight were measured. BMD and body composition were measured for each participant using DXA. Each of the studies excluded individuals taking medications known to affect BMD. Each study was approved by their respective university Institutional Review Board.
Combining the study databases involved a careful and rigorous procedure. Names, definitions, and methods of collection of variables from each study were reviewed. Variables that were measured and defined similarly among databases were merged. For variables that represented a similar data construct, but were not defined and/or measured identically, a new variable, with a definition that combined the representations of the data construct, was created. For example, among the databases, amenorrhea was defined as missing three or more consecutive menstrual cycles in the past year and reporting a frequency of less than four menstrual cycles in the past year. In these situations, a new variable combining both definitions was used (i.e. amenorrhea defined as either missing three or more consecutive menstrual cycles in the past year OR reporting less than four menstrual cycles in the past year). Categorical variables that were collected and defined similarly but coded differently among databases were re-coded to ensure consistency.

**Rationale for Experimental Design/Research Methodology**

**BMD and Body Composition:** BMD (areal BMD, bone mineral content, and bone area) and body composition (percent body fat, fat mass (kg), fat-free mass (FFM) (kg), and LBM (kg)) were analyzed by a certified technician using DXA. Participants were scanned on either a GE Lunar (GE Lunar Corporation, Madison, WI) DPX-NT (n=272), Prodigy (n=34), iDXA (n=114), or a Hologic QDR4500 (n=17, Hologic Inc., Bedford, MA) DXA scanner. Since no cross calibration study was completed, data were compared using standardized scores only.

**Menstrual Status Categorization:** Menstrual function was determined in all participants using a questionnaire. Our criteria for menstrual status considered the number of self-reported menstrual cycles in the past 12 months or menstrual cycle length prior to study enrollment. Categories of menstrual status included, 1) amenorrhea defined as the absence of menses for >3 consecutive months in the past year or <4 cycles in the past year, 2) oligomenorrhea defined as a
menstrual cycle length >35 days or between 4 and 9 cycles in the past year, or 3) eumenorrhea defined as a menstrual cycle length between 24-35 days or 10 to 12 cycles in the past year. Age of menarche (yr) was reported and LAOM was defined as onset of menses at age 15 yr or older (98).

Definitions of High DR and Pathological Weight Control Behavior (PWCB): DR was obtained using the TFEQ (112), the EDE-Q (84), or a pre-participation examination questionnaire corroborated with clinical interview with a physician. The TFEQ is a 51-item questionnaire that measures three dimensions of human eating behavior: (1) DR, (2) disinhibition, and (3) hunger (112). A cut-off of ≥9 was used to identify an elevated value for the DR subscale. A TFEQ DR score of ≥9 was chosen because prior reports have reported it as the median score for premenopausal women (122,123). The EDE-Q measures four subscales: (1) weight concern, (2) shape concern, (3) eating concern, and (4) DR. Scores ranging from 0 to 6 on a Likert scale correspond to the number of days over the past 4 weeks the respondent experienced a particular attitude, feeling, or behavior. An EDEQ DR score of ≥3 was chosen to identify an elevated value as per previous publications (8,91). Since all participants in the current study were assessed for dietary restraint using either the TFEQ, the EDE-Q, or an eating attitudes/behavior survey, elevated DR was defined/identified as either a score of ≥9 on the TFEQ, a score of ≥3 on the EDE-Q, or reporting elevated DR on a pre-participation examination questionnaire corroborated by clinical interview with a physician. PWCB was defined as reporting at least one prior episode of self-induced vomiting or use of laxatives, diuretics, and/or diet pills (91,120).

Sport/Activity Participation Categorization: Sport/activity participation was obtained using a questionnaire wherein the participants reported either the type, frequency (day·wk⁻¹), duration per session (min·day⁻¹), number of years and/or months per year of the sport or activity in which they participated from age 10 yr to current year (UCLA and SDSU) or in the past six months (PSU). Participants were categorized based on primary mode of sport/activity (lean vs. non-lean), using a classification system modified from Torstveit and Sundgot-Borgen (121). Lean
sports/activities included endurance (cycling, middle and long-distance running (>800m), rowing, swimming, triathlon, cross-country skiing), aesthetic (figure skating, gymnastics, rhythmic gymnastics, dance), weight class (boxing, weight-lifting, karate, judo, tae kwon do, wrestling, kickboxing, jujitsu), anti-gravitation sports (indoor and outdoor rock climbing, high jump, long jump, pole vaulting, triple jump), and aerobic (aerobics, gym-related cardio such as elliptical or stair climber); whereas, non-lean sports/activities included technical (bowling, curling, fencing, golf, horseback riding, sailing, snowboarding, water skiing), ball game (badminton, basketball, ice hockey, field hockey, soccer, lacrosse, softball, baseball, table tennis, squash, team handball, tennis, rugby, and volleyball), and power sports (alpine skiing, discus, hammer, hurdle, javelin, shot put, speed skating sprint, sprint (≤800 m) (121).

**Definitions of Low BMD:** Participants were categorized as low BMD if their values at the lumbar spine (L1-L4) or total body in the adolescent population (15 to 17 yr) (n=268) or the lumbar spine (L1-L4) or total hip in the premenopausal female population (18 to 35 yr) (n=170) were 1 or 2 SD or more below the age-matched, gender-specific reference data from the GE/Lunar and Hologic databases (Z-score<-1 or ≤-2, respectively). The ISCD defines low BMD for chronological age as an age, gender, and body size-adjusted BMD Z-score ≤-2 (58), whereas the ACSM 2007 Triad Position Stand (90) defines a low BMD as BMD Z-score<-1, particularly if accompanied by secondary clinical risk factors for fracture such as amenorrhea. We chose to examine both Z-score cut-offs (ACSM and ISCD criteria) (102) because exercising women are expected to demonstrate BMD values approximately 10% higher vs. sedentary controls (90). As such, a BMD Z-score between -1 and -2 requires attention.

**Triad Risk Factors For Low BMD:** Prior to the initiation of data analysis, investigators identified potential risk factors of low BMD associated with the Triad (i.e. factors associated with the Triad, such as low body weight (BW) or BMI, current OA, LAOM, elevated DR, PWCB, and lean sport/activity participation). Definitions and/or cut-off points used for each risk factor were
decided upon based on their recognition as criteria for an established clinical condition, or evidence linking the factor to the Triad and related clinical sequelae. The risk factors assessed included: low BMI (BMI < 18.5 kg/m²); low BW (BW < 90% ideal body weight (IBW)); elevated DR; PWCB; participation in a lean sport/activity; LAOM (≥ 15 yr); and current OA (irregular cycles ≥ 36 d in length or < 9 cycles in past yr). Other risk factors that were considered, but not included in each database (and, therefore, could not be assessed in the current study) were prior history of a clinical eating disorder, menstrual disturbances prior to the past year, history of cumulative menstrual dysfunction, subclinical menstrual disturbances, weekly training volume, and low energy availability.

The criteria used in the definition of the clinical eating disorder, anorexia nervosa, from the Center for Disease Control cites a BMI < 18.5 kg/m² as underweight. Torstveit and Sundgot-Borgen (120) also defined BMI < 18.5 kg/m² as an at-risk criterion of the Triad in large population of competitive female athletes by assuming that a very low BMI may indicate chronic energy deficiency. Additionally, a BW less than 10% of ideal, using the IBW equation (49), is used to define a moderate underweight status (99). Amenorrhea or oligomenorrhea are the two most severe clinical menstrual disturbances identified in the 2007 ACSM triad Position Stand (90). Primary or secondary amenorrhea is one of the required criteria in WHO and DSM-IV definitions of anorexia nervosa and also represents a well-documented factor associated with compromised bone mineral accretion and/or bone loss in exercising women (35,70). PWCB are unhealthy dieting strategies which may be utilized by female athletes to achieve a low BW for performance or appearance-related reasons (91,120). LAOM was included as a risk factor, indicative of a history of primary amenorrhea and a negative impact on bone mineral accrual during adolescence (80). Investigators have previously reported associations between two or more of the following factors associated with the Triad and low BMD in exercising women such as: elevated DR
(measured by the EDEQ, TFEQ, and clinical interview), participation in a lean sport/activity, and OA (8,91,102,120,123).

**Dataset**

Study Four consisted of data from four prospective multi-center studies completed in the United States and Canada (PSU, UT, UCLA, and SDSU) (Table 1.1). Data collection from UCLA, collected between the fall of 1996 and spring of 2001, consisted of female Division I collegiate track and field and cross-country athletes. Participants were followed prospectively for five years. Data collection for the SDSU study occurred during the 2003-2004 academic year. Female participants were between 13-18 yr with initial onset of menarche or, if not menstruating, were between 15-18 yr. Participants were evaluated through their respective 3-4 month sport season. Data collection from PSU, collected at two sites, the UT and the PSU, between 2005 and 2011, consisted of data from a cross-sectional study designed to assess cardiovascular status in exercising women and data from the baseline period of a prospective study designed to assess the effects of a 12-month intervention of increased energy intake on indices of bone health and menstrual status in women with EAMD, including amenorrhea (absence of menses for >90 days) and oligomenorrhea (long, inconsistent menstrual cycles of 36-90 days), vs. exercising women with ovulatory cycles. Participants were young, healthy adult women primarily from the general population of college-aged women (18-35 yr). These participants engaged in at least two or more hours·week⁻¹ of purposeful exercise.

**Study Groups Description for Specific Aims**

The specific aims of this study were tested in the total sample of exercising women (n=437). Descriptive comparisons were performed between the following groups:

1. Exercising women with BMD Z-Score<-1
Description of Participants

This study sample included 437 women (ages 13-35 yr), with 17 from the UCLA site, 272 from the SDSU site, 34 from the UT site, and 114 from the PSU site. Participants were either recreational- or competitive-level exercising women who were involved in a variety of sport and exercise training. Recreational exercising women (n=117) participated in at least 2 hours·week⁻¹ of “purposeful exercise”. Competitive exercising women (n=320) were current members of a high-school or collegiate athletic team. Inclusion criteria for this study were: 1) age 13-35 yr, 2) no history of any chronic illness, 3) not taking any medications that affect BMD, and 4) had an initial onset of menarche or, if not menstruating, age 15-18 yr. Written consent from participants and/or parents was obtained prior to participation.

Statistical Analyses

All statistical analyses were conducted using SPSS version 19.0 (SPSS, Inc., Chicago, IL). All hypothesis tests were two-sided and P<0.05 was considered significant. Data screening was conducted prior to analysis, involving outlier detection, evaluation of assumptions of normality, and regression diagnostics. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percentages for categorical data between participants with BMD Z-Score<-1 and those participants with BMD Z-Score≥-1. Clinical characteristics (i.e., age, ethnicity, height, body mass, BMI, body composition, age of menarche, gynecological age) and percent of Triad risk factors were determined to describe the study sample. Psychometric survey data was recoded and scored to determine subscale or total scores. All group comparisons were performed using independent student’s t-tests for continuous data and chi-square tests for categorical data.
To test hypothesis 1: Chi-square tests were performed to determine the association between Triad risk factors (individually and in combination) and low BMD (Z-Score\(<-1\) and \(\leq -2\)) in exercising women. Crude odds ratios (OR) and 95% confidence intervals (95% CI) were determined to compare the risk of low BMD (Z-Score \(<-1\) and \(\leq -2\)) in women with Triad risk factors (individually and in combination) compared to those women without those risk factors (individually and in combination).

To test hypothesis 2: Chi-square tests were performed to determine the association between the number of Triad risk factors (0, any 1, 2, 3, 4, or 5) and low BMD (Z-Score\(<-1\) and \(\leq -2\)) in exercising women. Adjusted ORs with 95% CI were generated using multivariate logistic regression analyses to determine the strongest predictors of low BMD in exercising women. Items in the logistic regression model analyses included current OA, LAOM, low BMI, elevated dietary restraint, and lean sport/activity participation. Adjusted models were conducted for low BMD Z-Score\(<-1\) and low BMD Z-Score\(\leq -2\).

Sample Size Calculations and Justification

All sample size calculations were performed using G Power 3.1.2 (Universitat Kiel, Germany, 2009). We based our sample size calculations on preliminary data (means and SDs) obtained from the literature. Sample size calculations were performed to determine the number of participants per group required: (1) to determine the association between Triad risk factors (i.e., current OA) and low BMD (Z-Score\(<-1\) and \(\leq -2\)) in exercising women and (2) to determine the strongest predictors of low BMD in exercising women by performing multivariate logistic regression analyses to generate adjusted OR with 95% CI.

Sample size calculations to test hypothesis 1: To date, this is the first study to determine the association between Triad-related risk factors (individually and in combination) and low BMD (Z-Score\(<-1\) and \(\leq -2\)) in exercising women. Four studies have been completed wherein
investigators determined the association between clinical menstrual disturbances and low BMD (Beals and Hill, 2006; Burrow et al., 2007; Hoch et al., 2009; Torstveit and Sundgot-Borgen, 2005b). In these studies, the percent of combined low BMD (Z-Score≤-2) and clinical menstrual disturbances (amenorrhea or oligomenorrhea) ranged from 0-7.5%. We expected to observe approximately 14% of participants to present with low BMD (Z-Score<-1) and current OA in our large sample of exercising women (n=437). This percent was above the upper limit of the prevalence of low BMD and clinical menstrual disturbances in the literature (7.5%) but below the mean prevalence of low BMD alone (20%) in samples of exercising women from the literature. In studies of clinical menstrual disturbances in exercising women, investigators have determined the prevalence of secondary amenorrhea and oligomenorrhea to range from 1-60% and 1-53% respectively.

We expected 50% of women to present with clinical menstrual disturbances (current OA) vs. 50% of women to present with current eumenorrhea. As such, we anticipated to observe the following percentages of women with a clinical menstrual disturbance (OA) or without a clinical menstrual disturbance (eumenorrhea) in a sample of exercising women categorized by BMD status: 14% (61/437) with a clinical menstrual disturbance and low BMD and, 36% (157/437) with a clinical menstrual disturbance and normal BMD, 14% (60/437) with eumenorrhea and low BMD, and 36% (157/438) with eumenorrhea and normal BMD. Using the above percentages (14%, 36%, 14%, 36%) for the alternative hypothesis (pH1) and 0.25 for each of the accompanying cells for the null hypothesis (pH0) for the total sum of the expected probabilities to equal 1.0, an effect size w of 0.44 was computed using the G*Power statistical program. An effect size of 0.44, an alpha error probability of 0.05, a power of 0.95, and 1 degree of freedom, as a 2 x 2 chi square analysis was performed, required a total sample size of 68 participants to test hypothesis 1 using a goodness-of-fit test for contingency tables.
Sample size calculations to test hypothesis 2: Using data from Barrack et al. (9) in 93 adolescent runners wherein a multivariate logistic regression analysis was performed (items in the logistic regression model analyses included: menstrual irregularity, lifetime seasons of running, elevated dietary restraint, lean tissue, BMI, gynecological age to predict low BMD Z-Score $\leq -1$ and low BMD Z-Score $\leq -2$, we determined a similar adjusted OR for low BMD (Z-Score $<-1$) in participants with current OA vs. eumenorrhea. As such, we assumed that the event rate under H0 was $p_1 = 0.086$ and that the event rate under H1 was $p_2 = 0.914$. The OR was 
\[(0.914/0.086)/(0.5/0.5) = 10.6 \text{ and } \beta_1 = \log(OR) = 2.36.\] Using the procedure of Hsieh et al. (56), an OR of 10.6, Pr(Y=1)H0 of 0.09, an alpha probability of 0.05, and a power of 0.95 required a total sample size of 327 participants to test hypothesis 2 using logistic regression analyses. Additionally, we assume that 20% of participants in the total sample will demonstrate low BMD (Z-Score $<-1$). We planned to include five risk factors in our multivariate logistic regression analysis to predict low BMD (Z-Score $<-1$) (current OA, LAOM, low BMI, leanness sport/activity participation, and elevated dietary restraint) and as such, five degrees of freedom multiplied by 15 observations per degree of freedom is equal to 75. The minimum of “events” (has low BMD) and “non-events” (normal BMD) was expected to be 20% and 80%; 75 is 20% of 375. Therefore, we would need approximately 375 participants in our sample.

Sample size justification: Thus, our sample size calculations indicated that 375 women provided sufficient power (1-$\beta = 0.95$) to detect significant relationships.

Expected Findings

We expected that the findings from this study would reinforce prior associations between Triad-risk factors and low BMD in exercising women such as: current OA, LAOM, low BMI, lean sport/activity participation, and elevated DR. All of these factors represented well-established indicators of menstrual dysfunction and/or chronic energy deficiency/low EA.
Furthermore, all of these factors are potentially etiological to the development of low BMD in exercising women. Additionally, women with varying combinations of these Triad risk factors would likely demonstrate a higher percent and risk for low BMD compared to those women without varying combinations of Triad risk factors (i.e., individual or no present risk factors). Specifically, we predicted that current OA or LAOM in combination with low BMI would be associated with the highest percent and risk for low BMD in exercising women. We also anticipated observing a cumulative effect of Triad risk factors on the percent of low BMD (Z-Score≤-1 and ≤-2) in exercising women such that there would be a dose-response relationship between Triad risk factors and BMD. Lastly, we presumed that current OA, LAOM, low BMI, elevated DR, and lean sport/activity participation would represent significant risk factors associated with low BMD.
References


CHAPTER 2
REVIEW OF THE LITERATURE- PART ONE


**ABSTRACT**

The Female Athlete Triad (Triad) is a syndrome linking low energy availability (EA) with or without disordered eating (DE), menstrual disturbances (MD), and low bone mineral density (BMD) in exercising women. The prevalence of Triad conditions (both clinical and subclinical) has not been clearly established. **Purpose:** The purpose of this review is to evaluate the studies that determined the prevalence of clinical or subclinical Triad conditions (low EA, DE, MD, low BMD) in exercising women and in women participating in lean (LS) vs. non-lean sports (NLS) using self-report and/or objective measures. **Methods:** A review of publications using MEDLINE and PubMed was completed. Randomized controlled trials and observational studies that evaluated the prevalence of clinical and subclinical Triad conditions (MD, low BMD, low EA, and DE) in exercising women were included. **Results:** 65 studies were identified for inclusion in this review (n=10,498, mean age: 21.8±3.5yr, mean BMI: 20.8±2.6kg/m²). A relatively small percentage of athletes (0-15.9%) exhibited all three Triad conditions.
The prevalence of any two or any one of the Triad conditions in these studies ranged from 2.7-27.0% and 16.0-60.0%, respectively. The prevalence of all three Triad conditions in LS athletes vs. NLS athletes ranged from 1.5-6.7% and 0-2.0%, respectively. LS athletes demonstrated higher prevalence rates of MD and low BMD (3.3% vs. 1.0%), MD and DE (6.8-57.8% vs. 5.4-13.5%), and low BMD and DE (5.6% vs. 1.0%) than the NLS athletes. **Conclusion:** Although the prevalence of individual/combined Triad conditions is concerning, our review demonstrates that additional research on the prevalence of the Triad using objective and/or self-report/field measures is necessary in order to more accurately describe the extent of the problem.

**INTRODUCTION**

The Female Athlete Triad (Triad) was first recognized two decades ago based on the association of disordered eating (DE), functional hypothalamic amenorrhea (FHA), and osteoporosis observed in recreational and elite-level exercising women (41). In 2007, the American College of Sports Medicine published a position stand (41) with updated scientific information and recommendations for screening, diagnosis, prevention, and treatment of the Triad. The most recent conceptual model of the Triad is a syndrome linking low energy availability (EA) (with or without DE), menstrual disturbances (MD), and low bone mineral density (BMD) across a continuum of healthy (optimal EA, normal and regular menstrual cycles, and optimal BMD) to unhealthy and increasingly severe clinical presentations of each component (41). The Triad is a
detrimental consequence of the failure to ingest adequate energy to compensate for energy expended during exercise, a condition referred to as low EA. As such, the Triad is commonly observed in exercising women (14,41), particularly those women involved in leanness, aesthetic, and/or endurance sports and activity (63). Low EA with or without DE may be induced for a variety of reasons to include: 1) intentional, i.e., modifying body size and composition to achieve appearance or performance goals; 2) compulsive, i.e., demonstrating DE and/or pathological weight control behavior; or 3) inadvertent, i.e., failing to match energy intake to exercise-induced energy expenditure (37). Low EA often results in an energy deficiency, which when sustained for prolonged periods of time, translates to metabolic and reproductive suppression (72). The result is the development of both subclinical (luteal phase defects (LPD) and anovulation) and clinical MD (FHA and oligomenorrhea), musculoskeletal complications, i.e., low BMD and stress fractures (41), and other clinical sequelae, i.e., endothelial dysfunction (29,45).

A large body of literature exists wherein the prevalence of individual disorders of the Triad has been determined (MD, low BMD, and DE/eating disorders). Several publications have reported the prevalence of clinical MD (FHA and oligomenorrhea) in female athletes, to include both high school (4,30,42,43) and premenopausal women (6,7,14,18,19,40,49,51,53,66,70). The earliest prevalence estimates of clinical MD were determined in the most at risk populations to include runners (17,24,28,58,60,73) and dancers (1,12). In general, clinical manifestations of MD have been shown to range from 1 to 61% in exercising women, and are documented at much higher rates than in
non-athletic, premenopausal women (3,47,61). Subclinical MD have only been assessed in a few studies (8,18,19,22,76), but there is evidence suggesting that approximately half of exercising women experience subclinical MD (LPD and anovulation) and that self-reported menstrual history alone does not provide the appropriate information to indicate presence of subclinical MD (LPD and anovulation) (19). Since the method of self-report can only identify those MD readily apparent to women as an absence of menses for greater than 3 months (FHA) or an irregularity in menstrual cycle length (oligomenorrhea), investigators reporting on the prevalence of MD as a component of the Triad, determined using self-report methods, are likely to have underestimated the percent of women with the Triad. Additionally, the majority of Triad prevalence reports have included clinical eating disorders and/or DE as a component of the syndrome (6,43,49,59,68,70). Previous findings indicate a higher proportion, up to 70%, of clinical eating disorders and/or DE are present in elite female athletes compared to controls, particularly in athletes participating in sports focused on leanness, aesthetic appearance, and weight control (11,62,64,67). However, it is important to acknowledge that despite a higher frequency of DE in female athletes, clinical DE is not always observed in exercising women presenting with Triad-related clinical sequelae.

A notable absence in Triad prevalence investigations is the assessment of EA, except for a prospective study in high school athletes compared to age-matched controls (30). Since EA is suggested to be one of the key components of the Triad (41), further evaluation of its prevalence alongside other Triad conditions is warranted. Last, the prevalence of low BMD in female athletes is not as well-documented, particularly due
to recent technological advancements in measurement tools and the inconsistency in
criteria used to identify low BMD. In earlier reports, investigators inappropriately used
the World Health Organization (WHO) criteria to define low BMD and osteoporosis in
premenopausal exercising women. Since the WHO criteria are intended for
postmenopausal women, these prevalence estimates for low BMD and osteoporosis are
suggested to be inaccurate. The definition of low BMD in the 2007 ACSM Position
Stand on the Triad includes a Z-score of <-1.0 (specifically between -1.0 and -2.0) in
the presence of additional clinical factors such as hypogonadism. This criterion is based
on the premise that athletes in weight-bearing sports should present with BMD 5-15%
higher than non-athletes (30,41). Therefore, a BMD Z-score between -1.0 and -2.0
warrants attention. To date, the prevalence of low BMD in amenorrheic athletes ranges
from 1.4-50.0% (4,6,10,30,43,48,49,51,57,59,77) and estimates of osteoporosis are
lower (0-13.0%) (10,48,57,77). As such, few investigators have examined the
prevalence of low BMD in high school (4,30,43,51,59) and premenopausal exercising
women (6,49) and its prevalence alongside other Triad conditions requires further
investigation.

Establishing the prevalence of the Triad and its components is a difficult task.
Concerns exist related to the methods of detection used in reports, lack of appropriate
definitions and criteria utilized for each Triad component, and limitations in
experimental design and methods of assessment (20,41). Recent investigators have
demonstrated that large discrepancies in prevalence exist between the presentation of all
three Triad conditions compared to estimates of two or one of the Triad disorders.
However, this information has yet to be summarized and as such, the magnitude of the syndrome has not been thoroughly evaluated. Additionally, the prevalence of two or three of the Triad conditions across the continuum (subclinical vs. clinical) is not well documented and requires examination. We will investigate these factors in our review and discuss such limitations related to the study of prevalence related to the Triad. To date, this is the first review examining the prevalence of any two or all three of the Triad conditions in a systematic manner, specifically evaluating the prevalence of both clinical and subclinical conditions. This review will also represent an updated examination of prevalence reports on individual disorders of the Triad as there have been several investigators that have determined the prevalence of MD, low BMD, or DE/eating disorders in exercising women. Furthermore, several studies have been completed (6,42,50,68) wherein the investigators have categorized the participants based on sport type (lean vs. non-lean) and reported the prevalence of the Triad conditions. To this end, a summary of those findings would be valuable in better understanding the Triad etiology and provide a comparison in prevalence of the Triad disorders between athletes in lean sports vs. those in non-lean sports. Preventive and treatment policy decisions rely on accurate estimates of the Triad and as such, a better understanding of the clinical relevance of the Triad is warranted. The purpose of this systematic review is: (1) To evaluate the prevalence of Triad conditions (low EA with or without DE, MD, and/or low BMD) (clinical or subclinical) using self-report and/or objective measures and (2) To evaluate the prevalence of Triad conditions (low EA with
or without DE, MD, and/or low BMD) (clinical or subclinical) in women participating in lean vs. non-lean sports.

SEARCH STRATEGY

**Data Sources:** An electronic search of the computerized databases PubMed and MEDLINE was performed for the period 1975-2011 using the search terms: Female Athlete Triad, low EA, DE, eating disorders, FHA, athletic amenorrhea, anorexia athletica, MD, energy deficiency, low bone mass, low BMD, osteoporosis, female athletes, and exercising women. In addition, when relevant articles were not included in the electronic search of PubMed and MEDLINE, the first author hand searched for the key articles and cited references. We excluded studies not published in English. We did not search for abstracts nor did we evaluate any case studies or unpublished studies. No contact with authors was made for this review.

**Study Inclusion and Exclusion Criteria:** Studies of the prevalence of at least one of the clinical and/or subclinical Triad disorders were included in this review. We included all published randomized controlled trials, observational (prospective and retrospective) cohort and cross-sectional studies that assessed the prevalence of one or more of the Triad conditions in high school and premenopausal exercising women using self-report and/or objective measures. We acknowledge that there are distinct physiological and developmental differences between high school and premenopausal women, however, based on previous research (4,30,42,43,65), it is clear that the Triad is a significant
clinical problem in high school female athletes, similar to premenopausal exercising and athletic women. Reports with or without control groups were included. The Triad was operationally defined as: (1) low EA (with or without DE) or if EA was not evaluated, the presence of clinical eating disorders or DE; (2) FHA (primary or secondary), oligomenorrhea, or subclinical MD to include LPD and anovulation; and (3) low BMD. Methods of evaluation and definitions of the clinical and subclinical presentations of the Triad used by studies included in this review are outlined in Table 1. Reports on the clinical and/or subclinical Triad disorders between lean and non-lean sport athletes were also included in this review. Lean sport athletes were defined as participants in sports that place an emphasis on endurance training, low body weight, lean physique, and aestheticism (68); whereas, non-lean sport athletes were defined as participants in sports emphasizing technical and/or ball-related skill and/or power training (68). Torstveit and Sundgot-Borgen outlined the specific sports that meet these criteria in another manuscript (68). Data on the incidence of musculoskeletal injuries and endothelial dysfunction were not included in this review.

**Independent Review and Analysis:** Independent extraction of articles by the first author was completed in a standardized manner. Information extracted from each article included characteristics of study participants (including age (yr), body mass index (BMI) (kg/m²), geographical source, and type of sport or exercise group) and the prevalence of the Triad conditions, both clinical and subclinical.
Table 1. Methods of evaluation and clinical/subclinical definitions of the components of the Triad used in studies included in this review.

<table>
<thead>
<tr>
<th>Components of the Triad</th>
<th>Method of Evaluation</th>
<th>Subclinical Definition</th>
<th>Clinical Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low EA with or without DE/ED</td>
<td>EI obtained using prospective 3-d (2 week days and 1 weekend day), 7-d food logs or 24hr food recall</td>
<td>EI – EEE normalized to FFM or LBM Cut-off : &lt;45 kcal/kg/FFM or LBM</td>
<td>EI – EEE normalized to FFM or LBM Cut-off : &lt;30 kcal/kg/FFM or LBM</td>
</tr>
<tr>
<td>DE</td>
<td>Clinical Interview or survey using DSM-III or DSM-IV criteria, EDI or EDI-2, TFEQ, EDEQ, EAT-26 or EAT-40, BITE, BSQ</td>
<td>Elevated scores on the EDI/EDI-2 (DT≥7; BD≥9), EDI (≥23), TFEQ (DR≥9), or an energy deficit (&gt;200 kcal)</td>
<td>Eating disorder: Met one of the criteria from the DSM-III or DSM-IV for AN, BN, or EDNOS Disordered eating: Severe elevated scores on the EDI/EDI-2 (DT≥15, BD≥14), EDI (&gt;30), EAT-26 (≥10), EAT-40 (≥30), EDEQ (≥4), TFEQ-18 (upper quartile on 3 subscales), BITE (≥10), BSQ (&gt;140), reported use of diet pills, laxatives, diuretics, or vomiting</td>
</tr>
<tr>
<td>MD</td>
<td>Daily urinary hormone analysis quantifying LH, E1G and PdG metabolites and/or other hormonal analysis and/or self-reported menstrual cycle patterns and menstrual history questionnaires</td>
<td>LPD: Short luteal phase (&lt;10 days) or inadequate PdG in luteal phase Anovulation: Suppressed E1G and PdG with no rise in LH</td>
<td>FHA: absence of menses for at least 90d or &lt; 4 menses/past yr Primary Amenorrhea: no onset of menses at 15 yr in presence of normal secondary sexual development Oligomenorrhea: menses at irregular intervals of &gt;35d or 4-9 menses/past yr Z-score≤ -2.0</td>
</tr>
<tr>
<td>Low BMD</td>
<td>DXA A positive diagnosis in at least one of the following measurement areas: total body, lumbar spine (L₁-L₄), femoral neck, or total hip as evidence of low BMD</td>
<td>Z-score&lt;1.0</td>
<td></td>
</tr>
</tbody>
</table>

EA = Energy Availability; DE = Disordered Eating; ED = Eating Disorder; EI = Energy Intake; EEE = Exercise Energy Expenditure; FFM = Fat-Free Mass; LBM = Lean Body Mass; DSM-III or DSM-IV = Diagnostic and Statistical Manual of Mental Disorders; EDI = Eating Disorder Inventory; TFEQ = Three Factor Eating Questionnaire; EDEQ = Eating Disorder Examination Questionnaire; EAT = Eating Attitudes Test; BITE = Bulimic Investigatory Test Edinburgh; BSQ = Body Shape Questionnaire; DT = Drive for Thinness; BD = Body Dissatisfaction; PWCM = Pathological Weight Control Methods; DR = Dietary Cognitive Restraint; AN = Anorexia Nervosa; BN = Bulimia Nervosa; EDNOS = Eating Disorder Not Otherwise Specified; MD = Menstrual Disturbances; LH = Luteinizing Hormone; E1G = Estrone-1-Glucuronide; PdG = Pregnandiol Glucuronide; LPD = Luteal Phase Defect; FHA = Functional Hypothalamic Amenorrhea; BMD = Bone Mineral Density; DXA = Dual Energy X-Ray Absorptiometry
RESULTS

Summary of Studies on the Prevalence of the Triad Conditions in Exercising Women: A total of 65 studies were identified for inclusion in this systematic review. The search of MEDLINE and PubMed databases provided a total of 169 citations. Of these, 47 studies were discarded because after reviewing the abstracts it appeared that these papers clearly did not meet our established criteria. The full text of the remaining 122 citations was examined. Of those citations examined, 57 studies did not meet our inclusion criteria as described. Among all of the included studies, there were 10,498 participants with a mean age of 21.8±3.5yr with a BMI of 20.8±2.6 kg/m². The source population for the studies varied geographically; 39 studies from the United States, eight studies from Norway, six studies from the United Kingdom, three studies from Australia, two studies from Germany, and one study each from Turkey, Malaysia, Iran, Sweden, Croatia, South Africa, and Brazil. Twenty-nine studies focused on competitive athletes from multiple sports, 15 studies on competitive runners, nine studies on competitive dancers, three studies on endurance athletes, three studies on recreationally active women, two studies on competitive gymnasts, and one study each on recreational triathletes, competitive swimmers, competitive figure skaters, and recreational weight-lifters. Twenty-three studies included nonathlete/sedentary controls in their analyses.

Nine studies were identified wherein the investigators evaluated the prevalence of all three Triad conditions individually or in combination (6,10,31,43,49,59,68,70) (Table 2) (see Table 1 for the definition of subclinical and clinical conditions for each Triad condition). A more specific evaluation of prevalence was completed wherein
investigators determined the prevalence of two Triad conditions (MD and low BMD; MD and DE; low BMD and DE; MD and low EA; low EA and low BMD) and revealed, 1) four studies on clinical MD and low BMD (6,10,30,68), 2) eight studies on clinical MD and clinical DE (9,14,26,43,50,65,68,70), 3) two studies on clinical DE and low BMD (6,68), 4) one study on low EA and clinical MD (30), and 5) one study on low EA and low BMD (30). Three studies on subclinical DE and clinical MD were also included (27,44,71).

In terms of clinical presentation of individual Triad conditions, there were: 1) 35 studies on clinical MD, 2) eight studies on low BMD (Z-score ≤ -2.0), 3) 29 studies on clinical eating disorders/DE, and 4) one study on low EA (<30 kcal/day/kg lean body mass (LBM)). In terms of subclinical presentation of individual Triad conditions, there were 1) four studies on subclinical MD, 2) six studies on low BMD (Z-score between -1.0 and -2.0), 3) six studies on subclinical DE, and 4) one study on low EA (<45 kcal/day/kg LB M). The prevalence estimates from these published reports are summarized in Tables 3, 4, and 5.

We included five studies wherein the investigators determined the prevalence of two or three Triad conditions in lean vs. non-lean sport athletes and thirteen studies wherein the prevalence of individual Triad conditions were evaluated. The prevalence estimates from these published reports are summarized in Table 6.
Prevalence Studies on All Three Triad Conditions Presenting Simultaneously: The prevalence of all three, any two, and any one of the Triad conditions in exercising women is presented in Table 2. A relatively small percentage of athletes (0-15.9%) exhibited all three Triad conditions in the published studies (9 studies; n=991). In reports on solely high school athletes, the prevalence of all three Triad conditions ranged from 1.0-1.3% (3 studies; n=328). The prevalence of any two Triad conditions in these studies ranged from 2.7-27.0% (7 studies; n=865) (Table 2). The prevalence of any one of the Triad conditions in these studies ranged from 16.0-60.0% (6 studies; n=537) (Table 2).

Prevalence Studies on the Different Combinations of Two Components of the Triad: The specific evaluation on the prevalence of the different combinations of two Triad conditions revealed, 1) the prevalence of MD and low BMD was 0-7.5% (4 studies; n=460), 2) the prevalence of MD and DE was 2.7-50.0% (8 studies; n=1,136), 3) the prevalence of low BMD and DE was 0.9-3.2% (2 studies; n=298), 4) the prevalence of MD and low EA was 17.5% (1 study; n=80), and 5) the prevalence of low BMD and low EA was 3.75% (1 study; n=80).
Table 2. Studies on the prevalence of all three, any two, and any one of the Triad conditions in exercising women.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Participants</th>
<th>Prevalence of all Triad Conditions (%)</th>
<th>Prevalence of any two Triad Conditions (%)</th>
<th>Prevalence of any one Triad Conditions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High school Athletes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoch et al. (30)</td>
<td>80 varsity female high school athletes</td>
<td>1.0</td>
<td>4.0-18.0</td>
<td>16.0-54.0</td>
</tr>
<tr>
<td></td>
<td>80 sedentary controls</td>
<td>1.0</td>
<td>5.0-10.0</td>
<td>21.0-39.0</td>
</tr>
<tr>
<td>Nichols et al. (43)</td>
<td>170 varsity female high school athletes</td>
<td>1.2</td>
<td>5.9</td>
<td>45.9</td>
</tr>
<tr>
<td>Schtscherbyna et al. (59)</td>
<td>78 female swimmers</td>
<td>1.3</td>
<td>15.4</td>
<td>47.4</td>
</tr>
<tr>
<td><strong>Premenopausal Exercising Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beals and Hill (6)</td>
<td>112 collegiate female athletes</td>
<td>0.9</td>
<td>9.0</td>
<td>52.7</td>
</tr>
<tr>
<td>Burrows et al. (10)</td>
<td>82 physically active females</td>
<td>0.0</td>
<td>NR</td>
<td>22.0</td>
</tr>
<tr>
<td>Hoch et al. (31)</td>
<td>15 women from club triathlon team</td>
<td>0.0</td>
<td>27.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Pollock et al. (49)(a)</td>
<td>44 elite female endurance athletes</td>
<td>15.9</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Torstveit and Sundgot-Borgen (68)(a)</td>
<td>186 female elite athletes</td>
<td>4.3</td>
<td>5.4-26.9</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>145 control participants</td>
<td>3.4</td>
<td>12.4-15.2</td>
<td>NR</td>
</tr>
<tr>
<td>Vardar et al. (70)(a)</td>
<td>224 female athletes</td>
<td>1.4</td>
<td>2.7</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR = Data Not Reported
\(a\) = Study included prevalence data on both high school and premenopausal female participants
**Prevalence Studies on MD:** The studies wherein the investigators evaluated the prevalence of MD are summarized in Table 3. All studies used self-report methods, except three studies which utilized hormonal analyses (8,18,19) and one study wherein pooled serum hormone levels for early follicular and midluteal phases of the cycle were used (76).

Prevalence of secondary amenorrhea ranged from 1.0-60.0% (34 studies; n=5,607). Prevalence of primary amenorrhea ranged from 0-56.0% (13 studies; n=2,216). The range in prevalence of oligomenorrhea was 0.9-52.5% (23 studies; n=4,044). In the four studies that assessed the prevalence of subclinical MD (LPD and anovulation), the prevalence of LPD and anovulation ranged from 5.9-43.0% (n=118) and 12.0-30.0%, (n=101) respectively.

**Prevalence Studies on DE:** Table 4 presents the studies wherein the investigators evaluated the prevalence of clinical eating disorders and DE (subclinical and clinical presentations) in exercising women. The prevalence of clinical eating disorders ranged from 0-48.0% (17 studies; n=2,869); whereas, the prevalence of clinical and subclinical DE ranged from 7.1-89.2% (17 studies; n=2,867) and 2.9-60.0% (6 studies; n=1,363), respectively.
Table 3. Studies on the prevalence of clinical menstrual disturbances in exercising women.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Prevalence of Oligomenorrhea (%)</th>
<th>Prevalence of Primary Amenorrhea (%)</th>
<th>Prevalence of Secondary Amenorrhea (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daily Urinary Reproductive Hormone Measures</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>De Souza et al. (18)</td>
<td>7.0</td>
<td>NR</td>
<td>37.2</td>
</tr>
<tr>
<td><strong>Self-Report Menstrual History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abraham et al. (1)</td>
<td>NR</td>
<td>13.8</td>
<td>37.9</td>
</tr>
<tr>
<td>Dadgostar et al. (16)</td>
<td>4.2</td>
<td>0.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Robinson et al. (54)</td>
<td>15.0-19.0</td>
<td>NR</td>
<td>15.0-28.0</td>
</tr>
<tr>
<td>Thompson (66)</td>
<td>17.7</td>
<td>NR</td>
<td>5.3</td>
</tr>
<tr>
<td><strong>Menstrual History Survey</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barrack et al. (4)</td>
<td>5.4</td>
<td>3.2</td>
<td>17.2</td>
</tr>
<tr>
<td>Beals and Hill (6)</td>
<td>NR</td>
<td>8.9</td>
<td>21.4</td>
</tr>
<tr>
<td>Beals and Manore (7)</td>
<td>11.9</td>
<td>NR</td>
<td>1.0</td>
</tr>
<tr>
<td>Brooks-Gunn et al. (9)</td>
<td>39.6</td>
<td>56.0</td>
<td>18.9</td>
</tr>
<tr>
<td>Burrows et al. (10)</td>
<td>20.0</td>
<td>NR</td>
<td>2.0</td>
</tr>
<tr>
<td>Calabrese et al. (13)</td>
<td>NR</td>
<td>NR</td>
<td>44.0</td>
</tr>
<tr>
<td>Cobb et al. (14)</td>
<td>26.0</td>
<td>NR</td>
<td>10.0</td>
</tr>
<tr>
<td>Cohen et al. (15)</td>
<td>6.7</td>
<td>23.3</td>
<td>36.7</td>
</tr>
<tr>
<td>Dusek (21)</td>
<td>NR</td>
<td>8.3</td>
<td>29.9</td>
</tr>
<tr>
<td>Feicht et al. (24)</td>
<td>NR</td>
<td>NR</td>
<td>6.0-43.0</td>
</tr>
<tr>
<td>Frisch et al. (25)</td>
<td>NR</td>
<td>NR</td>
<td>19.0</td>
</tr>
<tr>
<td>Glass et al. (28)</td>
<td>NR</td>
<td>NR</td>
<td>19.0</td>
</tr>
<tr>
<td>Hoch et al. (30)</td>
<td>18.0</td>
<td>6.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Lutter and Cushman (38)</td>
<td>19.4</td>
<td>NR</td>
<td>3.4</td>
</tr>
<tr>
<td>Martinsen et al. (39)</td>
<td>NR</td>
<td>0.5</td>
<td>9.2</td>
</tr>
<tr>
<td>Meyer et al. (40)</td>
<td>52.5*</td>
<td>NR</td>
<td>52.5*</td>
</tr>
<tr>
<td>Study</td>
<td>Prevalence (%)</td>
<td>Other Precipitating Factors</td>
<td>Note</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>-----------------------------</td>
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</tr>
<tr>
<td>Nichols et al. (43)</td>
<td>17.1</td>
<td>1.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Nichols et al. (42)</td>
<td>18.0</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>O’Connor et al. (44)</td>
<td>NR</td>
<td>NR</td>
<td>60.0</td>
</tr>
<tr>
<td>Pollock et al. (49)</td>
<td>38.0</td>
<td>NR</td>
<td>25.0</td>
</tr>
<tr>
<td>Quah et al. (50)</td>
<td>17.9</td>
<td>3.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Rauh et al. (51)</td>
<td>25.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NR</td>
<td>25.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Raymond-Barker et al. (52)</td>
<td>NR</td>
<td>NR</td>
<td>28.8</td>
</tr>
<tr>
<td>Sanborn et al. (58)</td>
<td>NR</td>
<td>NR</td>
<td>12.1-25.7</td>
</tr>
<tr>
<td>Schtscherbyna et al. (59)</td>
<td>19.2</td>
<td>21.8</td>
<td>NR</td>
</tr>
<tr>
<td>Shangold and Levine (60)</td>
<td>NR</td>
<td>NR</td>
<td>6.0</td>
</tr>
<tr>
<td>Thein-Nissenbaum et al. (65)</td>
<td>18.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NR</td>
<td>18.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vardar et al. (70)</td>
<td>0.9</td>
<td>NR</td>
<td>9.8</td>
</tr>
<tr>
<td>Wakat et al. (73)</td>
<td>46.0</td>
<td>NR</td>
<td>5.0</td>
</tr>
<tr>
<td>Walberg and Johnston (74)</td>
<td>28.6</td>
<td>NR</td>
<td>3.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Prevalence of secondary amenorrhea and oligomenorrhea grouped together  
NR = Data Not Reported
Table 4. Studies on the prevalence of clinical eating disorders (ED) and disordered eating (DE) (subclinical and clinical) in exercising women.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Prevalence of Subclinical DE (%)</th>
<th>Prevalence of Clinical DE (%)</th>
<th>Prevalence of Clinical ED (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beals and Hill (6)</td>
<td>NR</td>
<td>20.0</td>
<td>AN: 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BN: 0.9</td>
</tr>
<tr>
<td>Beals and Manore (7)</td>
<td>32.4</td>
<td>15.2</td>
<td>AN: 3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BN: 2.3</td>
</tr>
<tr>
<td>Beals (5)</td>
<td>26.0-35.0</td>
<td>NR</td>
<td>0.0</td>
</tr>
<tr>
<td>Brooks-Gunn et al. (9)</td>
<td>NR</td>
<td>NR</td>
<td>AN: 17.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BN: 21.0</td>
</tr>
<tr>
<td>Burrows et al. (10)</td>
<td>NR</td>
<td>15.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Byrne and McLean (11)</td>
<td>NR</td>
<td>NR</td>
<td>AN: 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BN: 4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDNOS: 8.0</td>
</tr>
<tr>
<td>Cobb et al. (14)</td>
<td>NR</td>
<td>25.6</td>
<td>NR</td>
</tr>
<tr>
<td>Evers (23)</td>
<td>NR</td>
<td>NR</td>
<td>AN: 33.0</td>
</tr>
<tr>
<td>Gadpaille et al. (26)</td>
<td>NR</td>
<td>NR</td>
<td>AN: 25.0</td>
</tr>
<tr>
<td>Hoch et al. (31)</td>
<td>60.0</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Holderness et al. (32)</td>
<td>NR</td>
<td>16.0</td>
<td>AN: 31.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BN: 12.0-26.0</td>
</tr>
<tr>
<td>Johnson et al. (34)</td>
<td>2.9-9.2</td>
<td>NR</td>
<td>AN: 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BN: 1.1</td>
</tr>
<tr>
<td>Martinsen et al. (39)</td>
<td>NR</td>
<td>44.7</td>
<td>NR</td>
</tr>
<tr>
<td>Nichols et al. (43)</td>
<td>NR</td>
<td>10.0-18.0</td>
<td>NR</td>
</tr>
<tr>
<td>Nichols et al. (42)</td>
<td>NR</td>
<td>20.0</td>
<td>NR</td>
</tr>
<tr>
<td>Petrie and Stoever (46)</td>
<td>NR</td>
<td>NR</td>
<td>4.1</td>
</tr>
<tr>
<td>Quah et al. (50)</td>
<td>NR</td>
<td>89.2</td>
<td>NR</td>
</tr>
<tr>
<td>Rauh et al. (51)</td>
<td>NR</td>
<td>16.0</td>
<td>NR</td>
</tr>
<tr>
<td>Raymond-Barker et al. (52)</td>
<td>NR</td>
<td>10.2</td>
<td>NR</td>
</tr>
<tr>
<td>Reinking and Alexander (53)</td>
<td>NR</td>
<td>7.1</td>
<td>NR</td>
</tr>
<tr>
<td>Rosendahl et al. (55)</td>
<td>26.7</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Rucinski et al. (56)</td>
<td>NR</td>
<td>NR</td>
<td>AN: 48.0</td>
</tr>
<tr>
<td>Study</td>
<td>AN</td>
<td>BN</td>
<td>EDNOS</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>Schtscherbyna et al. (59)</td>
<td>NR</td>
<td>44.9</td>
<td>NR</td>
</tr>
<tr>
<td>Sundgot-Borgen (62)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Sundgot-Borgen (63)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Sundgot-Borgen and Torstveit (64)</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Thein-Nissenbaum et al. (65)</td>
<td>NR</td>
<td>35.4</td>
<td>NR</td>
</tr>
<tr>
<td>Torstveit et al. (67)</td>
<td>NR</td>
<td>46.2</td>
<td>AN: 4.8, BN: 8.1, EDNOS: 19.9</td>
</tr>
<tr>
<td>Vardar et al. (70)</td>
<td>NR</td>
<td>16.8</td>
<td>NR</td>
</tr>
<tr>
<td>Walberg and Johnston (74)</td>
<td>12.3-25.1</td>
<td>NR</td>
<td>AN: 1.0, BN: 3.6</td>
</tr>
<tr>
<td>Weight and Noakes (75)</td>
<td>NR</td>
<td>14.0</td>
<td>NR</td>
</tr>
</tbody>
</table>

AN = Anorexia Nervosa; BN = Bulimia Nervosa; AA = Anorexia Athletica; EDNOS = Eating Disordered Not Otherwise Specified; NR= Data Not Reported
**Prevalence Studies on Low EA:** Hoch et al. (30) demonstrated that athletes (n=80) had a significantly lower prevalence of low EA (≤45 kcal/kg LBM) (p<0.05) in comparison to sedentary students/control subjects (n=80), 36% vs. 39%. Furthermore, in the athletic group with low EA (≤45 kcal/kg LBM), 6% had an EA less than 30 kcal/kg LBM compared to 4% in sedentary students/control subjects.

**Prevalence Studies on Low BMD:** Table 5 presents the studies wherein the investigators evaluated the prevalence of low BMD (defined as both Z-Score between -1.0 and -2.0 and Z-Score ≤ -2.0) in exercising women. The prevalence of low BMD defined as Z-score ≤ -2.0 ranged from 0-15.4% (8 studies; n=755); whereas, the prevalence of low BMD defined as Z-score between -1.0 and -2.0 ranged from 0-39.8% (7 studies; n=677).

**Prevalence Studies on Triad Conditions: Lean vs. Non-Lean Sport Athletes:** The prevalence estimates from the investigations comparing lean vs. non-lean sport athletes for the prevalence of individual and combined Triad conditions are summarized in Table 6. From the investigation on the prevalence of all three Triad conditions in female athletes categorized by sport type (lean vs. non-lean), there were no investigators that reported the prevalence of all three Triad conditions defined as low EA with or without DE, MD, and low BMD. The prevalence of all three Triad conditions defined as DE, MD, and low BMD in lean sport athletes ranged from 1.5-6.7% vs. non-lean sport athletes wherein the prevalence ranged from 0-2.0% (3 studies; n=365).
Table 5. Studies on the prevalence of low bone mineral density (BMD) in exercising women.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Study Population</th>
<th>Prevalence of low BMD (Z-score &lt; -1.0) (%)</th>
<th>Prevalence of low BMD (Z-score ≤ -2.0) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrack et al. (4)</td>
<td>93 female adolescent endurance runners</td>
<td>39.8</td>
<td>11.8</td>
</tr>
<tr>
<td>Beals and Hill (6)</td>
<td>112 female collegiate athletes</td>
<td>9.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Hoch et al. (31)</td>
<td>15 recreational female triathletes</td>
<td>0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0</td>
</tr>
<tr>
<td>Hoch et al. (30)</td>
<td>80 high school female athletes</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>Nichols et al. (43)</td>
<td>170 female high school athletes</td>
<td>21.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Pollock et al. (49)</td>
<td>44 elite female endurance athletes</td>
<td>34.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3</td>
</tr>
<tr>
<td>Rauh et al. (51)</td>
<td>163 female athletes</td>
<td>22.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Schtscherbyna et al. (59)</td>
<td>78 elite female swimmers</td>
<td>NR</td>
<td>15.4</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density; NR = Data Not Reported

<sup>a</sup> = Low BMD was defined as Z-Score between -1.0 and -2.0
Table 6. Studies on the prevalence of individual and combined conditions of the Triad in lean sport vs. non-lean sport athletes.

<table>
<thead>
<tr>
<th>Prevalence of the Triad</th>
<th>Lean Sport Athletes(^a) (%)</th>
<th>Non-Lean Sport Athletes(^a) (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>All three Triad conditions simultaneously (Low EA, MD, and Low BMD)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>All three Triad conditions simultaneously (DE, MD, and Low BMD)</td>
<td>1.5-6.7</td>
<td>0.0-2.0</td>
<td>(68), (50), (6)</td>
</tr>
<tr>
<td>MD and Low BMD</td>
<td>3.3</td>
<td>1.0</td>
<td>(68)</td>
</tr>
<tr>
<td>DE and MD</td>
<td>6.8-57.8</td>
<td>5.4-13.5</td>
<td>(68), (50), (43), (65)</td>
</tr>
<tr>
<td>Low EA and MD</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>DE and Low BMD</td>
<td>5.6</td>
<td>1.0</td>
<td>(68)</td>
</tr>
<tr>
<td>Low EA and Low BMD</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

**MD**

<table>
<thead>
<tr>
<th></th>
<th>Lean Sport Athletes(^a) (%)</th>
<th>Non-Lean Sport Athletes(^a) (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amenorrhea</td>
<td>1.4-27.7</td>
<td>0.0-12.8</td>
<td>(6,49,50,64)</td>
</tr>
<tr>
<td>Primary Amenorrhea</td>
<td>0.7-9.5</td>
<td>0.0-0.4</td>
<td>(50), (42)</td>
</tr>
<tr>
<td>Oligomenorrhea</td>
<td>22.3-24.7</td>
<td>14.3-16.5</td>
<td>(50), (42)</td>
</tr>
<tr>
<td>Anovulation/Luteal Phase Defects</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

**Eating Disorders/DE**

<table>
<thead>
<tr>
<th></th>
<th>Lean Sport Athletes(^a) (%)</th>
<th>Non-Lean Sport Athletes(^a) (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Eating Disorder</td>
<td>1.5-28.1</td>
<td>0.0-15.1</td>
<td>(6,7,11,62,63,66)</td>
</tr>
<tr>
<td>Clinical DE</td>
<td>6.0-89.2</td>
<td>2.9-89.2</td>
<td>(6,49,50,52,54,62,64,66,68)</td>
</tr>
<tr>
<td>Subclinical DE</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Low EA</strong></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

**Low BMD**

<table>
<thead>
<tr>
<th></th>
<th>Lean Sport Athletes(^a) (%)</th>
<th>Non-Lean Sport Athletes(^a) (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-score≤2.0</td>
<td>3.1</td>
<td>0.0</td>
<td>(6)</td>
</tr>
<tr>
<td>Z-score&lt;1.0</td>
<td>15.4</td>
<td>0.0</td>
<td>(6)</td>
</tr>
</tbody>
</table>

\(^a\) Lean sport athletes = participants involved in sports emphasizing endurance training, low body weight, lean physique, and aestheticism; Non-lean sport athletes = participants in sports emphasizing technical and/or ball-related skill and/or power training (68).
Similar findings were observed when evaluating the studies wherein the investigators assessed the prevalence of two Triad conditions with the lean sport athletes demonstrating higher prevalence of MD and low BMD (3.3% vs. 1.0%) (1 study; n=186), MD and DE (6.8-57.8% vs. 5.4-13.5%) (4 studies; n=987), and low BMD and DE (5.6% vs. 1.0%) (1 study; n=186) than the non-lean sport athletes. No studies measured any combination of subclinical Triad components.

In terms of the individual Triad disorders, lean sport athletes demonstrated a higher prevalence compared to non-lean sport athletes for MD (0.7-27.7% vs. 0-16.5%) (5 studies; n=1,032) and low BMD (3.1-15.4% vs. 0%) (1 study; n=112). However, prevalence of DE was similar between groups, 1.5-89.2% in lean sport athletes and 0.0-89.2% in non-lean sport athletes (12 studies; n=2,186). Notably, the prevalence of EA has yet to be determined in female athletes categorized by sport type (lean vs. non-lean).

**DISCUSSION**

To date, our review is the first to evaluate the prevalence of Triad conditions (clinical or subclinical) occurring individually and in combination in high school and premenopausal exercising women. We also address the notable discrepancy in the prevalence of the Triad conditions occurring simultaneously or in combination vs. individual presentations (MD, low EA, eating disorders/DE or low BMD). Our review demonstrates that despite a large body of evidence determining the prevalence of the individual Triad disorders in exercising and athletic women, the prevalence of Triad conditions presenting simultaneously or in varying combinations is not well
documented. The lack of good documentation is attributable to the methodological difficulties in measuring the Triad in a research setting to include the lack of established and consistent definitions and criteria utilized for each Triad component, selection bias in limiting studies to female athletes with the exclusion of recreational exercising women, and limitations in experimental design and methods of assessment. Ongoing research into the prevalence of EA is advancing our understanding of the etiology of MD and bone loss in exercising and athletic women. However, the calculation of EA is limited by the error associated with some methods of assessing its components, to include, energy intake, and non-objective assessments of exercise energy expenditure (EEE), and fat-free mass (FFM). Methods with greater precision for assessing FFM, one of the determinants of EA, i.e. DXA scans, are costly and difficult to access, and therefore, this challenge may prevent the proper measurement of EA in a research or field setting.

Prevalence estimates of all three Triad conditions presenting simultaneously are generally low in female athletes, ranging from 0-16%. A greater percentage of these female athletes present with any two of the Triad conditions, ranging from 3-27%, and an even greater proportion present with any one of the Triad conditions ranging from 16-60%. There are many reasons for this apparent discrepancy observed in the literature, such as inconsistencies and limitations in study methodology, criteria defining the Triad, and experimental design. In general, a substantially greater number of female athletes present with MD, low EA, eating disorders/DE, or low BMD alone compared to those female athletes presenting with multiple disorders of the Triad. In
particular, the prevalence of low BMD is lower in comparison to estimates of MD or clinical eating disorders/DE, which may impact the prevalence of combinations of the Triad (6,30,43,68). However, low EA may also have a detrimental impact on bone (33) and without an appropriate nutritional intervention; bone loss may continue to occur despite pharmacological treatment and the resumption of normal menses. To date, the prevalence of low EA is not well-established and further investigation of the prevalence of this Triad component may offer insight regarding the discrepancy in prevalence estimates for the Triad conditions presenting in combination vs. individually. Lastly, prevalence study of subclinical Triad disorders are notably absent from the literature. Further evaluation of the prevalence of all Triad conditions prior to the presentation of clinical severity is warranted.

Prevalence Studies of the Triad Conditions Presenting Simultaneously

One of the earliest studies measuring the prevalence of the simultaneous occurrence of the Triad conditions was completed in female soldiers (35). Lauder et al. (35) demonstrated that none of the 423 active-duty military personnel in their 12-month prospective cross-sectional study met the criteria for all Triad conditions presenting simultaneously despite a higher prevalence of participants (26%) being identified as at risk for the Triad. Of those women identified as at risk for the Triad, only 3.3% presented with low BMD according to the WHO criteria (T-score value between -1.0 and -2.5 SDs below average) at the lumbar spine, whereas none presented with osteoporosis (T-score less than or equal to -2.5 SDs). Although the study by Lauder et
al. (35) was conducted in military women, this work initiated interest in determining the prevalence of Triad conditions presenting simultaneously in female athletes and it was clear that the clinical relevance of the Triad needed to be further investigated. Numerous cross-sectional studies of the simultaneous presentation of all Triad conditions in collegiate/elite female athletes were subsequently completed (6,10,30,31,43,49,50,59,68,70). Consistent with the findings of Lauder et al. (35), the prevalence of all Triad conditions presenting simultaneously was low in the majority of these studies, except for a study completed in female cross-country runners (49). Notably, none of these studies objectively measured menstrual status, determined the prevalence of low EA, or evaluated the presence of subclinical MD.

**Prevalence Studies of the Triad Conditions Presenting In Varying Combinations**

Several investigators have determined the prevalence of any of the combinations of the Triad conditions and these reports support the notion of interrelatedness among clinical manifestations of this syndrome. Cobb et al. (14) were the first to examine the interrelationships among MD, low BMD, and DE in female athletes. These investigators demonstrated several key findings with relevant implications for Triad research, such as exhibiting associations between both DE and oligo/amenorrhea and low BMD and oligo/amenorrhea. They also observed a link between DE and low BMD, specifically in eumenorrheic athletes (14). However, Cobb et al. (14) did not report whether all Triad conditions presented simultaneously in any of the athletes they studied.
Torstveit and Sundgot-Borgen (68,69) were the first to assess the simultaneous existence of the Triad components in a population of female athletes and compare their findings to a control group. Their findings demonstrated that a significant percent of female athletes presented with varying combinations of Triad conditions and additionally, they exhibited that recreationally-active women (controls) also presented with Triad conditions. The relevance of these findings extends beyond elite female athletes to recreationally-active women with 12-15% of controls presenting with varying combinations of Triad conditions. Further analyses of these control participants suggested that it was their use of pathological weight control methods in attempt to lose weight that may have contributed to prolonged periods of low EA. However, it is important to highlight that Torstveit and Sundgot-Borgen (68) did not directly measure the physiological processes underlying the Triad and presented associations among risk factors rather than causal relationships among Triad conditions (36). Hoch et al. (30) demonstrated in their sedentary/control group that 5-39% of controls presented with any two of Triad conditions. Interestingly, 20% and 10% of these participants met the criteria for low BMD (Z-score between -1.0 and -2.0 and Z-score ≤-2.0, respectively). It is clear that a lack of weight-bearing exercise combined with low EA increases the risk of developing Triad disorders. Unlike Torstveit and Sundgot-Borgen (68), Hoch et al. (30) measured EA in their participants, however, the control participants may have under-reported energy intake and/or over-reported EEE to result in such a high prevalence of low EA. To this end, the clinical representation of the incidence of the Triad in female athletes in comparison to controls is still unclear and furthermore,
additional investigation into the prevalence of the Triad conditions in recreationally-active women is required.

Prevalence of the Subclinical Triad Conditions Presenting Simultaneously or In Varying Combination

To date, the prevalence of subclinical Triad conditions presenting simultaneously and in varying combinations have only been determined by a few investigators, predominantly assessing subclinical DE or MD. One particular concern involves the use of self-report menstrual history to categorize menstrual status in Triad prevalence studies. The method of self-report only indicates MD that is readily apparent to women by the absence or irregular intervals of menses. De Souza et al. (18,19), using daily urinary reproductive hormonal analyses, have shown that approximately half of exercising women present with subclinical MD (LPD and anovulation) despite experiencing cycles of regular inter-menstrual intervals and seemingly “normal” menstrual cycles. These findings confirm that investigators may underestimate the incidence of MD in exercising women using self-report. This is especially concerning considering the wide range of estimates of MD (0-60%) in published reports. Therefore, in order to objectively measure menstrual status as a component of the Triad and report the prevalence, daily urinary reproductive hormone analyses should ideally be utilized in order to determine accurate estimations of both subclinical and clinical MD. However, it is important to consider the feasibility of self-report methods as field measures or screening tools that may indicate the need for future, more comprehensive
evaluation of menstrual status using daily urinary measures of reproductive hormones in certain exercising and athletic women.

Three studies have included an evaluation of the prevalence of MD in exercising women with subclinical DE behavior, such as a high drive for thinness (27,44) and high dietary cognitive restraint (71). These reports demonstrated that clinical MD (FHA and oligomenorrhea) are significantly more prevalent (17-23%) in women with high drive for thinness or dietary cognitive restraint compared to those women without such subclinical DE behaviors. However, the prevalence of subclinical DE has not been investigated alongside presentations of low EA and/or low BMD.

Prevalence Studies of the Triad Conditions in High School Female Athletes

The Triad is not restricted to premenopausal women and the magnitude of this syndrome in adolescent and high school female athletes is not well documented. Nichols et al. (43) were the first to report prevalence estimates of MD and low bone mass in adolescents. It is important to mention that menstrual irregularity is common after menarche and cycles may regulate within approximately two years (2). Upon establishing that true MD exists, it is critical to evaluate bone mass using DXA to have a baseline measurement for comparative purposes and as such, the lack of exposure to estrogen may result in a failure to achieve peak bone mass. Since adolescence is such a critical time for bone mineral accrual, the negative impact of MD on bone mass during this phase of the lifespan may be severely detrimental and may impact the attainment of peak bone mass (43). Prevalence studies in younger populations of exercising women
are necessary to bring awareness to the number of young girls at risk for the Triad. Additionally, preventive strategies are necessary in youth to avoid development of potential long-term health consequences of the Triad, such as FHA, low BMD/osteoporosis, and stress fractures.

**Prevalence Studies of the Triad Conditions Presenting Simultaneously and In Varying Combinations: Lean vs. Non-Lean Athletes**

It is well-documented that Triad-related conditions predominate in sports emphasizing leanness (7,11,42,53,55,62,67-69). These types of athletes are suggested to be at a greater risk of demonstrating low EA and/or DE based on the high prevalence of energy restriction and/or dieting behavior reported in these athletes. Such behavior may lead to MD and subsequently, pathological bone loss.

Torstveit and Sundgot-Borgen (69) found that a higher percent of athletes competing in lean sports (70%) were categorized as “at risk” of the Triad vs. athletes competing in non-lean sports (55%). Beals and Hill (6) examined the prevalence of all three, any two or one of the Triad conditions (defined as DE, MD, and low BMD using a Z-score below -2.0) between lean sport and non-lean sport athletes. The only athlete that met the criteria for all three Triad disorders (using the criterion of a Z-score below -2.0 for low BMD) was notably in the lean sport group (a cross-country runner). The identification of two more athletes (both lean sport participants) with all three Triad conditions resulted when using a less conservative definition of low BMD (Z-Score below -1.0). However, there were no significant differences in the prevalence of any
two of the Triad conditions between lean sport and non-lean sport athletes in this study. Torstveit and Sundgot-Borgen (68) also demonstrated that the prevalence of all three Triad conditions presenting simultaneously was more common in lean sport athletes. These findings differed from Beals and Hill (6) such that Torstveit and Sundgot-Borgen (68) observed more athletes participating in lean sports with any two of the Triad conditions. Notably, 77% of those lean sport athletes presented with any two of the Triad conditions vs. 39% of non-lean sport athletes. Accordingly, these investigators reported that six of the eight athletes diagnosed with all three of the Triad conditions were lean sport athletes. More specifically, 58% of lean sport athletes met the criteria for eating disorders/DE and MD vs. 29% of their non-lean sport counterparts, supporting the notion that a higher frequency of DE occurs in athletes competing in sports that emphasize leanness and a low body weight (62). The majority of investigators report an increased incidence of DE behavior among athletes competing in lean sports (11,62,67), although there are investigators that failed to find differences between lean sport and non-lean sport groups (39,55). It is notable that there are potential limitations in comparing the prevalence of DE behavior between lean and non-lean sport athletes, to include under-reporting of DE symptoms in elite athletes, selection bias, small subsamples of athletes in certain sport types, and the lack of sport-specific instruments designed for athletes in different kinds of sports and competition levels (39,55,67).
Limitations of Prevalence Studies of the Triad Conditions Presenting Simultaneously or In Varying Combinations

This review suggests that there are notable methodological limitations associated with the study of the Triad. As such, further investigation into the prevalence of the simultaneous presentation of the Triad disorders needs to be completed. In order to obtain more reliable estimates of the prevalence of the Triad, consistent definitions and criteria must be used and employment of optimal experimental design and methods of assessment for each Triad condition is necessary.

One of the major limitations in Triad research is the failure to evaluate both subclinical and clinical outcomes of the Triad (41). FHA, osteoporosis, and clinical DE are the most studied conditions of the Triad based on their severity and notable impact on the health and quality of life of exercising and athletic women. However, it must be stressed that the “less” severe conditions related to the Triad are associated with similar negative outcomes and may result in long-term clinical consequences if appropriate treatment does not take place. Thus, accurate prevalence estimates of these subclinical conditions are required to fully appreciate the impact of the Triad from a clinical standpoint (20,41). Future research is required to identify the point at which each of the subclinical Triad conditions can reliably predict risk of future negative health consequences in order to effectively prevent the development of clinical Triad conditions (20).

Additionally, it is clear that the study of exercising and athletic women is difficult, particularly due to personal and sensitive nature of the research relating to
eating behavior and menstrual history. It is difficult to infer any conclusions from data with respect to MD and DE behavior in this population using self-report methods alone. Self-report can only identify those women with clearly recognizable MD and unless objective measures are used, subclinical disturbances would not be captured and prevalence of the Triad would be underestimated. Self-report also relies on the honest and accurate recall of data. Data using hormone analyses would improve the accuracy of measuring the prevalence of the Triad conditions presenting simultaneously and in varying combinations. Under-reporting of DE behavior and response bias on questionnaires is common and may distort prevalence estimates for eating disorders and DE in this population. As such, a clinical interview may represent an optimal approach.

To date, only one study (30) has measured EA as a component of the Triad in a prevalence study. EA is notably difficult to measure and the calculation of EA is limited by the error associated in determining its components, to include, energy intake, EEE, and FFM. EA is often measured using 3- or 7-day diet records to calculate energy intake and a physical activity compendium or heart rate monitors to calculate EEE. There are limitations to this approach and under- or over-reporting of energy intake alone or in combination with poor compliance in recording EEE constitute challenges to determining the prevalence of low EA. Methods with greater precision for assessing FFM, a necessary component of EA, i.e. DXA scans, are costly and difficult to access, and therefore, this challenge may prevent the measurement of EA in a research or field setting. Moreover, there is debate whether the concept of EA is more useful than the concept of energy balance for managing the diets of athletes (37), and future research
should determine whether EA or energy balance is more superior as an index of energy status for athletes in a field setting and for research/clinical purposes. Nonetheless, prevalence study on EA alone and in combination with other Triad conditions would improve our understanding of the etiology and interrelationships of the Triad components (41).

**CONCLUSIONS**

This review provides a comprehensive summary of the relevant publications that have determined the prevalence of the Triad conditions (subclinical and clinical), occurring simultaneously, in combination, and individually. It is clear that by establishing accurate prevalence estimates of the Triad, we could further our understanding of the physiological mechanisms and clinical relevance of this interrelated syndrome. Our review demonstrates that additional investigation using objective measures, and self-report/field measures as necessary, is required to determine the prevalence of the Triad. Since objective measures may not always be feasible, self-report/field measures may be necessary in evaluating some components of the Triad to include: menstrual status using self-report menstrual history (if daily urinary analyses of reproductive hormones are not accessible); energy intake using 3- or 7-day diet logs; EEE using physical activity compendium (if heart rate monitors or another validated, objective technique is not obtainable); body composition using field techniques or surrogate measures (i.e., body weight) (if DXA is not available to measure FFM); and eating behavior using validated questionnaires and self-report (if a clinical interview is
not feasible). It is notable that to measure bone density, DXA or other appropriate imaging assessment must be utilized. Regardless, further prevalence research on the Triad conditions alone or in combination in exercising and athletic women would enable more accurate estimation of the magnitude of this problem. In addition, prevalence estimates are important for developing effective preventive measures, screening criteria, reliable field assessment tools, and treatment strategies for the Triad conditions.

Lastly, it must be underscored that in order for any improvement in Triad preventive and treatment policies to ensue, the prevalence of this syndrome, both subclinical and clinical outcomes, must be accurately measured in a research setting. To date, the clinical representation of the incidence of the Triad in female athletes in comparison to controls is still unclear and furthermore, additional investigation into the prevalence of the Triad conditions in recreationally-active women is required. Moreover, there has yet to be comprehensive comparison between: (1) those exercising women presenting with all three conditions of the Triad compared to those with one or two conditions, and (2) those women with subclinical presentations vs. those with clinical presentations of the Triad. These comparisons would be insightful for this area of research and also, useful for identifying potential risk factors for the most severe presentation of Triad-related clinical sequelae. Also, participants must be evaluated longitudinally to better describe the development or recovery of all Trial clinical and subclinical conditions.
References


Chapter 2

REVIEW OF THE LITERATURE- PART TWO


ABSTRACT

Exercise-associated menstrual disturbances (EAMD) are frequently observed in women participating in recreational and competitive-level training and sport. The etiology of EAMD is linked to inadequate energy intake relative to high energy expenditure, also referred to as an energy deficiency. Chronic energy deficiency promotes compensatory alterations in resting energy expenditure (REE) and metabolic hormones (i.e., total triiodothyronine (TT₃), ghrelin, peptide YY (PYY), leptin) to conserve fuel for vital physiological processes. The cumulative effect of these energetic/metabolic disturbances translate to suppression of the normal release of reproductive hormones, i.e. gonadotropin-releasing hormone, luteinizing hormone, and follicular stimulating hormone, thereby altering the production of estrogen and progesterone to result in subclinical (luteal phase defects and anovulation) or clinical EAMD (functional hypothalamic amenorrhea (FHA) and oligomenorrhea). REE and TT₃ often present in a dose-response manner across the continuum of EAMD such that increases in energy
conservation (reductions in REE and TT₃) occur in association with increases in severity of EAMD. Ghrelin and PYY are gastrointestinal peptides that influence central mechanisms regulating energy homeostasis and reproductive function. An anorexigenic profile of elevated ghrelin and PYY is commonly observed in exercising women with FHA. Mechanistically, this profile may prevent compensatory increases in energy intake secondary to psychological markers of subclinical disordered eating and increased exercise energy expenditure in exercising women with FHA. Leptin, an adipocyte-secreted hormone, has been proposed to play a role in the hormonal regulation of both reproductive function and energy homeostasis in humans. Leptin has also been implicated in the maintenance or restoration of normal menstrual cyclicity in exercising women with FHA. Dietary strategies to restore menstrual function (i.e., by increasing energy intake) appear to be optimal approaches such that the focus of the treatment is on improvement of the full metabolic hormone profile concomitant with recovery of menstrual function.

INTRODUCTION

Menstrual disturbances are frequently observed in exercising women participating in recreational and competitive-level training and sport (31). The etiology of these menstrual disturbances is linked to inadequate energy intake relative to high energy expenditure, also referred to as an energy deficiency (81,134). Exercising women may induce an energy deficit for several reasons: 1) intentional, i.e., to improve performance by modifying body size and composition; 2) compulsive, i.e., as a result of
disordered eating behavior or pathological weight control; or 3) inadvertent, i.e., failing to match energy intake to exercise-induced energy expenditure (77). Exercising women often adopt restrictive eating behavior to maintain or reduce their body weight as a means to achieve optimal performance or physical attractiveness (118). Consequently, chronic energy deficiency may develop which is characterized by hallmark changes in resting energy expenditure (REE) and an endocrine panel of metabolic hormones (i.e., total triiodothyronine (TT₃), ghrelin, peptide YY (PYY), leptin) that if sustained for a prolonged period, the cumulative effect of these energetic/metabolic disturbances will translate to EAMD (33).

Chronic energy deficiency promotes compensatory mechanisms to conserve metabolic fuel for vital physiological processes (128). As already mentioned, these energy conservation mechanisms are associated with changes in energetic and metabolic signals to restore energy homeostasis (33) and to suppress growth-related functions, the least critical physiological process (128). In the presence of a sustained energy deficiency, the next physiological process considered least vital for survival is reproduction. Consequently, reproductive function is suppressed (128) and typically, reproductive dysfunction results in subclinical (luteal phase defects (LPD), anovulation) or clinical EAMD (functional hypothalamic amenorrhea (FHA), oligomenorrhea) (31) (Figure 1). In turn, both the energy deficient and estrogen deficient environment contributes to loss of bone mass or the failure to achieve peak bone mineral density (BMD) (32). This constellation of sequelae (chronic energy deficiency, menstrual disturbances, and low BMD) is a syndrome known as the Female Athlete Triad (97).
Figure 1. Association between chronic energy deficiency and exercise-associated menstrual disturbances. REE = resting energy expenditure; TT$_3$ = total triiodothyronine; PYY = peptide YY; FHA = functional hypothalamic amenorrhea; LPD = luteal phase defects.
The Female Athlete Triad was first defined in 1997 by the American College of Sports Medicine (ACSM) as a condition consisting of three components: disordered eating, FHA, and osteoporosis (96,101,137). These clinical consequences are most commonly observed in exercising women participating in sports that emphasize a lean physique or low body weight, such as distance running and gymnastics (117). As more research was completed in this area (20,66,123,124), our understanding of the etiology, pathophysiology, and clinical consequences of this syndrome was advanced. In 2007, a revision to the 1997 ACSM position stand (97) was published to present updated scientific information on this syndrome and to offer new recommendations for screening, diagnosis, prevention, and treatment.

The most recent conceptual model of the Female Athlete Triad presents energy availability (EA) (with or without disordered eating), menstrual function, and BMD across a continuum of healthy (optimal EA, normal menstrual cycles, and optimal BMD) to increasingly severe pathological presentations (low EA, FHA, and low BMD) (97) (Figure 2). Exercising women may exist anywhere along the continuum for any one component and can move in either direction (from health → disease and vice versa) at different rates depending on factors such as energy intake, energy expenditure, eating behavior, and type and amount of mechanical loading (97). The Female Athlete Triad is associated with significant health risks, such as FHA, eating disorders, infertility, premature osteoporosis, and stress fractures (97).
Figure 2. Model of the Female Athlete Triad characterizing an interrelated syndrome of low energy availability (EA) with or without disordered eating (DE), low bone mineral density (BMD), and clinical menstrual disturbances (functional hypothalamic amenorrhea (FHA) and oligomenorrhea) across a continuum of healthy/optimal conditions to subclinical and clinical disorders. LPD = Luteal phase defects; PBM = Peak bone mass. Modified from De Souza and Williams, Human Reproduction Update 2004; 10(5), 433-448 with permission.
Inadequate exposure to reproductive hormones, particularly estrogen (32,140), and metabolic hormones, such as insulin-like growth-factor-1 (IGF-1), TT₃, and leptin (68,132), impairs bone mineral accrual during adolescence (6,7,131) and promotes bone loss during adulthood (55). Alternatively, optimal energy status promotes both a normal ovulatory menstrual cycle and optimal BMD (32). This chapter will focus primarily on the metabolic hormonal and reproductive changes that occur in exercising women to result in clinical consequences such as chronic energy deficiency and EAMD.

**EXERCISE-ASSOCIATED MENSTRUAL DISTURBANCES: SUBCLINICAL AND CLINICAL DISTURBANCES**

The menstrual cycle is a repetitive process involving the interaction of the hypothalamic-pituitary-ovarian (HPO) axis with cyclic structural and hormonal changes in support of reproduction and fertilization (138). Each cycle is defined by the interval between the first day of menstrual bleeding in consecutive cycles. The length of the menstrual cycle varies and a regular cycle length in eumenorrheic women is generally 26-35 days (33). As mentioned earlier, menstrual disturbances occur across a continuum of severity from subclinical to clinical perturbations (33) wherein estrogen and progesterone exposure decreases with increasing severity (See Chapter 11 for more in-depth discussion of the changes in reproductive hormones and potential associated with physical activity and exercise).

Subclinical menstrual disturbances, which include LPD and anovulation, are the least severe along the spectrum of menstrual disturbances and represent perturbations
that occur in the presence of apparently regular cycle length (25). LPD are characterized by a short luteal phase (<10 days in length), an inadequate luteal phase (suppressed progesterone concentrations) or both (30). Anovulatory cycles, which represent the more severe form of subclinical menstrual disturbances, are typically characterized by the lack of a luteinizing hormone (LH) surge, absence of an ovulatory event, and subsequently suppressed progesterone concentrations (31). Thus, subclinical menstrual disturbances occur in cycles of regular length and are not readily apparent without hormonal assessment of an entire cycle (30,31,33).

Clinical menstrual disturbances, which include oligomenorrhea and FHA, are easily detected by a change in cycle length and represent more severe perturbations of menstrual function (31). Oligomenorrhea is characterized by long and inconsistent intermenstrual intervals of 36-90 days in length (31). This severe menstrual disturbance is perhaps the least understood and most difficult perturbation to interpret due to its inconsistent hormonal characteristics. Oligomenorrhea presents with or without ovulation and analysis of ovarian steroid profiles in oligomenorrheic women has revealed erratic yet overall suppressed estrogen production (4,31,33). The etiology of oligomenorrhea may or may not be hypothalamic in nature and moreover, oligomenorrheic cycles are often associated with hyperandrogenism (4) and polycystic ovarian syndrome (PCOS), causally linked to infertility in women (5,38). Recently, investigators have recommended performing rigorous screening of oligomenorrheic exercising women to rule out the presence of PCOS (4,116,129). FHA, the most severe form of EAMD and the disturbance associated with the most severe health
consequences, is typically defined as the absence of menses for at least 90 days (31).
FHA originates at the hypothalamus (specifically the arcuate nucleus) and is the outcome of reduced LH pulsatility, chronically suppressed ovarian steroid hormones (estrone-1-glucuronide (E1G) and pregnandiol glucuronide (PdG)) and unaltered pituitary responsiveness to gonadotropin-releasing hormone (GnRH) (33,138). FHA may be classified as either primary or secondary. Primary amenorrhea is defined as the failure to menstruate by 15 yr in girls with secondary sex characteristics (2); whereas secondary amenorrhea is the abnormal cessation of menses after menarche (2). The criteria defining FHA has varied in the literature (34,76), however, a conservative definition specifies no menses for at least 3 months (31,33).

**THE ETIOLOGY OF EXERCISE-ASSOCIATED MENSTRUAL DISTURBANCES IN EXERCISING WOMEN**

The etiology of EAMD in exercising women is linked to an energy deficiency where dietary energy intake is inadequate relative to energy expenditure (33). Chronic energy deficiency has been postulated as one of the key factors inducing alterations in metabolic and reproductive function (74,75,80,81,133,134). In a wide variety of mammalian species, reproductive function is dependent on cellular availability of oxidizable metabolic fuel (128) and in general, any experimental model of energy deficiency (i.e., famine, eating disorders, excessive exercise, and cold exposure) can present with aberrations in reproductive status (80,128). Alternatively, reproduction can be resumed when an optimal energy status is restored (128,134). Definitive work by
Wade et al. (128) in *syrian* hamsters demonstrated experimental models in support of the EA hypothesis wherein dietary energy is oxidized to metabolic fuel that is partitioned to five major physiological processes (thermoregulation, locomotion, cellular maintenance, reproduction, and growth). In circumstances of insufficient energy intake, less critical physiological functions, such as growth and reproduction (128), may be compromised to maintain energetic partitioning to vital functions, such as thermoregulation and cellular maintenance. Furthermore, classic work by Warren (130) illustrates that an “energy drain” is incurred in those women who experience an imbalance in energy input vs. output and as such, menarche may be delayed in adolescent girls and reproductive dysfunction may occur in premenopausal women.

Chronic energy deficiency translates to metabolic and reproductive changes via a cascade of energy conservation mechanisms (27,33,80). Once initiated, these compensatory responses may acutely affect energy expenditure, i.e., suppression of REE (27,93), and metabolic hormone concentrations, including reduced TT₃ (27,74,93), IGF-1/IGF binding protein-1 (67,68), leptin (69), and insulin concentrations (67,68), and elevated cortisol (29,68,79), growth hormone (67,68), PYY (110), and ghrelin (28). In turn, these metabolic indicators of energetic and nutritional status target the HPO axis during an energy deficiency, suppressing the normal release of reproductive hormones, i.e. GnRH, LH, and FSH, thereby altering the production of ovarian steroids, i.e. E₁G and PdG (33) (*Figure 3*). As such, an energy deficiency leads to menstrual disturbances characterized by altered release and production of several hormones from the level of the hypothalamus to the ovaries (61,138).
Figure 3. Changes in metabolism and reproductive hormones in exercising women across the continuum of energy and menstrual status. Modified from De Souza and Williams, Human Reproduction Update 2004; 10(5), 433-448 with permission.
Several hypotheses have been proposed to explain the mechanism underlying the induction of menstrual disturbances in exercising women. Past notion wherein “exercise stress” was deemed the primary factor in the induction of reproductive dysfunction has been refuted (81). A classic theory based on a study by Frisch and McArthur (39) suggested that reproductive function was compromised below a body fat percentage of 22% and that menarche occurs once body fat percentage is above 17% in young girls. The lack of causal evidence in support of this theory infers that these findings are merely associative and on the contrary, menstrual abnormalities have been shown to occur at various percentages of body fat above and below these proposed thresholds (73). In spite of these early hypotheses (39), the accumulating evidence in support of the role of energy status in reproductive function has suggested that those exercising women who are in an energy deficit (also referred to as “low EA” or “energy drain”) are more likely to experience metabolic alterations and menstrual disturbances (74,75,80,81,130).

Causal links between the energetic cost of exercise training and menstrual dysfunction have been provided in both human and animal studies wherein energy intake and expenditure have been manipulated. For example, in a prospective exercise training study by Bullen et al. (14), an abrupt increase in exercise training was imposed in 28 untrained, eumenorrheic women for two menstrual cycles wherein the women were randomly assigned to either a weight-loss or weight-maintenance group. Throughout the study, only four of the 28 women (three in the weight-maintenance group, one in the weight-loss group) maintained regular menses during training.
Specifically, strenuous exercise combined with weight loss resulted in a higher incidence of an absent LH surge (81% vs. 42%) and delayed menarche (75% vs. 8%) in previously untrained women compared to similar exercise in women in the weight maintenance group. Alternatively, the participants in the weight-maintenance group did not demonstrate similar changes in hormonal dysfunction over the course of the study. Notably, all participants regained normal menstrual function upon completion of the study. Taken together, Bullen et al. (14) demonstrated that women who completed strenuous exercise training and dieting for two menstrual cycles were more likely to present with menstrual disturbances than women who only exercised. In a follow-up study by Beitins et al. (11), investigators corroborated menstrual abnormalities with hormonal assessments of LH, FSH, estriol, and free progesterone (P₄) in these same 28 participants. In this sample, 18 of the 28 women demonstrated an inadequate or short luteal phase (20 out of 53 monitored cycles) between the two months of exercise training. To this end, these researchers provided evidence of an altered neuroendocrine regulation of key reproductive hormones associated with exercise training in previously ovulating, untrained women, specifically a delay in the LH peak and suppressed LH and P₄ in the luteal phase indicative of corpus luteum dysfunction [52].

Using an animal model, Williams et al. (134) investigated the induction and reversal of EAMD in female cynomolgus monkeys. The study protocol involved an increase in exercise volume (12.3±0.9 km/d of running) without changes in energy intake in 8 female monkeys. All 8 monkeys developed amenorrhea (defined as absence of menses for at least 100 days, with consistently suppressed LH, FSH, estradiol (E₂),
and P₄) within a 7-24 month period. Despite inter-individual variability, abrupt decreases in LH and FSH, average and peak E₂, and average and peak P₄ occurred within one or two cycles prior to the induction of amenorrhea. Moreover, the induction of amenorrhea was preceded by a decrease in TT₃. In a subsequent study, 4 of the monkeys were then fed supplemental calories (138-181% of energy intake during amenorrhea) without a reduction in exercise training volume. These monkeys resumed normal menstrual cycles in response to increased energy intake and accordingly, the recovery of menses correlated with volume of energy intake during refeeding. In other words, the monkeys that ate the most during refeeding regained menses the fastest.

To summarize, from data in human and animal models, changes in reproductive function secondary to energy deficiency in exercising women are proposed to reflect an energy deficient environment and present with a suppression of gonadotropin (LH, FSH) release followed by an increase in follicular phase length and a corresponding reduction in luteal phase P₄, which likely precedes the development of amenorrhea (11,133,134).

METABOLIC CONSIDERATIONS IN AMENORRHEIC EXERCISING WOMEN

Resting Energy Expenditure (REE)

REE is a major factor influencing total daily energy expenditure (103) in premenopausal women, and comprises 60-75% of an individual’s energy losses. REE is defined as the energy necessary to maintain physiological function and homeostasis
When energy intake fails to compensate for energy expenditure, mechanisms of energy conservation are initiated, including a reduction in REE, and concomitant adaptations in fasting circulating hormone concentrations, including reduced TT3, to restore homeostasis (26,27,99). As such, REE represents an indication of energy status and previous evidence in exercising women with FHA demonstrates an association between suppressed REE and chronic energy deficiency (27,32,110). Suppressed REE is often observed concomitant with alterations in metabolic hormonal profiles indicative of energy restriction (27,93). De Souza et al. (27) observed decreases in REE controlled for fat-free mass (FFM) alongside changes in key metabolic hormones (TT3, ghrelin, and leptin) reflective of an energy deficiency in exercising women with FHA vs. their ovulatory counterparts (sedentary and exercising). Furthermore, exercising women with subclinical menstrual disturbances (consistently anovulatory or inconsistent presentations of ovulatory, LPD, or anovulatory cycles) also demonstrated lower REE controlled for FFM compared to sedentary, ovulatory women (27).

In our laboratory, we have also calculated a ratio of measured REE compared to predicted REE (REE/pREE) to provide an estimate of energy deficiency in amenorrheic exercising women (26,27,32,41,110). A reduced ratio of REE/Harris-Benedict pREE (range of 0.60-0.80) has been observed in women with anorexia nervosa during periods of low body weight and prior to refeeding (63,84,104). We operationally defined an energy deficiency as an REE/Harris-Benedict pREE of <0.90 since we had previously determined that this cut-off best discriminated energy status (energy deficiency vs. energy replete) and menstrual status (amenorrheic vs. ovulatory) in exercising women.
Exercising women with FHA typically demonstrate REE/pREE between 0.80-0.88 (32,110) reflective of less severe under nutrition compared to women with anorexia nervosa who demonstrate REE/pREE between 0.60-0.80 (63,84,104).

**Total Triiodothyronine (TT₃)**

TT₃ is the most active form of thyroid hormone and is produced mostly in the peripheral tissues from thyroxine (T₄) (112). TT₃ is involved in the regulation of several physiological processes such as growth and development, metabolism, body temperature, and heart rate (112). TT₃ is tightly coupled with REE, oxygen consumption, and total energy expenditure (23). As such, there is evidence in animal and human experiments that supports the influence of energy and macronutrient intake directly on thyroid hormone status and indirectly on REE (15,107,135).

TT₃ is frequently used as an indicator of energy deficiency because reductions in TT₃ concentrations are suggested to initiate energy conservation mechanisms to restore homeostasis in underweight individuals (15,135) and in sedentary, regularly menstruating women exposed to low EA treatment (74,75). Onur et al. (100) demonstrated low plasma TT₃ concentrations in women with anorexia nervosa in conjunction with decreased REE. In addition, anorexic women who gained weight exhibited increases in TT₃ concentrations concomitant with increases in REE controlled for FFM (100).

Evidence of the effect of manipulations of EA on TT₃ has been demonstrated in a series of eloquent experiments in sedentary, regularly menstruating women (74,75). In
these studies, reductions in TT₃ were induced in response to low EA treatment and then restored to normal values alongside appropriate increases in EA (74). Furthermore, these changes in TT₃ were independent of exercise intensity and in particular, it was the energetic cost of exercise on EA that impacted TT₃ concentrations. Loucks et al. (75) revealed in a follow-up study that reductions in TT₃ were induced abruptly in participants with EA values between 19 and 25 kcal/kg lean body mass. Thus, TT₃ represents a sensitive marker of changes in EA associated with energy restriction and/or exercise training in women (74,75).

Low TT₃ has also been linked to reproductive dysfunction in women with anorexia nervosa (9) and exercising women with FHA (12,27,78). Loucks et al. (78) demonstrated suppressed TT₃ concentrations in amenorrheic female athletes compared to their eumenorrheic counterparts. In a study from our laboratory (27), TT₃ concentrations, similar to REE, were significantly lower in exercising women with FHA compared to exercising and sedentary ovulatory groups. These findings support the premise that REE and TT₃ are highly correlated. Furthermore, REE and TT₃ present in a dose-response manner across the continuum of menstrual disturbances such that increases in energy conservation (reductions in REE and TT₃) occur in association with increases in severity of EAMD (27) (Figure 4).

Causal links between thyroid hormones and reproductive function have been demonstrated in both human and animal experiments. In a study by Michaud et al. (86), thyroidarche (the maturation of the thyroid axis occurring between 9-11yr) has been shown to precede menarche (the maturation of the ovarian axis) in healthy adolescents.
Figure 4. Resting energy expenditure and metabolic hormone characteristics across the continuum of menstrual status. Panel A exhibits REE per kilogram (kg) fat-free mass (FFM) (kcal/d of REE per kg of FFM), total triiodothyronine (TT3) (ng/dL), ghrelin (pg/mL), and leptin (µg/L) in sedentary and exercising women categorized by menstrual status. Values are mean±SEM.
Figure 4 Caption (Continued)

Significant differences are denoted as follows: * ExAmen, ExAnov, ExIncon vs. SedOv; † ExAmen vs. ExOv; ‡ ExAmen vs. SedOv, ExOv, ExIncon, ExAnov; and § ExAmen, ExAnov, ExIncon, ExOv vs. SedOv. Panel B exhibits composite graphs of menstrual status depicted by daily estrone 3-glucuronide (E1G) (ng/mL) and pregnanediol 3-glucuronide (PdG) concentrations (µg/mL) in sedentary and exercising women categorized by menstrual status. E1G and PdG data for SedOv, ExOv, and ExIncon groups are aligned by LH peak, defined as day 0. The ExAnov and ExAmen participants’ E1G and PdG data are aligned by chronological day of daily urinary hormone collection. Number of days depicted for ExAmen participants is the mean cycle length of the menstruating participants. Values are mean±SEM. SedOv = sedentary ovulatory; ExOv = exercising ovulatory; ExIncon = exercising ovulatory, luteal phase defect and anovulatory inconsistent cycles; ExAnov = exercising anovulatory; ExAmen = exercising amenorrheic. Reprinted from De Souza et al. Fertil Steril 2007; 88:971-5 with permission.

Williams et al. (134) demonstrated that changes in circulating TT₃ were correlated with the induction and reversal of amenorrhea in female monkeys. Specifically, TT₃ concentrations dramatically decreased by 27% during the period of restricted dietary energy intake, whereas TT₃ concentrations increased significantly by 18% during the resumption of regular menses. Taken together, these findings (12,27,78,134) are in agreement that menstrual dysfunction is linked to energy conservation mechanisms underlying an energy deficiency with TT₃ and REE representing key markers of the induction of these mechanisms. Alternatively, recovery of menstrual function is associated with restoring an adequate energy intake relative to energy expenditure (133,134), which may also be associated with increases in TT₃ and REE.

Gastrointestinal Peptides: Ghrelin and Peptide YY (PYY)

Ghrelin and PYY are gastrointestinal peptides that are released into the periphery and travel through blood borne and neural pathways to influence central
mechanisms regulating energy homeostasis (53) and reproductive function (37,60,65,85,108,126). Ghrelin is an orexigenic hormone secreted from a distinct endocrine cell type, also known as X/A-like or ghrelin cells, in the stomach and gastrointestinal tract (24,62). PYY is an anorexigenic hormone secreted from the endocrine L cells of the intestine (10,53). Both ghrelin and PYY control the actions of neuropeptide Y (NPY) and agouti-related protein (AgRP) which interact with pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) in the arcuate nucleus of the hypothalamus (56).

Ghrelin and PYY have well-documented actions associated with the regulation of energy homeostasis and appetite in exercising and sedentary humans (51,95). Ghrelin is the only peripheral gut hormone known to stimulate appetite and per se, increases in ghrelin are proportional to increases in hunger and food intake (22,95,114). The mechanism whereby ghrelin regulates appetite is via the activation of NPY/AgRP and the suppression of POMC/CART (56). Ghrelin concentrations increase in the fasted state, and alternatively, decrease in the fed state (22). Consequently, changes in ghrelin act as a metabolic signal for meal initiation and meal termination by rising pre-prandial and falling post-prandial, respectively (22). Ghrelin is also associated with 24-hr energy intake (114) and when administered intravenously in nonobese men and women, ghrelin has been shown to be associated with a large increase in energy intake (136). PYY has well-recognized actions as a satiety factor (136). PYY converts to its active form, PYY3-36 (42,43), which subsequently binds to Y2 receptors initiating the opposite actions to ghrelin on the NPY/AgRP and POMC/CART pathway (46). This cascade of
responses at the level of the hypothalamus leads to decreases in energy intake and supposedly, decreases in body weight (46). Changes in ghrelin and PYY are proposed to act as homeostatic feedback mechanisms involved in the regulation of energy intake and appetite to return to a pre-determined set point and promote resultant changes in body weight (53,70,71,109). As such, alterations in these gut peptides are considered pivotal in weight loss and weight maintenance in healthy populations (70,71,109). These hormones are also implicated as critical factors in the development of clinical outcomes associated with reproductive dysfunction (13) as observed in models of severe, i.e., women with anorexia nervosa (89,119,122) and moderate energy deficiency, i.e., exercising women with FHA (33,110).

Ghrelin is a well-known marker of energy deficiency and chronic under nutrition (33,89,110,119,122). Investigators of women with anorexia nervosa (89,119,122) and exercise-associated FHA (28,110) have consistently observed elevated fasting ghrelin concentrations compared to control groups. Interestingly, women with anorexia nervosa (88,98,122) do not demonstrate the typical meal-induced decline in ghrelin and furthermore, total ghrelin secretion over a subsequent 12 hour period is in fact elevated compared to healthy controls (122). Similarly, De Souza et al. (28) observed elevations in fasting ghrelin in exercising women with FHA, 85% higher than exercising women with LPD/anovulation and exercising and sedentary ovulatory women. Changes in total ghrelin patterns, to include fasting, mealtime peak, and nocturnal peak, have been observed in premenopausal normal-weight women following a three-month diet and exercise intervention resulting in a negative energy balance
(70,71). Specifically, Leidy et al. (70) demonstrated that 24-hr ghrelin is elevated after diet- and exercise-induced weight loss and furthermore, these investigators demonstrated that circulating ghrelin is sensitive to changes in body weight. Such findings in energy-deficient populations are perplexing such that despite elevated fasting ghrelin concentrations, these women consistently report suppressed hunger, lower energy intake and/or weight loss (19,28,110,111). Thus, the mechanism whereby exercising women with FHA present with chronically low energy intake or lose body weight despite elevated ghrelin concentration is complex and involves both physiological and psychological explanations.

There is emerging interest in examining the role of PYY in the etiology of chronic energy deficiency and associated menstrual disturbances in exercising women with FHA. Notably, elevated PYY concentrations have been observed in anorexic women (90,94,102). As such, a metabolic profile wherein both fasting PYY and ghrelin concentrations are elevated is proposed to have an anorexigenic effect such that PYY may restrain the orexigenic effects of ghrelin in women with anorexia nervosa (90) and exercising women with FHA (110). In a study by Scheid et al. (110), elevated fasting ghrelin concentrations concomitant with elevated fasting PYY were shown in exercising women with FHA similar to women with anorexia nervosa (Figure 5). Additionally, fasting PYY concentrations were negatively correlated with REE/FFM indicative of the association between appetite-related hormones and energy conservation mechanisms (110). Taken together, chronically elevated fasting PYY may
Figure 5. Fasting total peptide YY (PYY) and ghrelin concentrations in sedentary (SedOv) (n=8) and exercising women with ovulatory cycles (ExOv) (n=20) compared to exercising women with amenorrhea (ExAmen) (n=20). ExAmen had higher fasting total log PYY (LogPYY, pmol/L) and ghrelin (pmol/L) compared to SedOv and ExOv groups. *p<0.05 vs. SedOv and ExOv. Results are expressed as mean±SEM. Reprinted from Scheid et al. Appetite 2009; 52(1):184-192 with permission.
represent a physiological mechanism in support of energy restriction in exercising women with FHA despite elevated fasting ghrelin concentrations.

From a psychological standpoint, subclinical and clinical disordered eating behavior is another pathway to chronic energy deficiency in exercising women with FHA (26,41). It is hypothesized that appetite-related hormones (i.e., ghrelin, PYY) might represent underpinnings of an eating behavioral phenotype associated with a greater susceptibility to chronic energy deficiency and EAMD (26,110). Researchers have recently examined the relationship between gut hormones and psychometric indicators of eating behavior, such as the DT subscale (26,110). Evidence of a link between subclinical, and if severely elevated clinical, elevations in DT score and a metabolic profile indicative of chronic energy deficiency has been exhibited in exercising women (26,41,110). Accordingly, De Souza et al. (26) demonstrated that women with high DT present with significantly higher ghrelin concentrations compared to women with normal DT. Thus, a high DT may contribute to the suppression of energy intake in the presence of elevated ghrelin concentrations. Scheid et al. (110) also found a positive correlation between fasting PYY concentrations and DT score in women categorized by exercise (exercising vs. sedentary) and menstrual status (FHA vs. ovulatory). Interestingly, despite consistently observing a positive correlation between DT and DR scores (26,41,125), DR has been notably unsuccessful at discriminating energy status (differences in REE controlled for FFM or REE/pREE and metabolic hormones such as TT₃, ghrelin, and PYY) (125). Women with higher DR scores do not differ from women with normal DR scores with respect to ghrelin and
PYY concentrations (125). In summary, elevated PYY concentrations, with simultaneously elevated ghrelin, are suggested to prevent compensatory increases in energy intake secondary to psychological markers of disordered eating (i.e., high DT) and increased exercise energy expenditure in exercising women with FHA (26,110).

Several researchers have explored the effect of acute exercise on ghrelin and PYY to better understand the regulation of energy balance in nonobese and obese populations (58,59,83). A well-documented suppression of appetite has been consistently observed in response to an acute exercise bout (52,58,59,83) and has been suggested to promote a decrease in relative energy intake in both women and men. Experimental findings by Hubert et al. (52) demonstrated that a diet-induced energy deficit increased hunger, whereas an exercise-induced energy deficit did not. These results suggest that a lack in initiative to match energy intake to exercise energy expenditure may be biologically driven (77). Furthermore, high carbohydrate diets, routinely prescribed in endurance athletes, may perpetuate the ad libitum energy deficit following exercise (49,50,115). This mechanism known as “exercise-induced anorexia” is complex, but appetite-related hormones have been proposed as contributing factors (59,83). Suppressed concentrations of acylated ghrelin have been demonstrated during aerobic exercise (58,59,83) and interestingly, ad libitum energy intake following an exercise bout has been shown to be similar to ad libitum energy intake following a rest condition (58). However, exercise-induced suppression of ghrelin seems to be temporary and it is elevations in circulating PYY concentrations that oppose the actions of ghrelin to promote satiety and chronic energy restriction (59,83). This anorexigenic
hormone profile following exercise has been proposed as the mechanism whereby gut hormones suppress appetite (59) and lead to an uncoupling between energy intake and energy expenditure. If sustained for prolonged periods, this phenomenon is thought to support “inadvertent under eating” where exercising women simply do not consume enough energy to compensate for exercise energy expenditure (77). “Inadvertent under eating” has been hypothesized to lead to the development of chronic energy deficiency in exercising women (77). Therefore, the cumulative effect of this anorexigenic appetite-related hormone profile at rest and following exercise is advanced as an explanation for the induction of a chronic energy deficiency and EAMD in exercising women.

Ghrelin and PYY are proposed as key metabolic signals in a complex network of mechanisms underlying disruption or restoration of energy homeostasis and reproductive function in exercising women (13,108) (Figure 6). As such, energy deficiency-induced changes in ghrelin and PYY are advanced as important factors involved in the suppression of the HPO axis (13,46) and ultimately, the transition to FHA. Ghrelin has been linked to reproductive function by directly acting on hypothalamic neurons in the arcuate nucleus or indirectly altering GnRH pulsatility (13,46) to result in suppressed LH secretion and pulsatility (108). Experiments in animals (37,40,126) and men (60,65) have demonstrated a direct relationship between elevated ghrelin and reproductive suppression (specifically via decreased LH secretion). In a recent study by Scheid et al. (108), investigators examined the impact of diet- and exercise-induced weight loss on LH pulsatility in premenopausal women. Scheid et al.
(108) found decreases in LH pulse frequency alongside increases in 24-hr mean ghrelin in the participants who underwent exercising training and lost weight. Change in LH pulse frequency was negatively associated with the change in mean 24-hr ghrelin and change in peak ghrelin at lunch (108). These findings infer that ghrelin is involved in the suppression of LH pulsatility associated with an energy deficiency in premenopausal trained women and to this end, elevated ghrelin concentrations may have a suppressive effect on reproduction. These findings extend to menstrual cyclicity as observed in women with anorexia nervosa and exercising women with FHA, both clinical populations that typically experience reproductive suppression concomitant with elevated ghrelin concentrations (19,28,111). Future prospective intervention research is necessary to determine the role of ghrelin and PYY in the induction and resumption of menstrual function in women with EAMD.

**Leptin**

Leptin, an adipocyte-secreted protein product of the *ob* gene, has been proposed as a factor involved in the hormonal regulation of both reproductive function and energy homeostasis in humans (35). Leptin is implied to play a role in the coordinated response of the HPO axis to metabolic fuel availability (35). Leptin regulates energy homeostasis via similar pathways as ghrelin and PYY and can also cross the blood-brain barrier to activate leptin receptor-bearing cells in the arcuate nucleus of the hypothalamus (91) (Figure 6). Specifically, leptin is a satiety factor and displays opposing actions to ghrelin (122).
Figure 6. A mechanistic overview of the actions of metabolic and gastrointestinal hormones, including ghrelin, peptide YY (PYY_3-36), and leptin. All of these hormones have the capacity to permeate the blood brain barrier and regulate appetite via interactions with neuropeptide Y (NPY) and agouti-related protein (AgRP) and pro-opiomelanocortin (POMC) and cocaine- and amphetamine regulated transcript (CART) located in the arcuate nucleus. These hormones also are proposed in the regulation of reproductive function via the hypothalamic-pituitary-ovarian axis affecting gonadotropin releasing hormone (GnRH) pulsatility and downstream release of luteinizing hormone (LH) and follicular stimulating hormone (FSH). Reprinted from Budak et al. Fertility and Sterility 2006; 85:1563-1581 with permission.
Mechanistically, leptin inhibits NPY/AgRP neurons that subsequently activate POMC/CART to decrease food intake (98). Experiments in animals (108) and humans (97) infer that hypoleptinemia plays a causal role in the development of obesity. Leptin resistance supposedly perpetuates an obesity-related metabolic profile characterized by a decrease in satiety alongside an increase in food intake and weight gain (115). Alternatively, leptin is relevant in the neuroendocrine adaptation to starvation, particularly as a protective mechanism to restore a eumetabolic state (17). Short-term fasting or severe energy restriction is associated with reduced leptin concentrations independent of declines in fat mass (17). Diet-induced weight loss also results in a decrease in circulating leptin concentration (86,140). As such, leptin represents a metabolic signal controlling short-term and long-term energy homeostasis at both ends of the weight spectrum (energy deprived and obese).

Leptin is a well-known indicator of chronic energy status based on a tight correlation between leptin concentration and body fat (18). Low leptin concentration acts as a metabolic signal to the hypothalamus communicating a state of energy deficiency; whereas, high leptin concentration promotes subsequent leptin resistance in support of suppressing appetite and increasing energy expenditure (18). Models of chronic energy deficiency, i.e., women with anorexia nervosa and women with FHA, consistently display suppressed leptin concentrations (23,61,73,93,130). As such, serum leptin concentrations in women with anorexia nervosa are typically lower than those of normal-weight controls (3,36,47,49,50,68,88). Tolle et al. (130) observed decreased leptin concentrations in women with anorexia nervosa across a 24-hr sampling period.
and alternatively, exhibited that leptin returns to control values following refeeding. Women with disordered eating and low energy intake, which are less severe presentations of abnormal eating behaviors compared to clinical eating disorders, also demonstrate significantly lower leptin concentrations, with both a lower baseline value and a blunted diurnal leptin pattern (69,130).

Findings in animal and human studies suggest that leptin plays a role in the regulation of reproductive function (3,64,132). In in vivo and in vitro experimental studies, investigators identified leptin receptors at all levels of the HPO axis (113,139). Furthermore, impaired GnRH pulsatility and secretion is demonstrated in leptin deficient animals and conversely, ovulation is restored by leptin administration (17,92). Leptin has also been linked to the maintenance of LH pulsatility (16,36,72) and the pre-ovulatory LH surge (105). Similar to women with anorexia nervosa, women with FHA typically present with lower leptin concentrations than their ovulatory counterparts (21). Miller et al. (87) observed suppressed leptin concentrations in women with FHA compared to age-, weight-, and body-fat-matched eumenorrheic controls. Similarly, Kaufman et al. (57) found a higher incidence of FHA in ballet dancers with suppressed leptin concentrations vs. age-, weight-, and body-fat-matched controls. Thus, suppressed leptin concentrations are likely associated with chronic energy restriction in women with FHA vs. controls. In exercising and non-exercising female adolescents, Ackerman et al. (1) reported a significant positive association between leptin and LH secretion. Additionally, leptin pulsatile secretion and area under the curve were significant predictors of LH pulsatile secretion and area under the curve, respectively
Therefore, these results suggest that suppressed leptin concentration may act as a metabolic signal communicating nutritional inadequacy from the periphery to the hypothalamus to initiate reproductive consequences.

To date, the relationship between leptin and reproductive function in exercising women with FHA is unclear. Since FHA is a hallmark condition presenting in women with chronic energy deficiency and these women often demonstrate low leptin concentrations, a critical leptin threshold hypothesis has been postulated in the literature (64,120,121). This hypothesis states that a specific leptin concentration (approximately 2.57 ng/mL) is necessary for regular menses to occur in underweight women. However, this hypothesis remains to be confirmed by intervention studies (3,8,47,64). On the contrary, there is evidence of variability in leptin concentrations in exercising women with FHA and these concentrations have been shown to overlap with concentrations observed in ovulatory women. Corr et al. (21) demonstrated that the ranges in leptin were similar in exercising women with FHA (Range: 0.30 – 16.98 ng/mL) and ovulatory cycles (Range: 2.57-18.28 ng/mL) and after adjusting for adiposity, the difference in leptin concentrations were no longer significant. These results conflict with the premise of a critical leptin threshold associated with menstrual cyclicity (64,120,121). As such, the regulation of menstrual function may not be associated with leptin alone and thus, other factors should also be considered in the etiology of FHA in exercising women (21). Notably, exercising women irrespective of menstrual status have been shown to present with decreased body fat compared to sedentary controls and to this end, significant differences in leptin concentration between exercising women
with FHA vs. ovulatory cycles may not be captured (27,28,69). De Souza et al. (27,28) and Laughlin and Yen (69) observed similar leptin concentrations in women with exercise-associated FHA compared to their ovulatory counterparts. However, definitive work by Laughlin and Yen (69) demonstrated an absence of the diurnal leptin rhythm in only the amenorrheic athletes. This finding indicates an association between nutritional and reproductive status that is not necessarily related to leptin concentration but the absence of a diurnal rhythm pattern in leptin.

The modulation of leptin in women with exercise-associated FHA is complex and represents a research area in need of further investigation. Certain investigators propose that other hormones and metabolic substrates may interact with leptin to promote normal cyclicity (67,120). Proposed modulators of leptin concentration include plasma insulin concentration (21,82), carbohydrate intake (54), glucose availability (44), sympathetic nervous activity (106), glycerol (21), and gonadal steroid environment (127). Laughlin and Yen (69) proposed that adaptations consistent with an energy deficiency in female athletes such as suppressed insulin and elevated cortisol concentrations may influence leptin regulation. Additionally, the modulation of leptin synthesis differs in exercising women based on menstrual status (21). In exercising women with FHA, insulin and glycerol explained an additional significant portion of the variance in leptin (21); whereas, percent body fat in both amenorrheic and ovulatory exercising women best predicted leptin. Therefore, additional research is necessary to describe the integrated control of reproductive function associated with several hormones and metabolic substrates in addition to leptin in exercising women with FHA.
Leptin has been implicated as a factor involved in the maintenance or restoration of normal menstrual cyclicity in women with FHA (18,132). Welt et al. (132) demonstrated that the administration of recombinant leptin restored normal ovulation in three of eight women with FHA. Additionally, leptin administration was associated with improvements in reproductive, thyroid, and growth hormone axes (132), suggesting that leptin represents an important metabolic signal relaying the adequacy of energy stores to the hypothalamus in support of normal reproductive function. In a study by Chou et al. (18), human recombinant leptin (metreleptin) was administered for 36 weeks in women with FHA. During the intervention, 7 of the 10 women receiving metreleptin resumed menses, and 4 of these women were considered to be ovulatory (18). In addition, E₂ and PdG concentrations increased significantly among the women treated with metreleptin compared to the women treated with placebo (18). Conversely, in a study by Audi et al. (3), investigators compared amenorrheic and eumenorrheic weight-recovered patients with anorexia nervosa and despite similar BMI and leptin levels in these groups, the eumenorrheic weight-recovered women presented with increased serum free E₂ and urinary GH compared to their amenorrheic counterparts. These findings infer that leptin plays a permissive role and is not necessarily a prerequisite signal in the restoration of menses (3). Further, it must be underscored that the other metabolic hormones associated with chronic energy deficiency also need to be fully normalized prior to the recovery of normal menstrual cyclicity (21). Treatment studies in women with anorexia nervosa present an increase in serum leptin concentrations as weight recovers and this rise in leptin also correlates with increases in gonadotropins
(45,47,48). The rise in gonadotropins is indicative of the sensitivity of leptin to changes in weight gain that are associated with activation of the HPO axis (45,47,48). Similar intervention research is required in women with EAMD to elucidate the effect of energy status on circulating leptin concentration and signaling patterns during the induction and resumption of EAMD in women.

**CONCLUSION AND FUTURE DIRECTIONS**

In conclusion, physical activity and exercise is associated with changes in metabolic and reproductive hormones in the presence of a chronic energy deficiency and menstrual dysfunction in women. Classic work in animal and human studies have illustrated that the etiology of clinical and subclinical EAMD is linked to a cascade of alternations in REE and metabolic hormones, such as TT₃, ghrelin, PYY, and leptin, indicative of a chronic energy deficiency. Alternatively, the restoration of an optimal energetic/metabolic and reproductive environment in exercising women is associated with favorable changes in REE and the metabolic hormone profile. An interrelated network of these hormonal responses is involved in the mechanisms associated with the induction and recovery of EAMD in exercising women. Dietary strategies to restore menstrual function (i.e., by increasing energy intake) appear to be optimal approaches such that the focus of the treatment is on improvement of the full metabolic hormone profile (occurs within weeks) concomitant with recovery of menstrual function (occurs within months). Future research on the effectiveness of these non-pharmacological treatment strategies associated with chronic energy deficiency-related EAMD is
necessary and additionally, the identification of metabolic biomarkers indicative of risk for or presence of EAMD in exercising women would be beneficial to prevent and manage EAMD and other Triad-related clinical sequelae.
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**Abstract**

A high drive for thinness (DT) score obtained from the Eating Disorder Inventory-2 is associated with surrogate markers of energy deficiency in exercising women. The purpose of this study is: (1) to confirm the association between DT and energy deficiency in a larger population of exercising women than that previously published and (2) to compare the distribution of menstrual status in exercising women when categorized as high vs. normal DT. A high DT was defined as a score $\geq 7$ corresponding to the 75th percentile for college-aged women. Exercising women aged 22.9±4.3yr with a BMI of 21.2±2.2kg/m$^2$ were retrospectively grouped as high DT (n=27) or normal DT (n=90) to compare psychometric, energetic, and reproductive characteristics. Chi-square tests were performed to compare the distribution of menstrual disturbances between DT groups. Measures of resting energy expenditure (REE) (4949±494 kJ/day vs. 5406±560 kJ/day, p<0.001) and adjusted REE (123±16 kJ/LBM vs. 130±9 kJ/LBM, p=0.027) were suppressed in exercising women with high DT vs. normal DT,
respectively. Ratio of measured REE compared to predicted REE (REE/pREE) in the high DT group was 0.85±0.10 meeting our operational definition for an energy deficiency (REE/pREE<0.90). A greater frequency of severe menstrual disturbances, such as amenorrhea and oligomenorrhea, was observed in the high DT group ($\chi^2=9.3$, $p=0.003$) compared to the normal DT group. In the current study, we confirm the association between a high DT score and energy deficiency in exercising women and demonstrate a greater frequency of severe menstrual disturbances in exercising women with high DT.

**Introduction**

Subclinical disordered eating behavior has been well-documented in premenopausal women engaging in recreational and competitive exercise regimens (26) and has been shown to promote chronic energy deficiency potentially leading to the development of clinical consequences associated with the Female Athlete Triad (Triad) (18,26). The Triad is a syndrome described by low energy availability (with or without disordered eating), functional hypothalamic amenorrhea, and low bone mineral density (18), alone or in combination, in exercising women. A tool often used to discriminate between disordered vs. healthy eating behavior and attitudes in college-aged women is the Eating Disorder Inventory-2 (EDI-2) (11). The drive for thinness (DT) subscale of the EDI-2 is used to predict the presence of a subclinical (if severely high, clinical) variant of disordered eating that is often observed in exercising women. Such
demonstration of high DT is characterized by a preoccupation with body weight and body shape, fear of gaining weight, and high dietary cognitive restraint (DR) (24).

A large body of evidence supports strong associations among subclinical disordered eating, DT, and amenorrhea in exercising women (5,18,27). Exercise-related menstrual disturbances are proposed to begin with intense pressures to achieve an unrealistic standard of thinness and also, to enhance weight control (2,26). A high DT has also been proposed as a keystone factor in the development of the Triad (5,26). Since a high DT promotes diet and exercise behavior consistent with the achievement of the thin ideal, we reasoned that a high DT might be associated with behavioral changes that reflect dietary energy restriction and/or high exercise volume, likely resulting in the development of an energy deficiency (5).

We have previously demonstrated that a high DT score may serve as an indicator of underlying energy deficiency based on the observation of a significant negative relationship between DT scores and resting energy expenditure (REE) when expressed as the ratio of measured REE to the Harris-Benedict predicted REE (REE/pREE) (5). The exercising women with a high DT in our previous study exhibited significantly suppressed REE controlled for fat-free mass and a lower ratio of REE/pREE. The ratio of REE/pREE has been utilized in published reports of energy deficient women with anorexia nervosa where a ratio of 0.60-0.80 is often observed (14,16,21). It is notable that we have previously reported a ratio of REE/pREE of 0.86±0.03 in exercising women with high DT, which met our operational definition of an energy deficiency (REE/pREE<0.90) (5,9).
Physiological evidence of an energy deficiency in exercising women can be demonstrated by a disruption in energetic and endocrine homeostasis, which triggers a reduction in REE, and alterations in fasting hormone concentrations, including reduced total triiodothyronine (TT₃) and elevated ghrelin concentrations (4-6). These energetic adaptations act as energy-conserving mechanisms translating effects to metabolism (5,6) and reproduction. As such, menstrual disturbances are often observed in energy deficient exercising women, and therefore, we hypothesize that these menstrual disturbances may be associated with a high DT (5,19,28).

The purpose of this study was two-fold (1) to confirm a high DT score as an indicator of energy deficiency in a larger population of exercising women (n=117) compared to our previous publication and (2) to compare the distribution of menstrual disturbances in exercising women when categorized as either high or normal DT. We hypothesized that (1) the exercising women with high DT will exhibit signs of energy deficiency (suppressed adjusted REE and a ratio of REE/pREE less than 0.90) and thus confirm our previous findings in a larger cohort and (2) the exercising women with high DT will present with a greater frequency of severe menstrual disturbances (amenorrhea and oligomenorrhea) as corroborated using measures of luteinizing hormone (LH), and daily urinary estrone-1-glucuronide (E1G) and pregnanediol glucuronide (PdG) metabolites.
Methods

Experimental design: This is a cross-sectional study comparing exercising women with high DT (n=27) to exercising women with normal DT (n=90) with respect to psychometric, energetic, and reproductive characteristics. Volunteers were retrospectively grouped according to their DT scores. Women were considered “exercising” if they were currently participating in 2 or more hours/week of purposeful exercise corroborated by a VO₂ peak ≥ 40 ml/kg/min (23). Exercising women (n=117) with high or normal DT were monitored for at least one complete menstrual cycle if eumenorrheic (menstruating) or at least one 28-day monitoring period if not regularly menstruating. Energy status was determined by measurement of REE and corroborated by serum measurement of TT₃. The reproductive evaluation included a description of menstrual history; confirmation of the presence of an LH peak, and quantification of daily urinary ovarian steroid metabolites, E₁G and PdG. The current investigation includes data from a cross-sectional study designed to assess cardiovascular status in exercising women and data from the baseline period of a prospective study designed to assess the effects of a 12-month intervention of increased energy intake on indices of bone health and menstrual status in exercising women with menstrual disturbances vs. exercising women with ovulatory cycles.

Volunteers: Eligibility criteria for this study included: (1) aged 18-35 yr; (2) good health as determined by a medical exam; (3) no chronic illness, including hyperprolactinemia and thyroid disease; (4) stable menstrual status over preceding 3 months; (5) currently participating in two or more hours/week of purposeful exercise
and corroborated by a VO2 peak ≥ 40 ml/kg/min; (6) non-smoker; (7) not currently dieting and weight stable for the preceding 3 months; (8) not taking any hormonal therapy for at least 6 months; (9) no current clinical diagnosis of eating disorders, and (10) no other contraindications that would preclude participation in the study. This study was approved by Institutional Research and volunteers signed an approved Informed Consent document.

**Study time period:** Volunteers completed psychometric measurements of eating attitudes and behaviors. This appointment was followed by a REE test and a fasting blood sample for the measurement of TT3. Eumenorrheic (menstruating, cycle length 26-35 days) women collected urine samples for at least one complete menstrual cycle, oligomenorrheic (inconsistent and irregular cycle length of 36-90 days) women collected urine samples for up to 90 days, and amenorrheic (no menses for at least 90 days) women collected urine samples for one 28-day monitoring period. The daily urinary samples permitted the characterization of menstrual status (ovulatory, anovulatory, oligomenorrheic, or amenorrheic).

**Groups categorization:** Participants were retrospectively grouped according to DT score, and categorized into one of two groups: (1) exercising women with high DT (n=27); or (2) exercising women with normal DT (n=90).

**Anthropometric data:** Total body mass was measured to the nearest 0.1 kg on at least two occasions (each measurement within a four week period), and the mean of these measurements was presented. Participants were expected to stay within ±2.5 kg of their first body mass measurement and were weighed in shorts and a t-shirt. Height
was measured to the nearest 1.0 cm. Body mass index (BMI) was calculated as the average body mass divided by height squared (kg/m²). Body composition was assessed using dual-energy x-ray absorptiometry (DXA) in a total of 99 subjects. It is notable that we did not standardize for time of day or food/fluid intake for the measurement of body mass and body composition. Subjects were scanned on either a GE Lunar Prodigy (n=76, enCORE 2002 software version 6.50.069) or a GE Lunar iDXA (n=23, enCORE 2008 software version 12.10.113). Consistent with the International Society of Clinical Densitometry guidelines, a cross calibration study was performed to remove systematic bias between the systems. Fourteen participants were scanned in triplicate on both machines. The values were highly correlated with no significant difference between the population mean values. Biases in the total BMD, total bone mineral content, total fat, and % fat relative to the magnitude of the variable were observed. Equations were derived using simple linear regression to remove these biases and report the Prodigy values calibrated to the iDXA.

**Resting energy expenditure (REE):** REE was measured on a single occasion during the study. REE was determined by methods previously published in detail (9). Predicted REE (pREE; kJ/day) was calculated using the Harris-Benedict equation (13). A ratio of the laboratory measured REE to predicted REE (REE/pREE) was calculated.

**Dietary energy intake:** Dietary energy intake was assessed on two occasions during the study from three day nutritional logs recorded for two weekdays and one weekend day, as previously described (5). Three day nutritional logs recording food intake have been demonstrated to provide comparable data to seven day records in
women who may underreport their food intake, including lean women (12). Daily dietary energy intake (kJ/day) over the three day recording periods was expressed as the mean value during the study. It is notable that some participants (n=18) completed only one diet record.

**Exercise logs:** Volunteers kept logs of their purposeful exercise on at least two separate seven day occasions during the study. These logs provided a measurement of exercise volume (min/wk).

**Peak oxygen uptake (VO₂ peak):** VO₂ peak was measured on a single occasion during the study using methods that have been previously published (5).

**Drive for thinness (DT) score:** DT score was obtained from the EDI-2 (11), which was completed once during the study. A normal DT score was defined as a score less than 7. About 75% of college-aged women have scores less than 7 (11). In the current study, a high DT score was defined as: (A) a DT score of 7 or greater; or (B) when the scores on the EDI-2 indicated a “fake profile” a strategy identified by O’Connor et al. (19). Those participants with fake profiles demonstrated a qualitative description of “low” scores on all of the EDI-2 subscales with the exception of a “high” score on the perfectionism subscale. We have modified this strategy herein to provide more specific criteria (**Figure 1**). The presence of a fake profile was determined when the DT and Body Dissatisfaction scores were less than or equal to 3 and the Bulimia score was less than or equal to 1 with a concomitant high score (greater than or equal to 9) on the Perfectionism subscale. The decision to utilize this strategy was intended to account for response bias. Women with “fake” profiles (n= 8) were grouped with the
high DT women, and their scores were adjusted to reflect the mean DT score of the high
DT group. **Figure 1** depicts an example of a typical “fake” profile we observed.
Athletes with subclinical eating disorders have been shown to have elevated scores on
the DT, Bulimia, and Body Dissatisfaction subscales (11). Thus, low scores on these
three subscales were considered, along with a high Perfectionism score, as paramount
indicators of a “fake” profile. The rationale for the cut-off of greater than or equal to 7
on the DT subscale was based on our previous publication (5). Other corroborative data
included data from Ramacciotti et al. (22) who defined a high DT score as 7 or greater.
Torstveit and Sundgot-Borgen (27) suggested the use of a DT score of 15 or greater for
classifying athletes “at risk” for the Triad; however, we concur that a score that high is
likely associated with clinical pathology, and our focus was on identifying indications
of subclinical disordered eating. Thus, in order to capture indications of subclinical
disordered eating, we chose a cut-off on the DT subscale of 7.
Figure 1. Graphical representation of a “fake” profile in two subjects in the high drive for thinness group. DT = Drive for Thinness; B = Bulimia; BD = Body Dissatisfaction; IE = Ineffectiveness; P = Perfectionism; ID = Interpersonal Distrust; IA = Interoreceptive Awareness; MF = Maturity Fears; A = Asceticism; IR = Impulse Regulation; SI = Social Insecurity. Reprinted with permission from Gibbs et al., IJSNEM. 2011; 21(4):280-90.
**Exercise status:** Volunteers were required to participate in two or more hours per week of purposeful exercise as documented in exercise logs during the study, and from exercise history review. We also utilized a VO$_2$ peak of $<40$ ml/kg/min to reflect sedentary status and 40 ml/kg/min or greater to reflect exercising status consistent with other reports (23).

**Energy status:** Energy status was defined using an objective laboratory-based measure, the measurement of REE, to identify individuals who experience energetic adaptations to an energy deficiency (6,9,17). Reductions in REE have been observed in several studies examining exercising women who present with amenorrhea (9). We compared lab assessed REE to a prediction equation for REE to estimate how much each individual’s measured REE deviated from the predicted REE. In women with anorexia nervosa (14,16,21), the majority of data published utilized the Harris-Benedict equation (13) to predict REE, and as such, we determined that this equation was most useful for our purposes. A previous study by our lab group provided our reasoning for using the Harris-Benedict equation over the Cunningham equation (9). In brief, it has been shown during periods of low body weight, and prior to refeeding in anorexic women (14,16,21), that a reduced ratio of measured REE to Harris-Benedict predicted REE (REE/pREE) (13) of 0.60-0.80 is often reported. The Cunningham equation has not been used in anorexic women. We have previously published data using operationally defined energy deficiency as a ratio of REE/pREE less than 0.90 (5,6,9). We chose to use this REE/pREE cut-off ($<0.90$) in the current study as our operational
definition of energy deficiency to best discriminate the exercising women who may present with an energy deficiency from those who are energy replete.

**Three factor eating questionnaire (TFEQ):** The TFEQ is a questionnaire that measures three dimensions of human eating behavior: (1) DR, (2) disinhibition, and (3) hunger (25). This questionnaire was administered once during the study primarily to identify restrictive eating behaviors.

**Blood sampling and storage:** Blood samples were collected between 0700 and 1000h on a single occasion during the study, stored and processed as previously described (5).

**Serum hormone measurements:** TT3 concentration was analyzed as previously described (5).

**Menstrual status:** Menstrual history was determined in all subjects and defined as the number of self-reported menstrual cycles in the past 3 months. Menstrual status was abnormal or severe if a volunteer was amenorrheic (reported no menses for the past 3 months), oligomenorrheic (reported irregular menses at intervals of 36-90 days) or normal or regular if a volunteer was eumenorrheic (reported regular menses at intervals of 26-35 days). Self-reported menstrual status was then confirmed prospectively by classifying menstrual cycles by length of the intermenstrual interval, length of follicular and luteal phases, the presence or absence of menses, and by ovulatory status (ovulatory or anovulatory) as described in previous publications in our lab (7,8). These determinations were made from the measurement of daily urinary E1G and PdG, as previously described (7,8). Eumenorrheic women collected daily urine samples for at
least one menstrual cycle, oligomenorrheic women for no more than 90 days, and amenorrheic women for at least one 28-day monitoring period. To determine estrogen and progesterone exposure, E1G and PdG urinary metabolites were compared among groups of menstrual cycles using the trapezoidal integrated area under the curve (AUC) and mean levels of E1G and PdG during the follicular and luteal phases, and across the entire cycle. Values from each repeated cycle or monitoring period were averaged. Composite graphs of menstrual cycles depicted by daily E1G and PdG concentrations were determined by taking the mean values from each repeated cycle or monitoring period. For graphing purposes, the E1G and PdG data for eumenorrheic, ovulatory women were aligned by the day of the LH peak defined as Day 0. The amenorrheic, oligomenorrheic, and anovulatory participants’ E1G and PdG data were aligned by chronological day of urinary collections. Both the high DT and normal DT group included oligomenorrheic, amenorrheic, anovulatory and eumenorrheic, ovulatory women and were as per our previous publication (28).

**Statistical Analysis:** All data sets were tested for non-normality, homogeneity of variance, and outliers before statistical hypothesis tests were performed. Data were expressed as mean ± SD. Since the TT$_3$ concentrations were not normally distributed, a logarithmic conversion of the TT$_3$ concentrations was used to normalize the data. Clinical characteristics (i.e., age, height, body mass, BMI, body composition, VO$_2$ peak, exercise volume, age of menarche, gynecological age) and data for all psychometric, energetic, and reproductive characteristics were compared between groups using an Independent Student’s $t$-test. Additionally, chi-square tests were performed as a cross-
tabulation between: (1) DT score and energy status to determine the association between a high DT score and our operational definition of an energy deficiency and (2) DT score and menstrual status to compare the distribution of menstrual disturbances in exercising women when categorized as high vs normal DT. All data were analyzed using SPSS for Windows (version 16.0, Chicago, IL) statistical software package.

Results

Clinical characteristics: The clinical characteristics of the volunteers are presented in Table 1. Age, gynecological age, age of menarche, height, weight, and BMI were similar (p>0.05) between the high DT and normal DT groups. Body composition was also similar (p>0.05) between groups. All of the women in this study were considered to be of “above average” physical fitness by ACSM classification (1). They exhibited an exercise volume greater than or equal to 120 min/wk and a VO₂ peak of ~46mL/kg/min.

Psychometric parameters: The psychometric profile data of the volunteers are presented in Table 2. By study design, DT scores were higher (p<0.001) in the high DT group compared to the normal DT group. Scores on the EDI-2 subscales for bulimia (p=0.007), body dissatisfaction (p=0.009), ineffectiveness (p=0.002), and interoceptive awareness (p=0.023) were also higher in the high DT group vs the normal DT group.
**Table 1.** Clinical characteristics of the exercising women grouped by drive for thinness (DT) score.

<table>
<thead>
<tr>
<th></th>
<th>Normal DT (n=90)</th>
<th>High DT (n=27)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>23.1±4.3</td>
<td>22.3±4.1</td>
<td>0.355</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.9±5.7</td>
<td>164.5±5.8</td>
<td>0.283</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>58.5±5.8</td>
<td>57.0±5.2</td>
<td>0.210</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3±1.9</td>
<td>21.0±1.5</td>
<td>0.546</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>25.2±4.5</td>
<td>26.5±4.9</td>
<td>0.269</td>
</tr>
<tr>
<td>Fat Mass (kg)*</td>
<td>14.9±3.4</td>
<td>15.4±3.6</td>
<td>0.591</td>
</tr>
<tr>
<td>Lean Body Mass (kg)*</td>
<td>42.0±4.5</td>
<td>40.5±4.1</td>
<td>0.176</td>
</tr>
<tr>
<td><strong>Exercise Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise Volume (min/wk)</td>
<td>385.4±236.2</td>
<td>439.1±289.8</td>
<td>0.376</td>
</tr>
<tr>
<td>VO₂peak (ml/kg/min)</td>
<td>46.7±6.9</td>
<td>44.8±6.3</td>
<td>0.303</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

BMI = Body Mass Index; High DT score (DT ≥ 7). Normal DT score (DT < 7).

*Subset of the population with body composition data- High DT (n=20) vs. Normal DT (n=79).
Table 2. Psychometric data of the exercising women grouped by drive for thinness (DT) score.

<table>
<thead>
<tr>
<th></th>
<th>Normal DT (n=90)</th>
<th>High DT (n=27)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EDI-2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drive for Thinness</td>
<td>1.2 ±1.8</td>
<td>10.7±3.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bulimia</td>
<td>0.3±1.0</td>
<td>1.6±2.3*</td>
<td>0.007</td>
</tr>
<tr>
<td>Body Dissatisfaction</td>
<td>4.0±4.5</td>
<td>8.3±7.8*</td>
<td>0.009</td>
</tr>
<tr>
<td>Ineffectiveness</td>
<td>0.7±1.5</td>
<td>3.5±4.3*</td>
<td>0.002</td>
</tr>
<tr>
<td>Perfectionism</td>
<td>5.9±3.5</td>
<td>8.4±3.7*</td>
<td>0.002</td>
</tr>
<tr>
<td>Interpersonal Distrust</td>
<td>1.6±2.5</td>
<td>3.4±4.4</td>
<td>0.057</td>
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<tr>
<td>Interoceptive Awareness</td>
<td>1.2±2.3</td>
<td>3.1±4.0*</td>
<td>0.023</td>
</tr>
<tr>
<td>Maturity Fears</td>
<td>2.2±2.5</td>
<td>2.9±3.2</td>
<td>0.077</td>
</tr>
<tr>
<td>Asceticism</td>
<td>2.8±1.9</td>
<td>3.7±2.2</td>
<td>0.246</td>
</tr>
<tr>
<td>Social Insecurity</td>
<td>1.6±2.1</td>
<td>3.0±3.0</td>
<td>0.052</td>
</tr>
<tr>
<td>Impulse Regulation</td>
<td>1.0±2.1</td>
<td>1.3±2.4</td>
<td>0.653</td>
</tr>
<tr>
<td><strong>TFEQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disinhibition</td>
<td>5.1±3.2</td>
<td>7.1±3.4*</td>
<td>0.004</td>
</tr>
<tr>
<td>Hunger</td>
<td>5.8±2.9</td>
<td>6.5±2.9*</td>
<td>0.236</td>
</tr>
<tr>
<td>Dietary Cognitive Restraint</td>
<td>8.1±4.5</td>
<td>13.9±5.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
EDI-2 = Eating Disorder Inventory-2; TFEQ = Three Factor Eating Questionnaire.
Normal DT score (DT<7); High DT score (DT≥7).
*High DT group vs Normal DT group; (p<0.05; Independent t-test).
The high DT group exhibited significantly higher scores on the perfectionism subscale (p=0.002) in comparison to the normal DT group. The groups were similar (p>0.05) on all the other EDI subscales, including interpersonal distrust, asceticism, maturity fears, social insecurity, and impulse regulation. Additionally, the high DT group had higher DR (p<0.001) and disinhibition (p=0.004) scores than the normal DT group. However, the groups had similar hunger scores (p>0.05).

**Energy status and metabolic hormones:** Energy status data are presented in Figure 2. REE, expressed as kJ/day, (p<0.001) and REE, expressed as a ratio of REE/pREE, (p<0.001), were both lower in our women with high DT compared to those with normal DT (Figure 2, Panel A). Adjusted REE, expressed as kJ/kg lean body mass (LBM), was lower (p=0.027) in the high DT group compared to our normal DT group (Figure 2, Panel B). Dietary energy intake was lower (p=0.014) in the high DT group vs. the normal DT group (Figure 2, Panel C). To corroborate the relationship between energy status (REE) and a hormonal marker of energy status (TT3), correlation analyses were performed and Log TT3 concentrations were positively correlated with REE/pREE (r=0.201, p=0.030). Log TT3 concentrations also demonstrated positive correlations with REE (r=0.210, p=0.023), and adjusted REE (r=0.295, p=0.003). Log TT3 values were lower (p=0.043) in the energy deficiency group compared to the energy replete group.
Figure 2. Data for energy status and dietary energy intake in high and normal DT groups. Panel A is the ratio of measured to predicted REE. Panel B is REE (kJ/day) adjusted for kilogram lean body mass (LBM). Panel C is dietary energy intake (kJ/day). Dietary energy intake conversion kcal x 4.186 = kJ, REE= resting energy expenditure, DT= drive for thinness, LBM=lean body mass. REE conversion kcal x 4.186 = kJ. Data were expressed as mean ± SD, except Panel C (mean ± SEM). Reprinted with permission from Gibbs et al., IJSNEM. 2011; 21(4):280-90.
**DT score and energy status:** A chi-square test of DT score and energy status demonstrated that more cases of energy deficiency were observed in women with high DT compared to women with normal DT ($\chi^2=5.119$, $p=0.024$).

**Menstrual Status:** Quantification of daily E1G and PdG metabolites and measurements of LH were completed in 102 volunteers. Note that certain volunteers with high DT (n=4) and normal DT (n=11) had incomplete daily urinary collection and were excluded from these particular analyses. Significantly more women with high DT, 73.9% (17/23), were categorized as having a severe menstrual disturbance (amenorrhea or oligomenorrhea) when compared with women with normal DT, 38.0% (30/79) ($\chi^2=9.260$, $p=0.002$). Among the women in the high DT group, 52.2% (12/23) and 21.7% (5/23) were amenorrheic or oligomenorrheic, respectively, and 26.1% (6/23) were eumenorrheic. Among the women in the normal DT group, 26.6% (21/79) and 11.4% (9/79) were amenorrheic or oligomenorrheic, respectively, and 62.0% (49/79) were eumenorrheic. The frequency of amenorrhea was significantly greater ($\chi^2=5.331$, $p=0.021$) in the high DT group in comparison to the normal DT group. The frequency of oligomenorrhea was similar between groups ($\chi^2=1.610$, $p=0.204$). When the eumenorrheic cycles were further categorized by ovulatory status, there was a greater frequency of eumenorrheic and ovulatory cycles in the normal DT group ($\chi^2=7.080$, $p=0.008$), 54.4% (43/79), in comparison to 21.7% (5/23) in the high DT group. A high DT subscale score did not discriminate individuals who displayed anovulatory cycles specifically, and there was no statistically significant difference between groups ($\chi^2=0.294$, $p=0.588$).
**Figure 3** illustrates these rates of frequency in both groups. Composite graphs of the daily urinary reproductive excretion in exercising women when categorized as high vs. normal DT are shown in **Figure 4**. There were no statistical differences observed among groups with respect to the menstrual cycle parameters or daily ovarian steroid excretion (**Table 3**).

**Correlations:** Pearson correlation analyses demonstrated negative correlations between DT score and REE parameters: REE ($r=-0.285$, $p=0.002$), adjusted REE ($r=-0.205$, $p=0.041$), and ratio of REE/pREE ($r=-0.290$, $p=0.002$). Additionally, DT score was inversely related to menstrual history ($r=-0.268$, $p=0.004$) and dietary energy intake ($r=-0.231$, $p=0.013$). DT score and DR score were positively correlated ($r=0.542$, $p<0.001$). DT score was positively correlated with perfectionism score ($r=0.322$, $p<0.001$), corroborating the strategy to account for “fake” profiles.
Figure 3. Frequency of menstrual disturbances among exercising women grouped by drive for thinness using daily hormone measures of estrogen and progesterone metabolites (E1G and PdG). Amen = amenorrheic cycle; Oligo = oligomenorrheic cycle; Eumen = eumenorrheic cycle; Ov = ovulatory cycle; E1G = estrone-1-glucuronide; PdG = pregnanediol glucuronide. Reprinted with permission from Gibbs et al., IJSNEM. 2011; 21(4):280-90.
Figure 4. Composite graphs of daily urinary reproductive excretion in exercising women when categorized as high or normal DT. Panel A demonstrates the mean daily E1G and PdG concentrations of exercising women with high DT (n=23). Panel B demonstrates the mean daily E1G and PdG concentrations of exercising women with normal DT (n=79). E1G = estrone-1-glucuronide; PdG = pregnanediol glucuronide. Data were expressed as mean ± SEM. Reprinted with permission from Gibbs et al., IJSNEM. 2011; 21(4):280-90.
Table 3. Reproductive profiles of the exercising women grouped by drive for thinness (DT) score.

<table>
<thead>
<tr>
<th></th>
<th>Normal DT (n=90)</th>
<th>High DT (n=27)</th>
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<tbody>
<tr>
<td><strong>Menstrual cycle characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of Menarche (yrs)</td>
<td>12.9±1.4</td>
<td>13.0±1.6</td>
<td>0.707</td>
</tr>
<tr>
<td>Gynecological Age (yrs)</td>
<td>10.2±4.5</td>
<td>9.2±4.8</td>
<td>0.337</td>
</tr>
<tr>
<td>Cycle Length (days)</td>
<td>31.4±6.7</td>
<td>32.3±4.5</td>
<td>0.702</td>
</tr>
<tr>
<td>Duration of Amenorrhea (days)</td>
<td>77.5±15.9</td>
<td>143.5±31.3</td>
<td>0.060</td>
</tr>
<tr>
<td><strong>Daily ovarian steroid excretion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1G Cycle AUC</td>
<td>1110.2±649.0</td>
<td>1094.6±624.6</td>
<td>0.919</td>
</tr>
<tr>
<td>E1G days 2-5 AUC</td>
<td>100.0±55.0</td>
<td>120.7±63.4</td>
<td>0.129</td>
</tr>
<tr>
<td>E1G days 2-12 AUC</td>
<td>298.1±158.3</td>
<td>334.5±166.5</td>
<td>0.340</td>
</tr>
<tr>
<td>PdG Cycle AUC</td>
<td>48.1±32.6</td>
<td>40.3±35.4</td>
<td>0.482</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
Normal DT score (DT<7); High DT score (DT≥7).
*Subset of the population with daily ovarian steroid excretion data- High DT (n=23) vs. Normal DT (n=79).

**Discussion**

In the present study, we compared 117 exercising women with high DT (scores≥7) and normal DT (scores<7) who displayed a range of psychometric, energetic, and reproductive characteristics. We report that a high DT is associated with energy deficiency in exercising women when energy deficiency is identified using a ratio of REE/pREE, confirming our previous publication (5). We also demonstrated that a high DT was associated with suppressed reproductive function, as corroborated by daily
urinary E1G and PdG metabolites. Interestingly, there was a significantly greater frequency of severe menstrual disturbances among women with high DT compared to women with normal DT (74% vs. 38%). Based on these results, a high DT score may provide valuable information to coaches, athletic trainers, and health practitioners attempting to get an indication of energy and menstrual status in large groups of exercising women where direct assessment is not feasible.

In exercising women with high DT, the average ratio of REE/pREE was 0.85, which is below 0.90, our operational cut-off for an energy deficiency (5,9,17). The ratio of REE/pREE was significantly associated with TT3, such that the lower the ratio, the lower the circulating concentration of this metabolic hormone. This observation is not surprising, since earlier reports from our laboratory (5) have confirmed an association between low TT3 and adaptive changes in energy expenditure. Low circulating TT3 concentrations contribute to energy conservation typically observed in undernourished or energy deficient individuals (20). In the current study, TT3 concentrations were clearly related to REE.

To date, this is the first study to compare ovarian steroid profiles of exercising women with high DT to exercising women with normal DT. Our findings support the notion that a high DT may successfully identify severe menstrual disturbances, given the high frequency of oligomenorrhea and amenorrhea in the high DT group (74%). Due to the inaccuracy of self-reported menstrual status and/or retrospective menstrual history questionnaires (7,8), these findings are undoubtedly important since menstrual status was objectively characterized by urinary measures of LH, E1G and PdG
metabolites and we observed clear qualitative differences in the hormonal profiles between our groups. Reproductive profiles of the normal DT group were indicative of ovulation and luteinization. In contrast the high DT group displayed chronically suppressed concentrations of E1G and PdG. These qualitative ovarian profiles coincide appropriately with the significant differences observed in menstrual status classifications (ovulatory vs amenorrheic) and energy status classifications (deficient vs replete).

Our findings also confirm that a high DT is associated with DR and menstrual disturbances, a finding we and others have previously reported (5,15,28). We also report that our women with high DT had significantly lower dietary energy intake compared to those with normal DT. However, it is notable that underreporting of energy intake is often suspected in exercising women (3,10) and it is possible that the women with high DT may be particularly susceptible to underreporting. It is also possible that self-reported physical activity was not a sensitive measure capable of detecting differences in exercise energy expenditure. We are, therefore, unable to conclude whether the energy deficit observed in the exercising women with high DT was attributable to differences in restrictive eating behavior or exercise energy expenditure.

Our study is not without limitations. One limitation of using the DT subscale is that some women may present with “fake profiles”. We chose to adopt the identification of “fake” profiles first described by O’Connor et al. (19) in order to effectively identify exercising women who may distort their answers. These authors provided a systematic approach for identifying a fake profile. The perfectionism score is rarely high in the
presence of normal eating attitudes and behavior (19). Based on these findings, our approach was to define more specific criteria than that proposed by O’Connor et al. (19), and to provide a useful operational definition of a “fake” profile. The mean perfectionism scores observed in exercising women were variable, ranging from 3 to 9 (11,19). We chose to define a high perfectionism score as greater than or equal to 9 in order to rigidly reflect the eating pathology motivating these subclinical disordered eating attitudes and behavior which may result in “risk” or the presence of an energy deficiency.

In conclusion, in the current study, we confirm the findings of a previous publication (5) that a high DT score was reflective of surrogate markers of energy deficiency in a large population of exercising women. Additionally, a greater frequency of severe menstrual disturbances (amenorrhea or oligomenorrhea) was observed in exercising women with high DT; whereas a greater frequency of eumenorrheic, ovulatory cycles was observed in exercising women with normal DT. Therefore, a high DT score may provide useful information to coaches, athletic trainers, and health practitioners when assessing energy and menstrual status in a large group of exercising women.

Acknowledgements

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References


**Abstract**

Dietary restraint (DR) is a key eating behavior associated with menstrual disturbances (MD) in exercising women. However, the association between DR and energy availability (EA) has not been examined. **purposes:** (1) To compare EA in women when categorized by DR score, to include an evaluation of the frequency of women with low EA and (2) to compare the distribution of subclinical and clinical MD between DR groups. **methods:** Exercising women (23±4 yr, BMI: 21.1±1.9 kg/m² and exercise volume: 333±198 min·wk⁻¹) were retrospectively categorized by DR score into two groups: (1) women with high DR (n=30) and (2) women with normal DR (n=56). DR scores were obtained from the Three Factor Eating Questionnaire. High DR score was defined as ≥13. Body composition was measured using dual-energy x-ray absorptiometry. EA was defined as energy intake–exercise energy expenditure/kg lean body mass (LBM). Low EA was defined as <30 kcal·kg⁻¹LBM. Menstrual status was determined using daily urinary samples assayed for reproductive hormones. **Results:** EA was lower in the high DR vs. the normal DR group (35.0±12.9 kcal·kg⁻¹LBM vs.
42.0±12.9 kcal·kg\textsuperscript{-1}LBM, p=0.018). There was no difference (p=0.866) in frequency of low EA between DR groups. There was a greater frequency of MD (amenorrhea, oligomenorrhea, anovulation or LPD) in the high DR group (75.0%, 21/28) vs. the normal DR group (51.1%, 24/47) ($\chi^2=4.2$, p=0.041). **Conclusion:** Our findings demonstrate that exercising women with high DR exhibited lower EA and a greater frequency of MD (subclinical and clinical) compared to women with normal DR. However, high DR was not associated with low EA in exercising women.

**Introduction**

Disordered eating behavior is notably prevalent in exercising women (7,10,15) and is often associated with restrictive eating and/or high exercise training volume (5,7,15). As such, inadequate energy intake (EI) relative to exercise energy expenditure (EEE) often contributes to the development of low energy availability (EA), a key factor underlying clinical outcomes which are unfavorable to reproductive health in exercising women. Furthermore, there is evidence for estrogen dependent and independent mechanisms for bone loss (14) and thus, low EA may promote potentially irreversible bone loss in women with or without exercise-associated amenorrhea. These detrimental health conditions are commonly referred to as the Female Athlete Triad (30), a syndrome first recognized in female athletes, wherein one or more of the following conditions may be present: low EA (with or without disordered eating), menstrual disturbances, and low bone mineral density.
Low EA promotes energy conservation mechanisms, reserving fuel for only the most critical physiological processes, i.e., thermoregulation and cellular maintenance (39). Reproductive function is not considered critical for survival, and as such, suppression of the reproductive axis may ensue and manifest as subclinical (luteal phase defects (LPD) or anovulation) or clinical menstrual disturbances (amenorrhea or oligomenorrhea) (11). Links between low EA and disruptions in luteinizing hormone (LH) pulsatility (23) and menstrual cyclicity (42) have been documented in human and animal experiments. However, the association between dietary restraint (DR), a psychometric indicator of eating behavior, and EA has not been clearly elucidated in exercising women.

DR is the conscious restriction of EI in an effort to achieve or maintain a certain body weight (36). A high DR is hypothesized to contribute to the development of low EA through the reduction of EI and/or increase in EEE (15,32). As a result, exercising women with high DR may be at a higher risk for the development of Female Athlete Triad-related clinical outcomes, such as menstrual disturbances. Several investigators have linked high DR to menstrual disturbances, such as amenorrhea, oligomenorrhea, and anovulation (3,38), in exercising and sedentary women. However, to date, the association between DR and EA has yet to be examined in exercising women. In brief, high DR may represent an underpinning eating behavior associated with low EA and thus, we propose a physiological link may exist between high DR and low EA contributing to the development of menstrual disturbances in exercising women. The purpose of this study is two-fold: (1) to compare EA in exercising women when
categorized by DR score, to include an evaluation of the frequency of women with low EA (defined as <30 kcal·kg⁻¹·LBM) and (2) to compare the distribution of menstrual dysfunction (subclinical and clinical menstrual disturbances) in exercising women categorized by DR score. We hypothesize that: (1) women with high DR will demonstrate lower EA and a greater frequency of low EA compared to women with normal DR and (2) exercising women with high DR will demonstrate a greater frequency of menstrual disturbances, to include subclinical (LPD or anovulation) and clinical perturbations (amenorrhea or oligomenorrhea), compared to women with normal DR.

Methods

Experimental Design: This is a cross-sectional study comparing exercising women aged 18-35yr with high DR (n=30) and normal DR (n=56) with respect to EA and menstrual characteristics. Participants were retrospectively categorized according to their DR scores obtained from the Three Factor Eating Questionnaire (TFEQ). Subjects with a DR score of ≥13 were classified as having a high DR. EA was defined as EI minus EEE relative to kg LBM [EA = (EI – EEE)/LBM (kg)] (22). EI was determined using three-day diet logs and EEE was obtained using Polar Heart Rate monitors and/or Ainsworth compendium over a seven-day period (2). Body composition was assessed using dual-energy x-ray absorptiometry (DXA). Participants who were eumenorrheic (normally menstruating) collected daily urine samples for at least one complete menstrual cycle; whereas women who were amenorrheic or oligomenorrheic
(experiencing menses at irregular intervals) collected daily urine samples for a least a 28-day monitoring period. Classification by menstrual status (amenorrheic, oligomenorrheic, anovulation, LPD, or ovulatory) involved the evaluation of self-reported menstrual history, confirmation of the presence of a LH peak, and quantification of daily urinary reproductive hormones, estrone-1-glucuronide (E1G) and pregnandiol glucuronide (PdG). This investigation includes data from a cross-sectional study referred to as the Active Women’s Study (10,31,38) and data from the baseline period of a prospective study designed to assess the effects of a 12-month intervention of increased EI on indices of bone health and menstrual status in exercising women.

Participants: To be eligible for the study, the following criteria needed to be met: (1) 18-35 yr; (2) good health status as determined by a medical professional; (3) free of any chronic medical conditions such as hyperprolactinemia or thyroid disease; (4) body mass index (BMI) of 16-25 kg·m⁻²; (5) stable menstrual status for the preceding three months; (6) ≥two hr·wk⁻¹ of purposeful exercise corroborated by a peak oxygen uptake (VO₂peak) ≥40 ml·kg⁻¹·min⁻¹; (7) non-smoker; (8) stable weight status (±2 kg) for the preceding six months and not presently dieting; (9) no hormonal therapy treatment for the last six months; (10) not pregnant or lactating or planning a pregnancy; (11) no current clinical diagnosis of eating or psychiatric disorders; (12) no medication use that would alter metabolic or reproductive hormone concentrations; and (13) no other contraindications that would prevent study participation. This study was approved by the Institutional Research Board and all participants signed an approved Informed Consent form.
Study Time Period: Eumenorrheic (menstruating, cycle length 26-35 days) women collected daily urine samples for at least one complete menstrual cycle, oligomenorrheic (inconsistent and irregular cycle length of 36-90 days) women collected daily urine samples for up to 90 days, and amenorrheic (no menses for at least 90 days) women collected daily urine samples for at least one 28-day monitoring period. All study procedures were completed within the monitoring period.

DR Score: DR score was obtained from the TFEQ (36), which was completed once during the study. A cut-off of $\geq 13$ on the DR subscale was based on the Eating Inventory (36) and also represents the 75th percentile in samples of premenopausal (37,38) and postmenopausal women (6). The participants were categorized into two groups based on their DR scores: (1) women with high DR and (2) women with normal DR.

TFEQ: The TFEQ (36) is a questionnaire that measures three dimensions of human eating behavior: (1) DR, (2) disinhibition, and (3) hunger. This questionnaire was administered once during the study primarily to categorize women by DR status, hypothesized to be associated with low EA and menstrual disturbances. We also measured flexible and rigid control, two subtypes of DR (41). Rigid control score indicates an “all or nothing” attitude to eating and dieting, whereas flexible control score is defined by a more sensible approach to eating and dieting (41). These subscales of the TFEQ were utilized to provide more specific information regarding the DR patterns observed in these exercising women. A cut-off of $\geq 3$ and $\geq 4$ on the flexible and
rigid control subscales, respectively, were representative of the 75th percentile of our sample of exercising women.

**Dietary EI:** EI (kcal·day\(^{-1}\)) was assessed using three-day diet logs recorded for two week days and one weekend day, as previously described (10). Three-day diet logs recording EI have been shown to provide comparable data to seven-day logs in women who may under-report their EI, including lean women (16). Additionally, three-day diet logs have been shown to reduce participant burden and improve compliance (25). 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. Subjects were also asked to record time and location of every eating episode. On-site registered dietitians met with the subjects to instruct them on how to accurately record EI. The nutrient data from the three-day logs were coded and analyzed using the Nutrition Data System for Research (NDSR 2008 Version; University of Minnesota; Minneapolis, MN, USA).

**EEE:** Participants completed exercise logs where all purposeful exercise sessions were recorded for a seven-day period. Purposeful exercise included activities such as elliptical, pilates, running or strength training, but not daily living activities such as house cleaning or walking a dog. Energy expended during these purposeful exercise sessions was measured using the OwnCal feature of the Polar S610 or RS400 heart rate monitors (Polar Electro Oy, Kempele, Finland) (9). The OwnCal feature has been validated for the use in calculating EEE from heart rate (17,19). This feature uses body weight, height, age, gender, VO\(_{2}\)peak, individual maximum heart rate, individual heart
rate in a sitting position, and heart rate during exercise to derive kilocalories from energy expenditure. Actual VO₂peak values were used to compute EEE. For purposeful exercise sessions in which subjects did not wear the heart rate monitors, the Ainsworth et al. (2) compendium of physical activities was used to determine the appropriate metabolic equivalent (MET) level for the exercise performed. To calculate the energy expended during the exercise session, the MET level was multiplied by the duration (min) of the exercise session. The MET value includes a resting component. To estimate only EEE, we therefore subtracted measured resting energy expenditure (REE) (kcal·min⁻¹) from this value. Determination of MET levels from exercise logs was made by the same member of the research team.

**Energy Availability (EA):** EA (kcal·kg⁻¹ LBM) was determined as EI minus EEE relative to kg LBM [EA = (EI – EEE)/LBM (kg)] (22). We calculated EA using the data described for EI, EEE, and body composition (LBM), which were determined within the same seven-day period. EEE represents only the energy (kcal) attributable to exercise such that the estimate of the energy (kcal) expended for REE throughout the duration of purposeful exercise sessions was subtracted from the estimate of EEE using the Polar heart rate monitor or physical activity logs. Low EA was defined as an EA <30 kcal·kg⁻¹ LBM based on previous evidence depicting a disruption in LH pulsatility at EA values below this threshold (23).

**VO₂peak:** VO₂peak (mL·kg⁻¹·min⁻¹) was measured during a progressive treadmill test to volitional exhaustion using indirect calorimetry on a single occasion during the study as per a previous publication (10).
**Exercise Logs:** Participants kept logs of their purposeful exercise on at least two separate seven-day occasions during the study as per a previous publication (10). These logs provided a measurement of exercise volume over a seven-day period (min·wk⁻¹).

**Anthropometric and Body Composition Data:** Total body mass was measured to the nearest 0.1 kg on at least two occasions during the study period, and the mean of these measurements was presented. Height was measured to the nearest 1.0 cm. BMI was calculated as the average body mass divided by height squared (kg·m⁻²). Body composition was assessed using DXA for use in the calculation of EA and for descriptive purposes. Subjects were scanned on either a GE Lunar Prodigy (n=49, enCORE 2002 software version 6.50.069), a GE Lunar iDXA (n=29, enCORE 2008 software version 12.10.113) or a Hologic QDR4500 DXA scanner (n=8, Hologic Inc., Bedford, MA). Consistent with the International Society of Clinical Densitometry guidelines, a cross-calibration study was performed to remove systematic bias between the systems. For the cross-calibration study between the Lunar Prodigy and Lunar iDXA, 14 participants were scanned in triplicate on both machines. The values for body composition obtained on each scanner were found to be highly correlated with no significant difference between the population mean values. Equations were derived using simple linear regression to remove biases, and body composition values obtained from both the Lunar Prodigy and the Hologic QDR-4500W were calibrated to the Lunar iDXA.
**REE:** REE was measured on a single occasion during the study. REE was determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA, USA) by methods previously published in detail (14).

**Menstrual Status:** Self-reported menstrual status at screening was confirmed prospectively by classifying menstrual cycles by length of the inter-menstrual interval, length of follicular and luteal phases, the presence of menses and by ovulatory status (ovulatory or anovulatory) as described in previous publications from our lab (12,13). These determinations were made from the measurement of daily urinary LH, E1G, and PdG, as previously described (12,13). Specific hormonal criteria for detecting ovulation and classifying subclinical menstrual disturbances (LPD and anovulation) were also previous described (12,13). Participants who were eumenorrheic (normally menstruating) collected daily urine samples for at least one complete menstrual cycle; whereas women who were amenorrheic or oligomenorrheic (experiencing menses at irregular intervals) collected daily urine samples for at least one 28-day monitoring period. Some women were monitored for more than one menstrual cycle, and in those cases, the data from cycles were averaged. For graphing purposes, the E1G and PdG data for ovulatory women were aligned by the day of the LH peak defined as Day 0. The amenorrheic, oligomenorrheic, and anovulatory participants’ E1G and PdG data were aligned by chronological day of urinary collections (15,38).

**Statistical Analysis:** All data sets were tested for non-normality, homogeneity of variance, and outliers before statistical hypothesis tests were performed. Data were expressed as mean ± SD. Descriptive characteristics (i.e., age, height, body mass, BMI,
body composition, VO_2peak, exercise volume, age of menarche, gynecological age, REE, TFEQ subscale scores) and data for EA and menstrual characteristics were compared between DR groups using Independent Student’s t tests. Chi-squares tests were performed to determine the association between: (1) DR status and EA status (defined as the presence or absence of low EA <30 kcal·kg^{-1}LBM) and (2) DR status and menstrual status (defined as (i) presence or absence of menstrual disturbances to include subclinical (LPD, anovulation) and clinical presentations (amenorrhea and oligomenorrhea) and (ii) presence or absence of amenorrhea). Secondary analyses included: (i) Pearson correlation analyses between psychometric indicators of DR (DR, flexible control, and rigid control scores) and EA parameters (EA, EI, and EEE) to examine the relationship between DR and EA and (ii) univariate and multivariate logistic regression analyses to determine whether DR (DR, flexible control, and rigid control scores) was associated with a greater risk for menstrual dysfunction (subclinical or clinical) or amenorrhea when adjusting for key covariates of menstrual status (percent body fat, age of menarche, and EA). All data were analyzed using SPSS for Windows (version 19.0, Chicago, IL) statistical software package. We performed a priori sample size calculations using G Power 3.1.2 (Universitat Kiel, Germany, 2009) based on data from previous publications (30,36). Sample size calculations were performed to determine the number of participants per group required to: (i) compare EA in exercising women when categorized by DR score and (ii) compare the frequency of menstrual disturbances in exercising women when categorized by DR score. A moderate effect size of 0.40, an alpha error probability of 0.05, and a power of 0.80
required a total sample size of 55 participants to test our hypotheses using the aforementioned statistical analyses. Thus, our sample size calculations indicated that 55 women provided adequate power (1-\( \beta \) = 0.80) to detect significant differences and associations at a moderate effect size for analyses.

**Results**

**Descriptive Characteristics:** The descriptive characteristics of the participants are presented in [Table 1](#). Descriptive characteristics were similar (p>0.05) between the DR groups. All of the women in this study were considered to be of “above average” physical fitness by ACSM classification (1). Participants exhibited an exercise volume greater than 120 min·wk\(^{-1}\) and presented with a mean VO\(_2\)peak of 45.5 mL·kg\(^{-1}\)·min\(^{-1}\).

**Psychometric Eating Behavior Characteristics:** The psychometric eating behavior data obtained from the TFEQ in our participants are presented in [Table 1](#). By study design, DR scores were higher (p<0.001) in the high DR group compared to the normal DR group. Flexible control (p<0.001) and rigid control (p<0.001) scores were higher in the high DR group vs. the normal DR group. Disinhibition and hunger scores were similar (p>0.05) between DR groups.
Table 1. Descriptive characteristics of exercising women categorized by dietary restraint (DR) score.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal DR group (n=56)</th>
<th>High DR group (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic/Anthropometric Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23.4±3.8</td>
<td>21.9±3.4</td>
<td>0.062</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.3±6.6</td>
<td>165.6±5.1</td>
<td>0.853</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>57.7±6.6</td>
<td>57.7±4.8</td>
<td>0.990</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>21.1±1.9</td>
<td>21.1±2.0</td>
<td>0.980</td>
</tr>
<tr>
<td>Reproductive Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of Menarche (yr)</td>
<td>13.1±1.8</td>
<td>12.8±1.5</td>
<td>0.453</td>
</tr>
<tr>
<td>Gynecological Age (yr)</td>
<td>10.3±4.2</td>
<td>9.1±4.0</td>
<td>0.193</td>
</tr>
<tr>
<td>Body Composition Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>25.0±5.3</td>
<td>25.1±5.5</td>
<td>0.939</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>14.4±4.0</td>
<td>14.4±3.9</td>
<td>0.978</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>41.0±4.5</td>
<td>40.9±3.4</td>
<td>0.915</td>
</tr>
<tr>
<td>Exercise Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Exercise Volume (min·wk⁻¹)ᵃ</td>
<td>322±199</td>
<td>356±197</td>
<td>0.482</td>
</tr>
<tr>
<td>VO₂peak (ml·kg⁻¹·min⁻¹)ᵇ</td>
<td>46.7±6.0</td>
<td>44.3±6.5</td>
<td>0.131</td>
</tr>
<tr>
<td>REE Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REE (kcal·d⁻¹)</td>
<td>1249±128</td>
<td>1215±123</td>
<td>0.231</td>
</tr>
<tr>
<td>REE/LBM (kcal·kg⁻¹·LBM)</td>
<td>30.7±2.7</td>
<td>29.9±3.2</td>
<td>0.234</td>
</tr>
<tr>
<td>TFEQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary Restraint</td>
<td>7.3±3.2</td>
<td>15.8±2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Flexible Control</td>
<td>1.3±1.1</td>
<td>3.7±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rigid Control</td>
<td>1.7±1.4</td>
<td>4.3±2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>4.5±2.9</td>
<td>5.4±2.6</td>
<td>0.151</td>
</tr>
<tr>
<td>Hunger</td>
<td>5.5±2.7</td>
<td>5.2±2.5</td>
<td>0.679</td>
</tr>
</tbody>
</table>

Values are mean ± SD. High Dietary Restraint Score (DR≥13); Normal DR score (DR<13); BMI = Body Mass Index; VO₂peak = Peak Oxygen Uptake; REE = Resting Energy Expenditure; REE/LBM = REE controlled for Lean Body Mass; TFEQ = Three Factor Eating Questionnaire; ⁿ = Total Exercise Volume (n=77); ⁿ = VO₂peak (n=69).
**Energy Availability (EA):** EA, EI, and EEE data of the exercising women categorized by DR score are presented in Figure 1. EA was lower (p=0.018) in the high DR group compared to the normal DR group. EI was lower (p=0.029) in women with high DR vs. those with normal DR; whereas, EEE and LBM were similar (p>0.05) between DR groups. There was no difference (p=0.866) in the frequency of low EA between the high DR group (8/30, 26.7%) and the normal DR group (14/56, 25.0%). DR score was negatively associated with EA (r=-0.323, p=0.002) and EI (r=-0.306, p=0.004) in exercising women. Flexible control and rigid control scores were also negatively correlated with EA (r=-0.295, p=0.006 and r=-0.324, p=0.002, respectively) and EI (r=-0.326, p=0.002 and r=-0.330, p=0.002, respectively). There were no significant associations (p>0.05) between DR (DR, flexible control, or rigid control scores) and EEE.

**Menstrual Status:** Figure 2 demonstrates the composite graphs of the daily urinary reproductive hormone excretion in exercising women when categorized by DR score. There was a greater frequency of menstrual disturbances (amenorrhea, oligomenorrhea, anovulation or LPD) in the high DR group (75.0%, 21/28) compared to the normal DR group (51.1%, 24/47) (χ²=4.2, p=0.041). Specifically, more women in the high DR group were amenorrheic (50.0%, 14/28) compared to the normal DR group (23.4%, 11/47) (χ²=5.6, p=0.018). Conversely, more women in the normal DR group (61.7%, 29/47) had ovulatory cycles compared to the high DR group (32.1%, 9/28) (χ²=6.1, p=0.013).
Figure 1. Energy availability (EA) data (EA, energy intake (EI), and exercise energy expenditure (EEE)) in exercising women categorized by dietary restraint (DR) status. Panel A is data for EA (kcal·kg⁻¹ lean body mass (LBM)) and demonstrates that EA is lower (p=0.018) in the high DR group compared to the normal DR group. This statistical finding is denoted by “a”. Panel B is data for EI (kcal·day⁻¹) and demonstrates that EI is lower (p=0.029) in the high DR group compared to the normal DR group. This statistical finding is denoted by “a”. Panel C is data for EEE (kcal·day⁻¹) and demonstrates there are no significant differences (p=0.419) in EEE between groups. Data were expressed as mean ± SD.
Figure 2. Composite graphs of daily urinary reproductive excretion in exercising women when categorized by dietary restraint (DR) status. Panel A demonstrates the mean daily E1G and PdG concentrations of exercising women with high DR group (n=28). Panel B demonstrates the mean daily E1G and PdG concentrations of exercising women with normal DR (n=47). E1G = estrone-1-glucuronide; PdG = pregnanediol glucuronide. Data were expressed as mean ± SEM.
Table 2. Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) for menstrual dysfunction (MD) associated with dietary restraint (DR) in exercising women (n=75).

<table>
<thead>
<tr>
<th></th>
<th>MD (Subclinical and Clinical)</th>
<th>Amenorrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR (95% CI)(^a)</td>
<td>AOR (95% CI)(^b)</td>
</tr>
<tr>
<td>High DR score(^c)</td>
<td>3.7 (1.2, 11.4)</td>
<td>5.5 (1.5, 19.7)</td>
</tr>
<tr>
<td>Age at onset of menarche (yr)</td>
<td>1.5 (1.1, 2.1)</td>
<td>1.5 (1.0, 2.3)</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>-</td>
<td>0.8 (0.7, 0.9)</td>
</tr>
</tbody>
</table>

\(^a\) = Final Model: \(R^2 = 0.209\) (Nagelkerke), 0.154 (Cox & Snell); This regression model is also adjusted for percent body fat and energy availability (kcal·kg\(^{-1}\) lean body mass).

\(^b\) = Final Model: \(R^2 = 0.393\) (Nagelkerke), 0.283 (Cox & Snell); This regression model is also adjusted for energy availability (kcal·kg\(^{-1}\) lean body mass)

\(^c\) = High dietary restraint score (DR \(\geq\) 13); normal DR score (DR < 13)

Exercising women with high DR were three times more likely to demonstrate menstrual dysfunction (subclinical or clinical) or amenorrhea than women with normal DR (OR, 2.9; 95% CI: 1.0, 8.1 and OR, 3.3; 95% CI: 1.2, 8.9, respectively). Multivariate logistic regression analysis revealed that high DR is a predictor of menstrual dysfunction (subclinical or clinical) or amenorrhea when adjusting for percent body fat, age of menarche, and EA (Table 2).

**Discussion**

Our findings provide evidence that exercising women with high DR presented with lower EA and a greater frequency of menstrual disturbances (subclinical and clinical) compared to women with normal DR. However, we did not observe an association between high DR and low EA, which is proposed to be a threshold indicative of an energy deficiency sufficient to cause menstrual disturbances (23). The
lack of association between high DR and low EA may be explained by the observation of only moderately low EA in the high DR group (mean EA of 35.0 kcal·kg⁻¹LBM) that is above the proposed EA threshold. As such, our study demonstrates that menstrual dysfunction in exercising women categorized by DR score also occurs above the purported EA threshold, (48% and 26% of women in the high and normal DR groups, respectively). It is possible that this threshold of EA established in a laboratory setting that predicts when LH pulsatility declines significantly (23) may not similarly manifest under free-living conditions using self-report and field methods to determine EA when changes in menstrual status, not LH are the outcome (33). Potentially, the threshold of EA associated with changes in menstrual status may be higher or vary among exercising women categorized by DR score. The threshold may be higher because a smaller energy deficit that is present over a longer time may be sufficient to disrupt the menstrual cycle.

In our study, the difference in EA observed between DR groups was primarily driven by the reduction in EI relative to EEE in the women with high DR. Few investigators have specifically explored the relationship between DR and EA in exercising women (32). A high DR is suggested to promote reductions in EA by way of persistent monitoring of EI (32) and as a result, energy restriction may occur in these women as an attempt to maintain or lose body weight. Our findings demonstrate that the women with high DR consumed 14% lower EI than women with normal DR. However, we did not observe any differences in mean EEE between the high and normal DR groups. Both DR groups presented with similar EI values as observed in exercising
women with low EA in the literature (1,326-1,992 kcal·d⁻¹); whereas, the mean EEE values in our DR groups were lower than the range of EEE values (272–537 kcal·d⁻¹) in these same studies (12,29,32,43). Therefore, reductions in EI relative to higher EEE values may be necessary to achieve low EA in exercising women categorized by DR score. Interestingly, we did not observe differences in exercise volume between DR groups. These findings are in contrast to evidence from a study by McLean et al. (28) wherein women with high DR exercised more than women with low DR. The relationship between DR and exercise volume has relevant implications for bone health outcomes and high DR and high exercise training volume should be explored as potential risk factors, alone or in combination, for low bone mineral density and prospective bone stress injury independent of low EA and menstrual dysfunction in large samples of exercising women.

Previous investigators suggest that individuals with high DR exert cognitive control over EI (18) and would be less likely to respond to the physiological cues of hunger initiated by an exercise-induced energy deficit. Accordingly, those women with high DR would avoid compensating for energy expended during exercise, and therefore, may be more likely to reduce EA. In a study by Lluch et al. (20), there was no energy compensation at a subsequent meal or throughout the remainder of the day in women with high DR following an exercise-induced energy deficit. To this end, women with high DR may avoid compensating for EEE by way of better appetite control translating to lower EI and EA. Thus, high DR may represent a successful strategy for reducing EA in combination with exercise. Notably, higher flexible control and rigid control scores
were also associated with lower EI, contributing to lower EA. Future research on the association between the subtypes of DR (flexible and rigid control) and EA in exercising women is required to describe whether exercising women with a specific type of DR are better able to restrict EI, resulting in low EA and potential menstrual dysfunction.

Previous findings by Vescovi et al. (38) demonstrated an association between high DR and clinical menstrual disturbances (amenorrhea or oligomenorrhea) in exercising women. Notably, Vescovi et al. (38) did not assess EA in their study. Since EA has been linked to disruptions in LH pulsatility (23,24) in short-term experiments in sedentary, regularly menstruating women and menstrual cyclicity (42) concomitant with exercise training in female monkeys, an objective of this study was to clarify whether an association between DR and EA exists in exercising women, potentially contributing to menstrual dysfunction. To this end, we demonstrated that exercising women with high DR demonstrated lower EA and a greater frequency of exercise-associated menstrual disturbances (amenorrhea, oligomenorrhea, anovulation or LPD), 75%, vs. women with normal DR, 51%. Specifically, more women in the high DR group were amenorrheic (50%) compared to the normal DR group (23%) and alternatively, more women in the normal DR group were ovulatory (62%) compared to the high DR group (32%). Similarly, more women with high flexible control were amenorrheic than women with normal flexible control, suggesting that women with a sensible approach to monitoring food intake are better able to restrict EI (40,41), resulting in lower EA that may lead to menstrual dysfunction. In addition, no differences in frequency of
menstrual disturbances were observed when comparing women with high vs. normal rigid control, wherein a high rigid control is indicative of an all or nothing approach to food intake and often cycles of lower EI followed by higher EI (40,41).

Notably, we did not observe an association between low EA and high DR. The percent of women with low EA was distributed evenly between the high DR and normal DR groups, 27% and 25%, respectively. Additionally, the mean EA values in our DR groups were higher than the range of EA in female amenorrheic runners (12-29 kcal·kg⁻¹ LBM) observed in the literature (12 studies) (21). In a prospective study of EA characteristics in female elite soccer players across a competitive season (approximately 3 months in length), Reed et al. (33) demonstrated that a significantly greater frequency of participants with menstrual disturbances (67%) exhibited an average EA below 30 kcal·kg⁻¹ LBM for the season compared to participants with regular menstrual cycles (0%). However, some participants with an average EA above 30 kcal·kg⁻¹ LBM for the entire season also demonstrated menstrual disturbances (23%). Thus, these findings suggest that low EA consistent with energy deficiency and suppressive effects on the reproductive axis may vary among exercising women. Taken together, the EA threshold associated with menstrual dysfunction in women with high DR may actually be higher than 30 kcal·kg⁻¹ LBM. As such, in our study, DR status was not associated with EA status, despite discriminating EA values and menstrual status in exercising women.

There are significant challenges associated with measuring EA and its components (EI, EEE and LBM) in free-living exercising women. Specifically, the measurement of EI and EEE data using self-reported logs and field measures may be
associated with a higher degree of variability, particularly in women with high DR. Previous investigators have shown that self-reported diet records are prone to inaccuracies in the form of under-reporting (26,35). Rennie et al. (34) demonstrated that under-reporting was more common in women with high DR than in those with lower DR. We took special precautions to prevent reporting inaccuracies by having a registered dietitian train each of the participants on food recording techniques. This same limitation applied to quantifying EEE in free-living women who self-reported purposeful exercise. In a study by Conway et al. (8), exercise logs overestimated EEE by 8% in comparison to doubly labeled water. Thus, it is reasonable to speculate that certain exercising women with high DR may underestimate EI and overestimate EEE resulting in an overall underestimation in EA. Furthermore, EA is often transient in exercising women and to this end, the utility of measuring EA as an indicator of energy deficiency and menstrual dysfunction in free-living participants is questionable. Since low EA is one of the main etiological factors associated with menstrual dysfunction identified in the 2007 Female Athlete Triad Position Stand (30), additional research on the role of EA in menstrual function is necessary in order to clarify low EA as an underpinning component of menstrual dysfunction in exercising women. To date, no one has examined whether an EA threshold of 30 kcal·kg\(^{-1}\) LBM, defined similarly as Loucks et al. (23,24), discriminates menstrual status when EA is measured with self-reported diet logs, exercise logs, and heart rate monitoring in trained, exercising women studied under free-living conditions. In addition, future studies are needed to: (i) assess the effect of varying levels of EA on menstrual function in premenopausal women and
(ii) examine the duration of ‘suboptimal’ EA that is required to induce menstrual disturbances (subclinical or clinical). Such investigation is necessary to clarify whether a dose-response relationship exists between severity of menstrual dysfunction and magnitude of low EA.

Despite high DR representing a key factor associated with menstrual dysfunction and reductions in EA, DR has been notably unsuccessful at discriminating energy status in exercising women (differences in REE and metabolic hormone concentrations indicative of energy deficiency, i.e., total triiodothyronine (TT₃) and ghrelin) (38). From a psychological standpoint, high DR is often tightly correlated with high drive for thinness (DT) (10,15). High DT has been shown to consistently demonstrate stronger associations with surrogate markers of chronic energy deficiency (suppressed REE and fasting TT₃ concentrations concomitant with elevated ghrelin concentrations) than high DR (10,15). As such, high DT is suggested to represent a better psychometric indicator of energy conservation and menstrual dysfunction in exercising women than high DR. In addition, women with high DR scores may exhibit more stress with respect to food intake than women with normal DR scores (27). Evidence of an association between high DR scores and urinary cortisol, a biological marker of stress, has been demonstrated in studies of healthy premenopausal women (4,27) and may represent another mediating mechanism underlying ovulatory disturbances (4). Further analysis of the directionality of these associations among psychometric indicators of eating behavior (DR and DT), energy status, and stress responsiveness focusing on models of causality (i.e., path analysis, discriminant
analysis) would allow for a better understanding of the etiology of Female Athlete Triad clinical sequelae, particularly low EA and menstrual dysfunction, in premenopausal exercising women.

In conclusion, our findings provide evidence that exercising women with high DR demonstrated lower EA and a greater frequency of menstrual disturbances (subclinical and clinical) compared to exercising women with normal DR. Interestingly, we did not observe a difference in the frequency of low EA in women categorized by DR score, which may be explained by the observation of only moderately low EA in the high DR group. However, low EA may simply not represent a useful marker of energy deficiency in free-living women categorized by DR score. The threshold of EA consistent with energy deficiency sufficient to cause menstrual dysfunction may be higher in these women or vary inter-individually. In this study, we demonstrated that women with high DR presented with menstrual disturbances despite exhibiting EA values above the proposed threshold (23). Since this was a cross-sectional study, future prospective research needs to be conducted to examine the interrelationships among psychometric indicators of eating behavior (i.e., DR and DT scores), EA, and menstrual function with a focus on determining models of causality for low EA and menstrual disturbances in exercising women.

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References


Chapter 5

STUDY THREE

Gibbs JC, Williams NI, Mallinson RJ, Scheid JL, Hill BR, Reed JL, Olmsted MP, De Souza MJ. REFUEL: The effect of a randomized controlled trial of increased energy intake on psychometric indicators of eating behavior in women with exercise-associated menstrual disturbances.

Abstract

Disordered eating attitudes and behavior, i.e., drive for thinness (DT), body dissatisfaction (BD), dietary cognitive restraint (DR), and bulimia symptoms, have well-known negative implications for clinical conditions associated with the Female Athlete Triad in exercising women. To date, no investigators have characterized the changes in psychometric indicators of eating behavior (DT, BD, DR, and bulimia scores) during a randomized controlled trial (RCT) of increased energy intake in women with exercise-associated menstrual disturbances (EAMD). **Purpose:** To determine the effect of an RCT of increased energy intake on DR, DT, BD, and bulimia scores in exercising women with EAMD compared to exercising controls with EAMD and ovulatory cycles. **Methods:** The current study includes prospective data in 54 exercising women with EAMD and ovulatory cycles who completed 6 months of an increased energy intake intervention (20-40% above baseline energy requirements) to reverse EAMD and increase bone density. EAMD participants were randomly assigned to a treatment (EAMD+Cal; n=14) or control group (EAMD Control; n=16). Ovulatory women were also recruited and acted as a control group (OV Control;
Measurements of psychometric indicators of eating behavior, energy intake, body weight, body composition, energy status, and metabolic hormones were completed at baseline, and repeated at weeks 9 and 21 (IWk9 and IWk21). A one-way ANOVA with repeated measures was performed to compare dependent variables over time. **Results:** Participants were 23.0±0.6 yr, weighed 58.2±0.9 kg with 25.0±0.6 percent body fat, exercised 497±49 min·week⁻¹, and had a VO2peak of 47.8±1.1 mL·kg⁻¹·min⁻¹. Baseline DR score was higher in the EAMD+Cal (p=0.006) and EAMD Control groups (p=0.017) vs. the OV control group (10.7±0.8 and 10.9±1.2 vs. 7.1±1.1, respectively). No differences were observed at baseline (p>0.05) among groups in DT, BD, and bulimia scores. A group x time interaction (p=0.004) and group effect (p<0.001) was observed for energy intake, such that the EAMD+Cal group increased energy intake from baseline to IWk21 (p=0.003); whereas, no change in energy intake from baseline to IWk21 (p>0.05) was observed in the OV Control and EAMD Control groups. A time (p<0.001) and group effect (p=0.030) was observed for DR score. Specifically, in the EAMD Control group, DR score was lower at IWk9 (p=0.001) relative to baseline. However, at IWk21, DR score in the EAMD Control group did not differ from baseline (p>0.05). Notably, by IWk21, there were no differences (p=0.054) in DR score among groups. There was no group x time interaction, time, and group effects (p>0.05) for DT, BD, and bulimia scores. **Conclusion:** In summary, refeeding did not exacerbate DR or promote adverse outcomes for DT, BD, and bulimia scores in exercising women with EAMD across the 6 month intervention. Thus, an RCT of increased energy intake with psychological and nutritional monitoring was associated with favorable outcomes for eating attitudes and behavior in exercising women with EAMD.
Introduction

Disordered eating attitudes and behavior have well-known negative implications for clinical sequelae in exercising women, including disturbances in menstrual function, bone loss, and fractures (21,40,71). Prior research findings in exercising women exhibit strong interrelationships among disordered eating behavior, chronic energy deficiency, and amenorrhea (21,40,71), all hallmark conditions associated with the Female Athlete Triad (Triad). Notably, disordered eating behavior is often associated with the progression from a healthy energy status to increasingly unhealthy presentations of low energy availability (EA) and chronic energy deficiency (21,26,40). To this end, the etiology of Triad-related clinical outcomes in exercising women frequently begins with conscious aberrant eating behavior, i.e., energy restriction and/or high exercise energy expenditure, motivated by sport-specific or sociocultural pressure to achieve often unrealistic standards of body size and physical attractiveness (67). Consequently, significant reductions in energy intake relative to energy expenditure may contribute to the development of a chronic energy deficiency in exercising women (26,40,62,74).

Exercising women with disordered eating consistently demonstrate higher scores on psychometric subscales assessing dietary cognitive restraint (DR), drive for thinness (DT), body dissatisfaction (BD), and bulimia symptoms (10,34,38,51,74). High DR is associated with restrictive eating behavior and lower EA (9,39,58) and thus, may play a role in the etiology of the Triad. As such, several investigators have linked high DR to menstrual disturbances (7,8,39,51,74) and compromised bone health (8,9,52). High DT is another cornerstone feature of disordered eating characterized by preoccupation with body weight and fear of weight gain (36). A high DT is shown to be associated with a disruption in
energy homeostasis translating to metabolic and reproductive dysfunction in exercising women (26,40). BD and bulimia-related symptoms are other behaviors associated with disordered eating (21,34,71,72) such that higher BD may be linked to weight loss or cycling and restrained eating; whereas, bulimia symptoms include purging, binge eating, and aberrant exercise behavior.

The primary aim of treatment in exercising women with menstrual disturbances with or without disordered eating is to reverse chronic energy deficiency via modifications in diet primarily, and if necessary, modifications in exercise behavior (57). This treatment approach focuses on improvement of overall energetic status by increasing energy intake, and perhaps reducing energy expenditure, or a combination (32,47,76). The efficacy of dietary interventions for chronic energy deficiency and related menstrual disturbances has been examined in studies of women with anorexia nervosa (17,46,54,55), retrospective study of female athletes with menstrual disturbances (5), case studies of amenorrheic exercising women (32,47), and experiments in female cynomolgus monkeys (75,76). However, the effect of increased energy intake on eating attitudes and behaviors in women with exercise-associated menstrual disturbances (EAMD) during an intervention to reverse menstrual disturbances and improve bone density has yet to be explored.

Findings in women with clinical eating disorders, such as, anorexia nervosa and bulimia nervosa, demonstrate positive outcomes for disordered eating behavior following treatment and refeeding (17,46,66). Specifically, improvements in DT and bulimia score without compromising weight gain have been well-documented (17,46,66). However, BD is often high even after weight restoration in women with anorexia or bulimia nervosa (23,37,46,69). To our knowledge, changes in eating attitudes and behaviors in women with
EAMD across a randomized controlled trial (RCT) of increased energy intake have not been investigated. The purpose of this study is to characterize changes in DR, DT, BD, and bulimia scores across an RCT of increased energy intake in exercising women with EAMD compared to exercising controls with EAMD and ovulatory cycles. First, we hypothesize that baseline DR, DT, BD, and bulimia scores will be higher in exercising women with EAMD vs. exercising women with ovulatory cycles. Second, we hypothesize that DR, DT, BD, and bulimia scores will decrease in exercising women with EAMD who increased energy intake during the 6 month RCT, such that there will be no differences in DR, DT, BD, and bulimia scores at 6 months between the exercising women with EAMD who increased energy intake and exercising controls with ovulatory cycles. Third, we expect that there will be no changes in DR, DT, BD, and bulimia scores in exercising controls with EAMD across the 6 month intervention, such that these psychometric indicators of eating behavior will be higher in this group at 6 months compared to the other two groups (exercising women with EAMD who increased energy intake and controls with ovulatory cycles).

**Methods**

**Experimental Design:** We used a prospective repeated measures design to determine the effect of increased energy intake on psychometric indicators of eating behavior (DR, DT, BD, and bulimia scores) in the exercising women who increased energy intake across the 6 month intervention (EAMD+Cal) vs. Control groups with EAMD (EAMD Control) and ovulatory cycles (OV Control). This study included data from an RCT that was designed to assess the effects of 12 months of increased energy intake (20-40% above baseline energy
requirements) on indices of bone health and menstrual status in women with EAMD, including functional hypothalamic amenorrhea (FHA) and oligomenorrhea vs. exercising control participants with (EAMD Control) and without menstrual disturbances (OV Control). The study was conducted at two sites, University of Toronto (UT) and the Pennsylvania State University (PSU) over 7 years. Participants (OV and EAMD women) were recruited on a rolling basis and observed for 12 months. All participants were “exercising” and currently participating in at least two hours·week\(^{-1}\) of purposeful exercise. All participants who completed 6 months of the RCT were eligible for our analysis.

**Participants:** Participants were young adult women (18-35 yr) at UT and PSU. Recruitment was accomplished through the university campus and community newspapers, television, radio, and bulletin fliers. Eligibility criteria for this study was: (1) aged 18-35yr; (2) good health as determined by a medical exam; (3) body mass index (BMI) 16-25 kg·m\(^{-2}\); (4) no chronic illness, including hyperprolactinemia and thyroid disease; (5) currently participating in at least two hours·week\(^{-1}\) of purposeful exercise; (6) non-smoker; (7) not currently dieting; (8) not taking any hormonal therapy for at least six months; (9) no current clinical diagnosis of eating or psychiatric disorders; (10) not pregnant or lactating or planning a pregnancy; (11) no medication use that would alter metabolic or reproductive hormone concentrations; and (12) no other contraindications that would preclude participation in the study. Women reporting regular menstrual cycles of 26-35 days were eligible for the OV Control group; whereas, women who reported no menses in the past three months or ≤6 cycles in the past 12 months were eligible for the EAMD groups.
**Figure 1** shows the progression of participants through the study. One hundred and eighty-five participants were assessed for eligibility for the study during screening procedures. Fifty-three women were excluded during screening, i.e., 6 for high BMI, 6 moved out of area/not in the area, 6 lost interest, 5 for time commitment, 3 lost to follow-up, and 27 for other reasons. One hundred and thirty-two women entered baseline. Twenty-four women were eliminated during baseline, i.e., 7 for time commitment, 2 were non-compliant with study protocol, 2 decided to go on oral contraceptives, 3 demonstrated cycle length inconsistent with initial self-reported menstrual category, and 10 reported other reasons. Thirty-nine women were assigned to the OV Control group. Thirty-five and 34 women were randomized to the EAMD Control and EAMD+Cal groups, respectively. Forty-eight women were eliminated during the intervention. Sixty women completed 6 months of the study and 54 women were included in our analyses. Of those excluded, 3 participants had missing data for dependent variables, 1 participant had a medical issue affecting reproductive health, and 2 participants demonstrated baseline menstrual status inconsistent with initial self-reported menstrual category.

**Screening Procedures:** During the screening period, participants were informed of the purpose, procedures, and potential benefits/risks of study participation prior to signing an Informed Consent approved by the Biomedical Institutional Review Board at PSU or the Human Ethics Boards at UT. Once consent was obtained, height (cm) and weight (kg) were measured, and questionnaires were completed to assess demographic information, medical and menstrual history, eating attitudes and behaviors (36,64), exercise participation, bone health, and psychological health (15,22,31,68). The EDI-2 and TFEQ were administered.
Figure 1. Flow diagram of progression of participants through the randomized controlled trial. OV Control = exercising controls with ovulatory cycles; EAMD Control = exercising controls with menstrual disturbances; EAMD+Cal = exercising women who increased energy intake; OCs = oral contraceptives.
A physical exam was performed to determine health status and to rule out any physical signs or symptoms of polycystic ovarian syndrome (i.e., acne, hirsutism) or eating disorders. A fasting blood sample was obtained and analyzed for complete blood count, CHEM-24, and an endocrine panel, which included follicular stimulating hormone, luteinizing hormone (LH), estradiol, prolactin, thyroid stimulating hormone, thyroxine, total and free testosterone, and dihydroepiandrosterone sulfate. Results of the blood test were used to rule out endocrine and metabolic disease. Participants completed a 3-day diet log for assessment of dietary energy intake and a 7-day exercise log for assessment of purposeful exercise energy expenditure. A research psychologist with trained expertise in clinical eating disorders completed a semi-structured interview with each participant to rule out current clinical eating disorders and other psychiatric disorders. Participants met with a registered dietician to determine the participants’ eating patterns, relationship with food, and food preferences. A dual-energy x-ray absorptiometry (DXA) scan was performed to assess body composition (percent body fat, fat mass (kg), and lean body mass (LBM) (kg)) and bone mineral density (BMD). Subjects were scanned on either a GE Lunar Prodigy (n=27, enCORE 2002 software version 6.50.069), a GE Lunar iDXA (n=24, enCORE 2008 software version 12.10.113) or a Hologic QDR4500 DXA scanner (n=3, Hologic Inc., Bedford, MA). Consistent with the International Society of Clinical Densitometry guidelines, a cross-calibration study was performed to remove systematic bias between the systems, as previously published (39).

**Baseline Procedures:** During baseline, participants collected daily urine samples for a 28-day monitoring period if in the EAMD group or for an entire menstrual cycle if in the OV
Participants began a calcium and vitamin D run-in period on day 1 of week 1 of baseline which coincided with the first day of menses for the Ov Control group and a random day for the EAMD groups. All groups received oral calcium and vitamin D3 supplements to ensure that they consumed the adequate intake (AI) of 1200 mg/per day of calcium and 400 IU of vitamin D (usual dietary intake was considered in achieving this goal and supplemented when necessary). Calcium and vitamin D3 were used as control measures similar to other studies of bone health (6,14,33,50). The dosage targeted was the current recommendation for AI for both calcium and vitamin D3 (43,44). During baseline week 3, participants arrived at the Women’s Health and Exercise Laboratory between 600-830hr (fasted and having refrained from exercise and caffeine for the prior 24hr and alcohol for the prior 12hr) and completed the following: (i) body weight and body composition measurement (via DXA), (ii) REE testing, (iii) blood sample measurement for the determination of metabolic hormone profile (specifically TT3), iv) peak oxygen uptake (VO2peak) test to evaluate physical fitness (often completed on a separate occasion) and v) a 3-day diet log and a 7-day exercise log (see below).

**i) Anthropometric and Body Composition Assessment:** Total body weight was measured to the nearest 0.1 kg. Height was measured to the nearest 1.0 cm. BMI was calculated as the average body weight divided by height squared (kg·m⁻²). Baseline values for body weight and BMI were reported as the average of all baseline and screening measurements. Baseline body composition was assessed using DXA to determine LBM for use in the calculation of resting energy expenditure (REE) controlled for LBM.

**ii) Resting Energy Expenditure Test:** REE was determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax Series, Yorba Linda, CA, USA) by methods
previously published in detail (30,40). REE was corrected for LBM (REE/LBM). We compared lab assessed REE to a prediction equation (42) for REE to estimate how much each individual’s measured REE differed from the predicted REE (REE/pREE) (26,27,40,62,74). In women with anorexia nervosa (46,53,59), the majority of data published utilized the Harris-Benedict equation (42) to predict REE, and as such, we determined that this equation was most useful for our purposes.

**iii) Dietary Energy Intake:** Energy intake (kcal·day\(^{-1}\)) was assessed using three-day diet logs recorded for 2 week days and one weekend day, as previously described (39,40). Baseline values for energy intake were reported as the average of baseline and screening measurements. Three-day diet logs recording energy intake have been shown to provide comparable data to 7-day logs in women who may under-report their energy intake, including lean women (41). Additionally, 3-day diet logs have been shown to reduce participant burden and improve compliance (49). On-site registered dietitians met with the participants to instruct them on how to accurately record energy intake. Participants were specifically instructed to measure (using standard measuring cups/tools) and record all food and beverages consumed in detail. Participants were also asked to record time and location of every eating episode. The nutrient data from the 3-day logs were coded and analyzed using the Nutrition Data System for Research (NDSR 2008 Version; University of Minnesota; Minneapolis, MN, USA).

**iv) Purposeful Exercise Energy Expenditure:** Participants kept logs of their purposeful exercise during the study as per a previous publication (39,40). These logs provided a measurement of exercise volume over a 7-day period (min·wk\(^{-1}\)). Purposeful exercise energy expenditure was estimated at baseline using a polar heart rate monitor. Energy expended
during these purposeful exercise sessions was measured using the OwnCal feature of the Polar S610 or RS400 heart rate monitors (Polar Electro Oy, Kempele, Finland) (24). The OwnCal feature has been validated for the use in calculating exercise energy expenditure from heart rate. The Polar S601 and RS400 heart rate monitors include rest in their estimation of energy expenditure. To estimate only exercise energy expenditure, we subtracted the most recently measured REE (kilocalories·min⁻¹) from the Polar heart rate monitors’ estimation of energy expenditure. For purposeful exercise sessions in which participants did not wear the Polar S610 or RS400 heart rate monitors, the Ainsworth et al. (2,3) compendiums of physical activities were used to determine the appropriate metabolic equivalent (MET) level for the exercise performed (1). To calculate the energy expended during the exercise session, the MET level was multiplied by the duration (min) of the exercise session and the measured REE (kcal·min⁻¹). The MET value includes a resting component. To estimate only exercise energy expenditure, we subtracted the most recently measured REE (kcal·min⁻¹) from this value.

v) VO₂peak Test: VO₂peak (mL·kg⁻¹·min⁻¹) was measured during a progressive treadmill test to volitional exhaustion using indirect calorimetry on a single occasion during the study as per a previous publication (40).

vi) Blood Sample and Serum Hormone Measurement: Blood samples were collected between 0700 and 1000h, stored, and processed as previously described (40). TT₃ was analyzed using a chemiluminescence-based immunoassay analyzer as previously described (40). The analytical sensitivity for the TT₃ assay was 0.54 nmol/L, and the intra-assay and inter-assay coefficients of variation were 13.2% and 15.6%, respectively.
**Classification of Baseline Menstrual Status:** Initial classification of menstrual status prior to the intervention was based on self-reported menstrual history, results of physical exam, and urinary estrone-1-glucuronide (E1G), pregnandiol glucuronide (PdG), and LH profiles. Participants recorded menses and/or other menstrual symptoms (i.e., cramps, spotting, discharge, etc.) daily on menstrual calendars. Eumenorrheic women collected daily urine samples for one menstrual cycle, oligomenorrheic women for no more than 90 days, and women with FHA for one 28-day monitoring period. Menstrual cycle length was defined as the number of days from the first day of menses up to the day preceding the next menses (28,29). Daily first morning void urine samples were assayed for LH, E1G, and PdG to assess ovulatory status. Ovulatory status was determined by the day of the urinary LH surge, identified as an LH peak on the day of or within a few days after the mid-cycle E1G peak (28,29). Specific hormonal criteria for detecting ovulation included a LH surge concentration above 25 mIU·mL⁻¹, an E1G peak concentration above 35 ng·mL⁻¹ and a peak PdG concentration above 5 µg·mL⁻¹ during the luteal phase (29,30,45,61).

Menstrual status was defined as EAMD if a participant was FHA (reported no menses for the past 3 months) or oligomenorrheic (reported irregular menses at intervals of 36-90 days). Menstrual status was defined as ovulatory if a participant was eumenorrheic (reported regular menses at intervals of 26-35 days) and ovulatory. Self-reported menstrual status was then confirmed prospectively by classifying menstrual cycles by length of the intermenstrual interval, length of follicular and luteal phases, the presence of menses, and by ovulatory status (ovulatory or anovulatory) as described in previous publications from our lab (28,29). For graphing purposes, the E1G and PdG data for eumenorrheic, ovulatory women were aligned by the day of the LH peak defined as Day 0. The FHA,
oligomenorrheic, and anovulatory participants’ E1G and PdG data were aligned by chronological day of urinary collections.

**Intervention Procedures**

Prior to the start of the intervention, participants with EAMD were randomly assigned to either a treatment group (EAMD+Cal) or a control group (EAMD Control).

**i) Energy Prescription:** The EAMD participants randomly assigned to the treatment group (EAMD+Cal) were provided an energy prescription of increased energy intake 20-40% above baseline energy requirements and asked to maintain their usual exercise training regimen for the intervention phase of the study. Baseline energy requirements for this study were operationally defined as the sum of laboratory-measured REE and purposeful EEE. Participants in the EAMD+Cal group were requested to increase their energy intake through the use of nutritional and sports energy supplements or with foods they typically eat. Energy bars (primarily PowerBars, Clif Bars) that contained approximately 220-300 calories were provided by the research staff and used as a strategy to increase energy intake throughout the day. Participants in the EAMD Control group were asked to maintain their baseline physical activity level and energy intake.

**ii) Nutritional Intake Monitoring:** The EAMD+Cal participants met with a registered dietician at screening and baseline then bi-weekly for the first 3 months and monthly for the remainder of the study. The dietician evaluated whether the participant was a good candidate for a study in which they may have to increase energy intake. The dietician also provided the participant with specific instruction on how to record a 3-day diet log to accurately assess their food and beverage intake during the study. Calcium intake was also assessed (56).
Those participants with dietary habits that did not comply with study protocol were excluded. Participants were monitored by the dietician for compliance to energy prescription (i.e., review participants’ diet logs and provide strategies to achieve prescribed energy intake) and changes in nutritional and eating behavior characteristics. The participants in the EAMD and OV Control groups met with the registered dietician at monthly and 3-month intervals throughout the study, respectively.

**iii) Psychological Status/Behavior Monitoring:** The EAMD+Cal participants met with a clinical psychologist bi-weekly for the first three months and then monthly for the remainder of the study to monitor general psychological and eating behavior status and provide assistance in implementing the energy prescription and other lifestyle changes to ensure compliance to the intervention. The participants in the EAMD and OV Control groups met with the clinical psychologist at monthly and 3-month intervals throughout the study, respectively.

**iv) Study Protocol:** Psychometric assessment of eating behavior (EDI-2, TFEQ), energy intake (3-day diet logs), body weight, body composition (via DXA), energy status (REE test), and metabolic hormones (fasting TT3) were repeated at weeks 9 and 21 (IWk9 and IWk21). OV Control women collected daily urine samples for an entire menstrual cycle on four occasions throughout the intervention, whereas EAMD+Cal and EAMD Control groups collected daily urine samples for the entire intervention. Menstrual bleeding/symptoms and exercise training were monitored throughout the study using menstrual calendars and seven-day purposeful exercise logs.
Primary Psychometric Measures

i) TFEQ: The TFEQ is a 51-item questionnaire that measures three dimensions of eating behavior: (1) DR, (2) disinhibition, and (3) hunger (64). This questionnaire is composed of two parts: the first 36-items use a dichotomous (true/false) response format, while the last 15-items use a 4-point Likert scale response format. The TFEQ has been shown to demonstrate good reliability, validity, and internal consistency (Cronbach’s=0.87) (64).

ii) EDI-2: The EDI-2 is a widely used self-report measure of symptoms typically associated with the psychopathology of clinical eating disorders (36). The EDI-2 is a 91-item questionnaire from which standardized subscale scores on 11 dimensions are generated. Reliability coefficients for this inventory are between 0.83 and 0.93, and test-retest reliability coefficients for all subscales range from 0.79 to 0.95, except for interoceptive awareness (0.67). We presented data from the three subscales used to assess attitudes and behaviors concerning eating, weight, and shape (DT, BD, Bulimia). The items are presented on a 6-point Likert scale requiring respondents to answer whether each item applies “always”, “usually”, “often”, “sometimes”, “rarely”, or “never”.

Statistical Analyses: All statistical analyses were conducted using SPSS version 19.0 (SPSS, Inc., Chicago, IL). All hypothesis tests were two-sided and p<0.05 was considered significant unless otherwise noted. Data screening was conducted prior to analysis, involving outlier detection and evaluation of assumptions of normality and sphericity. Descriptive statistics were expressed as mean and standard error mean and frequency and percentage for categorical data. Psychometric survey data was recoded and scored to determine subscale scores. Baseline measurements were examined using one-way analysis of variance analysis (ANOVA). When main effects were observed, post hoc analyses were
performed using least significance difference (LSD) procedures. Repeated measures ANOVA with one grouping factor (EAMD+Cal vs. EAMD Control vs. OV Control) was performed to compare psychometric indicators of eating behavior, energy intake, body weight, body composition, and metabolic characteristics over time (baseline, IWk9, and IWk21). When main effects were observed, post hoc analyses were performed using t-tests with a Bonferroni correction, comparing baseline to each time point for a total of two tests (p<0.025), and one-way ANOVA, comparing differences among groups. When variables were not normally distributed, the Kruskal-Wallis test was performed to compare differences among groups and Mann Whitney tests were then used to determine pairwise differences. The Wilcoxon rank sum test was used to confirm significant time effects.

Pearson bivariate correlation analyses were performed to determine: (i) the associations among changes (from baseline to IWk21) in DR, DT, BD, and bulimia scores in the total sample and (ii) the association between changes (from baseline to IWk21) in psychometric indicators of eating behavior (DT, DR, BD, bulimia) and change in anthropometric/body composition characteristics in the EAMD+Cal group. All sample size calculations were performed using G Power 3.1.2 (Universitat Kiel, Germany, 2009). We based our sample size calculations on data (means and SDs) obtained from the literature (16,46). Sample size calculations were performed to determine the number of participants per group required to detect differences in DT, BD, and DR scores across three time points (Baseline, IWk9, IWk21) using a Repeated Measures ANOVA with one grouping factor (EAMD+Cal vs. EAMD Control vs. OV Control). Our sample size calculations indicated that 30 women (10 women per group) will provide sufficient power (1-β = 0.95) to detect significant relationships at a large effect size of 0.78.
Results

Baseline descriptive characteristics: Participants were 23.0±0.6 yr and weighed 58.2±0.9 kg with 25.0±0.6 percent body fat. Participants exercised 497±49 min·week⁻¹ and had a mean VO₂peak of 47.8±1.1 mL·kg⁻¹·min⁻¹. The participants were primarily recreationally active women (83%) with a small proportion representing competitive athletes on a varsity/elite-level team (17%). Running was the most common primary mode of exercise in our women (52%), with 7% and 6% of women participating in multiple forms of cardiorespiratory fitness (i.e., running, gym-based cardio, cycling) and cycling as primary modes of exercise, respectively. Baseline descriptive characteristics are presented in Table 1.

All groups had similar (p>0.05) anthropometric, body composition, and exercise training characteristics. The OV control group was gynecologically more mature (p=0.004) than the EAMD control group. The EAMD+Cal and EAMD Control groups had a later age of menarche than the OV control group (p<0.05). Baseline REE (p=0.004), REE/LBM (p=0.004), and pREE/REE (p<0.001) were higher in the OV control group than the EAMD control group. Baseline fasting TT₃ concentrations did not differ among groups (p=0.188). Menstrual composite graphs of daily urinary reproductive excretion are shown in Figure 2.

Baseline psychological indicators of eating behavior: Baseline psychological indicators of eating behavior are presented in Table 2. Baseline DR score was higher in the EAMD+Cal (p=0.006) and EAMD Control groups (p=0.017) compared to the OV control group. No baseline differences in DT, bulimia, and BD scores were observed (p>0.05) among groups. Baseline DR was positively correlated with baseline BD (r=0.513, p<0.001) and DT (r=0.636, p<0.001). Baseline BD was positively correlated with baseline DT (r=0.747, p<0.001) and bulimia symptoms (r=0.322, p=0.019).
Table 1. Baseline descriptive characteristics of exercising women categorized by randomized controlled trial group (ovulatory (OV) control, exercise-associated menstrual disturbances (EAMD) control, and EAMD intervention (EAMD+Cal)).

<table>
<thead>
<tr>
<th></th>
<th>OV Control (n=24)</th>
<th>EAMD Control (n=16)</th>
<th>EAMD+Cal (n=14)</th>
<th>Main Effect of Group p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>24.0±1.1</td>
<td>21.1±0.8</td>
<td>23.5±1.0</td>
<td>0.100</td>
</tr>
<tr>
<td><strong>Anthropometric and Body Composition Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.9±1.2</td>
<td>166.2±1.5</td>
<td>165.5±1.7</td>
<td>0.808</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>58.8±1.0</td>
<td>58.2±2.0</td>
<td>57.1±1.8</td>
<td>0.730</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7±0.4</td>
<td>21.0±0.6</td>
<td>20.9±0.5</td>
<td>0.439</td>
</tr>
<tr>
<td>Percent Body Fat</td>
<td>25.3±0.8</td>
<td>25.6±1.5</td>
<td>23.7±1.2</td>
<td>0.532</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>14.9±0.6</td>
<td>15.1±1.1</td>
<td>13.5±0.8</td>
<td>0.406</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>41.9±0.8</td>
<td>40.4±1.2</td>
<td>41.8±1.4</td>
<td>0.554</td>
</tr>
<tr>
<td><strong>Reproductive Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of Menarche (yr)</td>
<td>12.2±0.2§</td>
<td>13.8±0.6</td>
<td>14.2±0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Gynecological Age (yr)</td>
<td>11.8±1.1§</td>
<td>7.4±0.9</td>
<td>9.1±1.2</td>
<td>0.013</td>
</tr>
<tr>
<td>Cycle Length (d)</td>
<td>29.7±2.3</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of Amenorrhea (d)</td>
<td>N/A</td>
<td>207±60</td>
<td>233±51</td>
<td>0.743</td>
</tr>
<tr>
<td><strong>Dietary and Metabolic Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary Energy Intake (kcal/d)</td>
<td>1989±116</td>
<td>1709±110</td>
<td>1987±98</td>
<td>0.167</td>
</tr>
<tr>
<td>REE (kcal/d)</td>
<td>1314±28§</td>
<td>1155±40</td>
<td>1238±56</td>
<td>0.016</td>
</tr>
<tr>
<td>REE (kcal/kg LBM)</td>
<td>31.4±0.5§</td>
<td>28.6±0.8</td>
<td>29.9±0.8</td>
<td>0.014</td>
</tr>
<tr>
<td>REE/pREE</td>
<td>0.94±0.02§</td>
<td>0.82±0.02</td>
<td>0.88±0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>TT3 (ng/dL)</td>
<td>88.8±1.9</td>
<td>82.1±5.4</td>
<td>77.6±6.8</td>
<td>0.188</td>
</tr>
<tr>
<td><strong>Exercise Training Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise Volume (min/wk)</td>
<td>476±77</td>
<td>424±52</td>
<td>616±121</td>
<td>0.563</td>
</tr>
<tr>
<td>VO2peak (mL/kg/min)*</td>
<td>48.6±1.7</td>
<td>44.5±2.4</td>
<td>49.5±1.9</td>
<td>0.218</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM. Significance of P<0.05 using LSD post hoc analyses (§ = p≤0.017 with Bonferroni correction); Kruskal-Wallis Tests were used to compare age and exercise volume.

a = p<0.05, OV Control vs. EAMD Control and EAMD+Cal
b = p<0.05, OV Control vs. EAMD Control
* = VO2 peak data (n=50)

BMI = body mass index; LBM = lean body mass; REE = resting energy expenditure; REE/pREE = ratio of measured REE compared to Harris Benedict-predicted REE; TT3 = total triiodothyronine; VO2peak = peak aerobic capacity
Figure 2. Menstrual composite graphs of baseline daily urinary reproductive excretion in exercising women when categorized by randomized controlled trial group. Panel A demonstrates the mean daily estrone-1-glucuronide (E1G) and pregnandiol glucuronide (PdG) concentrations of exercising controls with ovulatory cycles (OV Control) (n=24). Panel B demonstrates the mean daily estrone-1-glucuronide (E1G) and pregnandiol glucuronide (PdG) concentrations of exercising controls with exercise-associated menstrual disturbances (EAMD Control) (n=16). Panel C demonstrates the mean daily estrone-1-glucuronide (E1G) and pregnandiol glucuronide (PdG) concentrations of exercising women with exercise-associated menstrual disturbances who increased energy intake (EAMD+Cal) (n=14).
Table 2. Baseline psychometric indicators of eating behavior (obtained from TFEQ and EDI-2) in exercising women categorized by randomized controlled trial group (ovulatory (OV) control, exercise-associated menstrual disturbances (EAMD) control, and EAMD intervention (EAMD+Cal)).

<table>
<thead>
<tr>
<th></th>
<th>OV Control (n=24)</th>
<th>EAMD Control (n=16)</th>
<th>EAMD+Cal (n=14)</th>
<th>Main Effect of Group p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFEQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR score</td>
<td>7.1±1.1a§</td>
<td>10.9±1.2</td>
<td>10.7±0.8</td>
<td>0.008</td>
</tr>
<tr>
<td>EDI-2*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT score</td>
<td>2.2±1.0</td>
<td>2.7±0.9</td>
<td>2.4±0.7</td>
<td>0.061</td>
</tr>
<tr>
<td>Bulimia</td>
<td>0.5±0.2</td>
<td>0.3±0.2</td>
<td>0.7±0.4</td>
<td>0.570</td>
</tr>
<tr>
<td>BD Score</td>
<td>4.8±1.2</td>
<td>4.1±0.9</td>
<td>4.3±1.1</td>
<td>0.906</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM. Significance of P<0.05 (§ = p≤0.017 with Bonferroni correction). Kruskal Wallis Tests were used to compare DR, DT, and BD scores. Post-hoc Mann Whitney tests were used to determine pairwise differences in DR scores. a = p<0.05, OV vs. EAMD+Cal and EAMD Control; * = EDI subscale scores (n=53)

TFEQ = Three factor eating questionnaire; EDI-2 = Eating disorder inventory-2; DR = Dietary restraint; DT = Drive for thinness; BD = Body dissatisfaction.

Change in energy intake: Of the 14 EAMD+Cal group, only one participant was non-compliant to randomization. In the EAMD+Cal group, energy intake increased incrementally relative to baseline: 24.0±2.0% (prescribed energy intake: 2377±114 kcal·d⁻¹) at IWk1, 31.4±2.4% (prescribed energy intake: 2495±120 kcal·d⁻¹) at IWk9, and 37.2±2.9% (prescribed energy intake: 2580±123 kcal·d⁻¹) at IWk21. EAMD+Cal group consumed on average 93.4±4.4%, 99.5±3.9%, and 101.0±6.5% of prescribed energy intake at IWk1, IWk9, and IWk21, respectively (Figure 3). A group x time interaction (p=0.004) and group effect (p<0.001) was observed for energy intake. Specifically, the EAMD+Cal group increased energy intake from baseline to IWk21 (+528±154 kcal·d⁻¹; p=0.003); whereas, no change in energy intake from baseline to IWk21 was observed in the OV Control (+217±117 kcal·d⁻¹; p=0.117) and EAMD Control groups (+91±199 kcal·d⁻¹; p=0.756). Change (%) in energy intake across the 6 month intervention is shown in Figure 4A.
Figure 3. Comparison of actual energy intake (kcal·d⁻¹) (solid bars) vs. prescribed energy intake (kcal·d⁻¹) (open bars) in exercising women with exercise-associated menstrual disturbances who increased energy intake (EAMD+Cal group, n=13) across a 6 month intervention. Note, one participant in the EAMD+Cal group was non-compliant with the increased energy intake intervention.

**Change in anthropometric and body composition characteristics:** Change (%) in body weight across the 6 month intervention is shown in Figure 4B. Change in anthropometric and body composition characteristics across the 6 month RCT is shown in Table 3. There were group x time interactions (p<0.05) for body weight, BMI, percent body fat, and fat mass. No group effects were observed for any anthropometric and body composition variables (p>0.05) across the 6 month intervention. Time effects were observed (p<0.05) for percent body fat and fat mass. Specifically, the EAMD+Cal group increased body weight (+1.7±0.4 kg, p=0.001), BMI (+0.6±0.2 kg·m⁻², p=0.001), and fat mass (+1.2±0.4 kg, p=0.001) from baseline to IWk21; whereas, no change in BW, BMI, and fat mass was observed (p>0.05) in the EAMD Control and OV Control groups from baseline to IWk21. Both the EAMD+Cal and EAMD Control...
groups increased percent body fat (+1.6±0.5%, p=0.006 and +1.1±0.4%, p=0.005, respectively); whereas, no change in percent body fat was observed for the OV control group (-0.6±0.3%, p=0.099).

Figure 4. Change (%) in energy intake and body weight in exercising women categorized by randomized controlled trial (RCT) group across the 6 month intervention. In Panel A, change (%) in energy intake in exercising women categorized by RCT group is shown. In Panel B, change (%) in body weight in exercising women categorized by RCT group is shown. The symbol “a” denotes p<0.05 for OV Control group vs. EAMD+Cal group. The symbol “b” denotes p<0.05 for EAMD+Cal vs. EAMD and OV Control groups. Note, change in energy intake data was reported in 51 participants.
Table 3. Change in anthropometric/body composition characteristics across the 6 month RCT in exercising women categorized by RCT group (ovulatory (OV) control, exercise-associated menstrual disturbances (EAMD) control, and EAMD intervention (EAMD+Cal)).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>IWk9</th>
<th>IWk21</th>
<th>Time Effect p-value</th>
<th>Group x Time Interaction p-value</th>
<th>Group Effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OV Control</td>
<td>58.8±1.0</td>
<td>58.7±1.0</td>
<td>58.5±1.0</td>
<td>0.097</td>
<td>0.003</td>
<td>0.965</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>58.2±2.0</td>
<td>58.3±2.0</td>
<td>58.3±1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAMD+Cal</td>
<td>57.4±1.9</td>
<td>58.1±1.7a</td>
<td>58.9±1.7§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OV Control</td>
<td>21.7±0.4</td>
<td>21.6±0.4</td>
<td>21.5±0.3</td>
<td>0.112</td>
<td>0.003</td>
<td>0.644</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>21.0±0.6</td>
<td>21.1±0.6</td>
<td>21.1±0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAMD+Cal</td>
<td>20.9±0.6</td>
<td>21.1±0.6a</td>
<td>21.4±0.6§</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Percent body fat</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>OV Control</td>
<td>25.3±0.8</td>
<td>24.8±0.8</td>
<td>24.7±0.8</td>
<td>0.003</td>
<td>&lt;0.001</td>
<td>0.637</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>25.6±1.5</td>
<td>26.0±1.5</td>
<td>26.6±1.5§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAMD+Cal</td>
<td>23.7±1.2</td>
<td>24.5±1.1§</td>
<td>25.3±1.1§</td>
<td></td>
<td></td>
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<tr>
<td><strong>Fat mass (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OV Control</td>
<td>14.9±0.6</td>
<td>14.6±0.6</td>
<td>14.5±0.6</td>
<td>0.010</td>
<td>&lt;0.001</td>
<td>0.587</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>15.1±1.1</td>
<td>15.4±1.2</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAMD+Cal</td>
<td>13.5±0.8</td>
<td>14.1±0.8§</td>
<td>14.7±0.7§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LBM (kg)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OV Control</td>
<td>41.9±0.8</td>
<td>42.2±0.8</td>
<td>42.2±0.8</td>
<td>0.859</td>
<td>0.345</td>
<td>0.422</td>
</tr>
<tr>
<td>EAMD Control</td>
<td>40.4±1.2</td>
<td>40.3±1.2</td>
<td>39.9±1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EAMD+Cal</td>
<td>41.8±1.4</td>
<td>41.8±1.4</td>
<td>41.9±1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM.

*P*<0.05 vs. baseline using post hoc t-tests (§ = *p*<0.025 with Bonferroni correction).

BMI = Body mass index; LBM = Lean body mass.
Change in metabolic characteristics: Change in metabolic characteristics (REE, REE/pREE, fasting TT₃) is shown in Figure 5. A group effect was observed for REE (p=0.021), REE/LBM (p=0.041), and REE/pREE (p=0.001). REE was higher in the OV Control group vs. the EAMD Control group at IWk9 (p=0.004). At IWk21, the EAMD Control group had lower REE than both the EAMD+Cal (p=0.026) and OV Control groups (p=0.039) (Figure 5A). REE/pREE was lower (p<0.05) in the EAMD Control group compared to the EAMD+Cal and OV Control groups at IWk9 and IWk21 (Figure 5B). There was no time effect (p=0.113) and group x time interaction (p=0.128) for REE/LBM. No time (p=0.297), group x time interaction (p=0.948), and group effects (p=0.112) were demonstrated for fasting TT₃ concentration (Figure 5C).

Change in psychometric indicators of eating behavior: Change in psychometric indicators of eating behavior across the 6 month intervention is shown in Figures 6 and 7. A time (p<0.001) and group effect (p=0.030) was observed for DR score (Figure 6). Specifically, in the EAMD Control group, DR score was lower at IWk9 (p=0.001) relative to baseline. However, at IWk21, DR score in the EAMD Control group did not differ from baseline (p=0.027). By IWk21, there was no difference (p=0.054) in DR score among groups (9.5±1.2 vs. 8.4±1.1 vs. 6.2±1.0 in the EAMD+Cal, EAMD Control, and OV Control groups, respectively). There were no time, group x time interaction, and group effects (p>0.05) observed for DT, BD, and bulimia scores (Figure 7).
Figure 5. Change in metabolic characteristics (resting energy expenditure, REE (Panel A), ratio of measured REE compared to predicted REE, REE/pREE (Panel B), and fasting total triiodothyronine (TT₃) (Panel C)) in exercising women categorized by randomized controlled trial (RCT) group (exercised associated menstrual disturbances treatment group, EAMD+Cal; exercise associated menstrual disturbances control group, EAMD Control; ovulatory control group, OV Control) across the 6 month intervention (Baseline, Intervention Weeks 9 and 21). The symbol “a” denotes p<0.05 for OV Control group vs. EAMD Control groups. The symbol “b” denotes p<0.05 for OV Control group vs. EAMD+Cal and EAMD+Control groups.
Figure 6. Change in dietary restraint (DR) score obtained from the Three Factor Eating Questionnaire in exercising women categorized by randomized controlled trial group (exercised associated menstrual disturbances treatment group, EAMD+Cal; exercise associated menstrual disturbances control group, EAMD Control; ovulatory control group, OV Control) across the 6 month intervention (Baseline, Intervention Weeks 9 and 21). The symbol “a” denotes p<0.05 for OV Control group vs. EAMD+Cal and EAMD Control groups. The symbol “b” denotes p<0.05 for OV Control group vs. EAMD+Cal group.

**Associations with change in psychometric indicators of eating behavior from baseline to 6 months:** Change in DR score was positively correlated with change in DT (r=0.376, p=0.007). Change in DT score was associated with change in BD (r=0.570, p<0.001) and bulimia scores (r=0.595, p<0.001). Change in BD score was positively correlated with change in bulimia score (r=0.595, p<0.001).
Figure 7. Change in psychological indicators of eating behavior obtained from the Eating Disorder Inventory-2 (drive for thinness (DT) (Panel A), body dissatisfaction (BD) (Panel B), and bulimia scores (Panel C)) of exercising women categorized by randomized controlled trial group (exercised associated menstrual disturbances treatment group, EAMD+Cal; exercise associated menstrual disturbances control group, EAMD Control; ovulatory control group, OV Control) across the 6 month intervention (Baseline, Intervention Weeks 9 and 21).
Associations between change in psychometric indicators of eating behavior and change in anthropometric/body composition characteristics across 6 month RCT of increased energy intake: Associations between change in psychometric indicators of eating behavior and change in anthropometric/body composition characteristics in the EAMD+Cal group are shown in Table 4. Change in DR score was negatively correlated (p<0.05) with change in body weight and BMI. Change in BD score was negatively correlated with change in percent body fat (p=0.048). Notably, in a sub-analysis of the EAMD+Cal group, change in body weight was associated with changes in DR (r=-0.624, p=0.017), fasting TT3 (r=0.689, p=0.006), and fat mass (r=0.598, p=0.024).

Table 4. Association between change in psychometric indicators of eating behavior (DR, DT, and BD scores) and change in anthropometric/body composition characteristics associated with energy status in exercising women who increased energy intake (EAMD+Cal).

<table>
<thead>
<tr>
<th>Psychometric Indicators of Eating Behavior</th>
<th>ΔDT</th>
<th>ΔBD</th>
<th>Δ DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Body weight</td>
<td>-0.282</td>
<td>0.329</td>
<td>-0.486</td>
</tr>
<tr>
<td>Δ BMI</td>
<td>-0.272</td>
<td>0.346</td>
<td>-0.501</td>
</tr>
<tr>
<td>Δ Percent Body Fat</td>
<td>-0.374</td>
<td>0.188</td>
<td>-0.536</td>
</tr>
<tr>
<td>Δ Fat Mass</td>
<td>-0.337</td>
<td>0.238</td>
<td>-0.490</td>
</tr>
</tbody>
</table>

Significance of P<0.05 using Pearson bivariate correlation analyses.
DT = drive for thinness; BD = body dissatisfaction; DR = dietary restraint; REE = resting energy expenditure; REE/LBM = REE controlled for lean body weight; REE/pREE = measured REE compared to predicted REE; TT3 = total triiodothyronine.
Discussion

To our knowledge, this is the first study wherein investigators characterized the changes in psychometric indicators of eating behavior (DR, DT, BD, and bulimia scores) during an RCT of increased energy intake in women with EAMD. Most importantly, in the current study, increased energy intake (i.e., refeeding) did not exacerbate disordered eating attitudes and behavior (specifically DR) or promote adverse outcomes for DT, BD, and bulimia scores in exercising women with EAMD. In terms of clinical significance, following 6 months of increased energy intake, DR score in the EAMD+Cal group normalized and was similar to the OV Control group and consistent with norms in premenopausal women (4,73,74). Furthermore, DT, BD, and bulimia scores in our EAMD+Cal group remained within optimal ranges for eating attitudes and behavior across the RCT (below the 75th percentile), such that the mean values for DT, BD, and bulimia scores were similar with the majority of college-aged women (36). We speculate that our EAMD+Cal women considered the goal of menstruation more important than the control of energy intake and body weight, such that they effectively managed or improved DR concomitant with increased energy intake, weight gain, and favorable responses in REE compared to the EAMD Control group. In studies of women with clinical eating disorders (20,23,37,46,69), BD often remains high following treatment and weight restoration. However, in our EAMD+Cal group, wherein energy intake and body weight increased by 29% and 3% relative to baseline, reductions in BD and DR were observed in association with increases in percent body fat and body weight across the 6 month intervention. Overall, we suggest
that an RCT of increased energy intake with psychological and nutritional monitoring may be associated with favorable outcomes for DR, DT, BD, and bulimia scores during weight gain and improvements in metabolic status in exercising women with EAMD.

Several investigators have demonstrated that high DR is associated with disordered eating behavior (7,8,39) and energy restriction (39,60) subsequent to development of Triad clinical sequelae (9,39,52,74). Specifically, higher DR scores in women with EAMD may be associated with the desire to maintain or achieve a lower energy intake and/or body weight and as such, these women have been shown to demonstrate lower EA and a greater frequency of menstrual disturbances (39,60). Alternatively, we speculate that effective management of or improvements in DR may be linked to a cascade of favorable responses in eating behavior, body weight, and metabolic status. As such, we hypothesized that our RCT of increased energy intake with psychological and nutritional monitoring would be associated with improvements in DR score based on the additional guidance and support provided to the EAMD+Cal women via regular meetings with a nutritionist and a psychologist. Notably, following the 6 month RCT, the EAMD+Cal group demonstrated a DR score similar to the OV Control group and consistent with normative values in premenopausal women (DR score of 9) (4,73,74). This finding was in addition to greater weight gain and improved REE and REE/pREE in the EAMD+Cal women compared to EAMD Controls. Importantly, in 69% of the women (9 out of 13) in the EAMD+Cal group (1 woman was non-compliant), refeeding did not exacerbate DR score (i.e., the DR score stayed the same or improved). Interestingly and contrary to our hypothesis, the EAMD Control
group demonstrated significant reductions in DR score from baseline to IWk9. We speculate that the EAMD Control group may have desired randomization to the treatment group based on goals for menstrual recovery and upon allocation to the control group, these women likely initiated changes in eating behavior (i.e., reductions in DR) without the guidance and support that was provided to the EAMD+Cal group and without increasing energy intake (see Figure 4). As a result, the reductions in DR exhibited by the EAMD Controls were not associated with similar improvements in weight, body composition, and REE as observed in the EAMD+Cal group. Taken together, an RCT of increased energy intake may be associated with favorable outcomes for DR score in addition to improvements in REE and weight gain in exercising women with EAMD.

Several investigators have identified high DT, BD, and bulimia scores as markers of disordered eating behavior and predictive of the development of eating disorders or poor outcomes following treatment (higher DT, BD, and bulimia scores relative to baseline) in exercising women (13,34,38). In our study, refeeding did not adversely affect DT, BD, and bulimia scores in exercising women with EAMD, such that these psychometric indicators of eating behavior were consistent with or lower than norms for college-aged women (below the 75th percentile) (36) during the 6 month RCT of increased energy intake. As such, we propose that the EAMD+Cal women were well-supported by the intervention and accepted goals of menstruation as more important than rigid control of energy intake and body weight. Findings in women with clinical eating disorders (11,23,25,46,77) indicate that treatment and weight restoration may be
associated with positive outcomes for the EDI subscales (particularly, DT and bulimia scores). Konrad et al. (46) demonstrated an improvement in DT score concomitant with weight gain in women with anorexia nervosa who underwent refeeding during a 7-9 week hospitalization. Bratland-Sanda et al. (16) also observed decreases in DT and bulimia score alongside an increase in BMI in adult women with clinical eating disorders following inpatient treatment for 12-20 weeks. Other investigators (20,70) reported lower DT, bulimia, and BD scores in weight/symptom recovered women with anorexia nervosa compared to symptomatic women 5 to 14 yr after initial diagnosis. From a treatment standpoint, women with severe disordered eating may require additional cognitive and psychological support to promote symptom recovery, i.e., clinically significant decreases in DT, BD, and bulimia scores, concomitant with weight restoration and recovery of menses. However, as observed in this study, refeeding combined with psychological and nutritional support may be sufficient to prevent adverse outcomes for eating attitudes and behavior concomitant with increases in body weight and REE in exercising women with EAMD.

In studies of women with clinical eating disorders, BD is often high after weight restoration (23,37,46,69). Positive correlations between BD score and body weight have been observed in young women with and without disordered eating (19,35). To this end, high BD is often associated with relapse among anorexia nervosa patients (18) and weight preoccupation in women with subclinical disordered eating (10,38). Athletes with subclinical disordered eating behavior (mean DT, BD, and bulimia of 9.8, 13.2, 2.4) reported that weight gain (≥2.2 kg or 5 lb) would negatively impact their self-image
and perpetuate fears of becoming fat (10) In our study, in the EAMD+Cal group, we observed favorable psychological outcomes related to body image (i.e., decreases in BD and DR) in association with increases in percent body fat and body weight. We speculate that improvements in body image satisfaction and restrained eating behavior, promoted a greater acceptance of changes in body composition and weight in our exercising women who increased energy intake, and as a result, the importance of weight and shape likely reduced in these participants.

Exercising women are often considered at a greater risk for disordered eating behavior due to the higher incidence of restrained eating, BD, and chronic dieting (21,40,65). In studies of exercising women categorized by menstrual status (21,48,60,62), investigators reported higher DT, DR, BD, and/or bulimia scores in amenorrheic exercising women compared to regularly menstruating exercising women. Contrary to our hypothesis, no baseline differences in DT, BD, and bulimia scores were observed in the EAMD groups compared to the OV Control group. However, DR score was significantly higher in the EAMD women vs. OV Controls. Associations among high DR, restrictive eating, and menstrual disturbances are well-documented (8,39,51,63,74) and findings from our lab (39,60,74) support the observation of menstrual profiles of suppressed E1G and PdG concentrations in our EAMD groups with higher DR. Reed et al. (60) also demonstrated that exercising women with EAMD consume lower energy intake and density than their ovulatory counterparts and suggest that this reduction in kilocalories may be associated with DR scores. Alternatively, we hypothesize that increases in energy intake and density may be linked to a more flexible
approach to eating (reductions in DR) and may represent potential strategies for women with EAMD to reverse chronic energy deficiency (i.e., consume more energy-dense food or an additional snack). Future investigation into the dietary strategies associated with the recovery from chronic energy deficiency and menstrual dysfunction would be valuable in order to design optimal dietary interventions in women with EAMD and high DR.

Further understanding of the complex interrelationships among eating behavior, increased energy intake, and weight gain is necessary in order to implement effective preventive and treatment strategies for disordered eating behavior in exercising women with EAMD. In our intervention, the aim of the psychological semi-structured interview was to provide psychological support around food intake and body image issues, such that the women in the EAMD+Cal group were provided additional guidance to the increased energy intake prescription and monitored for changes in eating behavior. Notably, these sessions did not involve exploratory psychology or cognitive behavioral therapy (CBT). In a study by Berga et al. (12), the effect of CBT vs. observation on reproductive function was investigated in normal weight women with FHA over 20 weeks. The aim of the CBT intervention was to target disordered eating attitudes and behaviors hypothesized to impact reproductive function. Following the intervention, participants were followed for 8 weeks to monitor reproductive hormones (estradiol and progesterone) and 88% of those women who underwent CBT had evidence of ovulation vs. only 25% of those in the control group. However, Berga et al. (12) did not measure changes in energy intake and metabolic status in their amenorrheic women. Thus, it is
unclear if the improvements in menstrual function observed in this study are due to a psychological intervention of CBT or to physiological improvements in energy status linked to an increased energy intake. Nonetheless, CBT is a potential strategy to explore in exercising women with EAMD and disordered eating behavior to prepare women for refeeding and/or weight gain, to enhance their motivation for menstrual recovery, and to manage eating behavior, i.e., promote reductions or prevent adverse increases in DT, DR, BD, and bulimia scores, particularly in those women with higher initial psychometric scores.

The first aim of treatment of chronic energy deficiency and menstrual disturbances in exercising women is to normalize energy status via non-pharmacological strategies (i.e., increasing energy intake or decreasing exercise energy expenditure, or both) (57). In our study, we observed favorable responses in key outcomes such as energy intake (29% increase), body weight (3% increase), percent body fat (7% increase) and metabolic markers associated with energy status, i.e., REE/pREE (6% increase), in our EAMD+Cal women across the 6 month RCT. Taken together, our findings translate knowledge on dietary strategies to improve energy status in women with EAMD secondary to chronic energy deficiency.

In conclusion, our RCT of increased energy intake did not exacerbate DR or adversely impact DT, BD, and bulimia scores in women with EAMD. Based on these findings, an increased energy intake intervention with psychological and nutritional monitoring may be associated with favorable outcomes for disordered eating symptoms, weight gain, and REE. Future prospective investigation of the effect of an increased
energy intake intervention on eating attitudes and behavior in exercising women with higher DT, DR, BD, and bulimia scores is necessary. Cluster analysis to identify subgroups of women with different responses to the intervention would provide valuable information for developing optimal treatment strategies for exercising women affected by disordered eating behavior concomitant with Triad clinical sequelae. Last, evaluation of additional constructs related to values and priorities for weight control vs. physical health or self-esteem derived from weight, controlled eating, or athletic/exercise performance would also improve our understanding of changes in eating behavior in women with EAMD following non-pharmacological treatment. To this end, consensus on an optimal non-pharmacological approach for disordered eating behavior and recovery from the Triad clinical sequelae is required in order to establish and implement effective preventive and treatment strategies in exercising women with EAMD.
References


Chapter 6

STUDY FOUR

Gibbs JC, Nattiv A, Barrack MT, Williams NI, Rauh MJ, Nichols JF and De Souza MJ.

Abstract

The cumulative effect of the Female Athlete Triad (Triad) risk factors on the likelihood of low bone density (BMD) in exercising women is unclear. Purpose: To determine the risk of low BMD in exercising women with multiple Triad risk factors.

Methods: We retrospectively examined data from 437 exercising women (mean age of 18.0±3.5yr, weighed 57.5±7.1kg with 24.5±6.1% body fat) from 4 prospective cohort studies examining Triad risk factors. Questionnaires were completed to obtain information on demographic characteristics, self-reported eating attitudes/behaviors, menstrual function, sport/activity participation, and medication use. Height and body weight were measured. BMD was measured using dual energy x-ray absorptiometry. Low BMD was defined as Z-Scores of <-1 and ≤-2. Chi-square tests were performed to determine the percent of women with low BMD who met criteria for single (current oligo/amenorrhea, late menarche, low body mass index (BMI), elevated dietary restraint, lean sport/activity participation) or multiple (2, 3, 4, or 5) Triad risk factors. Results: Late menarche and low BMI were
associated with the highest percent of low BMD (Z-Score<-1), 55% and 54%, respectively and low BMD (Z-Score≤-2), 14% and 16%, respectively. The percent of participants with low BMD (Z-Score<-1 and ≤-2) increased from 21-62% and 3-18%, respectively, as women met criteria for an increasing number of Triad risk factors. **Conclusion:** A cumulative number of Triad risk factors were associated with an increasing risk of low BMD, suggesting a dose-response association between the number of Triad risk factors and BMD in exercising women. Further research should be conducted to develop a user-friendly algorithm integrating these indicators of risk for low BMD in exercising women (particularly factors associated with low BMI/BW, menstrual dysfunction, lean sport/activity participation, and elevated dietary restraint).

**Introduction**

The osteogenic impact of exercise on bone health in exercising women is well-documented (12,13) and weight-bearing exercise, in particular, may act as a preventive strategy against low bone mineral density (BMD) and osteoporosis (21). However, in the presence of inadequate energy intake relative to exercise energy expenditure, also known as low energy availability (EA), certain exercising women may be susceptible to low BMD (11) and impaired bone microarchitecture (1). Low EA and/or an energy deficiency may negatively impact longitudinal bone growth and maturation in adolescent female athletes (7), and may promote bone loss in premenopausal exercising women with or without oligo/amenorrhea (20). All of these clinical outcomes are associated with an interrelated syndrome known as the Female Athlete Triad (Triad) (26).
The Triad is a well-documented syndrome in exercising women and is characterized across a continuum of healthy to subclinical and clinical conditions of low EA (with or without disordered eating), menstrual disturbances, and low BMD (26). The Triad is most often observed in exercising women participating in leanness focused sport/activity (34) characterized by stringent weight control, such as long-distance running, gymnastics, and figure skating. Low EA and/or an energy deficiency are frequently associated with elevated dietary restraint and menstrual disturbances that often result in hypoestrogenism, a mechanism for bone loss in exercising women.

The relationship among Triad disorders in exercising women is well-established (19,27,33). Low EA acts to promote low BMD via an energy deficiency (10) and it is presumably the disruptions in metabolic hormones, including reductions in insulin-like growth factor-1 (IGF-1) and leptin (16,38), which suppress bone formation. Additionally, peak bone mineral accrual in girls is strongly associated with menarche (24). Thus, as adolescence is such a critical time period for bone acquisition, positive benefits of exercise on bone may be negated in the presence of a late menarche and menstrual dysfunction (11).

There is emerging interest in the evaluation of risk factors associated with the Triad, which may identify low BMD in exercising girls and women (6,19,30). To date, few investigators have evaluated whether certain combinations of risk factors are associated with a higher cumulative risk for low BMD in exercising girls and women, such that an increase in number of Triad risk factors could be associated with higher risk for low BMD. Thus, a comprehensive examination of the association between Triad risk factors and low BMD in exercising girls and women is warranted. Such data could also provide evidence in support
of preventive strategies for low BMD associated with the Triad in exercising girls and women.

The purpose of this study is two-fold: (1) to evaluate the association between Triad risk factors (individually and in combination) and low BMD in exercising women and (2) to determine whether an increase in the percent of exercising women with low BMD is associated with an increase in number of Triad risk factors. First, we hypothesized that exercising women with menstrual dysfunction (current oligo/amenorrhea or late menarche) and underweight status (low body mass index (BMI) or low body weight (BW)) in combination will demonstrate the highest risk for low BMD (Z-Score < -1 and ≤ -2). Second, we hypothesized that the percent of exercising women with low BMD (Z-Score < -1 and ≤ -2) will increase as women meet the criteria for an increasing number of Triad risk factors, such that the risk for low BMD will be higher in association with a greater number of Triad risk factors. Third, we predicted that current oligo/amenorrhea, late menarche, low BMI, elevated dietary restraint, and lean sport/activity participation will represent significant predictors of risk for low BMD (Z-Score < -1 and ≤ -2) in exercising women.

Materials and Methods

Study design: This retrospective study was designed to examine the percent of and the risk for low BMD associated with Triad factors among a large sample (n=437) of adolescent and adult exercising women. The study consisted of cross-sectional data obtained at baseline from 4 prospective cohort studies completed in the United States and Canada (Pennsylvania State University (PSU), University of Toronto (UT), University of California at Los Angeles (UCLA), and San Diego State University (SDSU)).
Data collection from UCLA, collected between the fall of 1996 and spring of 2001, consisted of female Division I collegiate track and field and cross-country athletes (n=77). Participants were followed, prospectively, for five years. Data collection for the SDSU study occurred during the 2003-2004 academic year. Female interscholastic high school athletes were between ages 13-18 yr (n=325). Prior to the 3-4 month sport season, participants were evaluated for risk factors for low BMD. Data collection from PSU and the UT, between 2005 and 2011, consisted of data from a cross-sectional study designed to assess cardiovascular status in exercising women (n=54) and data from the baseline period of a prospective study designed to assess the effects of a 12-month intervention of increased energy intake on indices of bone health and menstrual status in exercising women with severe menstrual disturbances vs. exercising women with regularly ovulatory menstrual cycles (n=202). Participants in the PSU and UT studies were young, healthy adult women primarily from the general population of college-aged women (18-35 yr). These participants engaged in two or more hours/week of purposeful exercise and included competitive and recreational-level exercising women.

For each study, one or more questionnaires were completed to obtain demographic and general background information including eating attitudes and behaviors, menstrual function, sports participation, exercise, and medication use. Height and weight were measured. BMD and body composition were measured for each participant using dual-energy x-ray absorptiometry (DXA). Each of the studies excluded individuals taking hormonal therapy and medications known to affect BMD. Each study was approved by their respective university Institutional Review Board.
Merging databases: Study databases were merged in a careful and rigorous manner. Definitions of terms and methodology for the collection of variables from each study were reviewed. Data for variables that were measured and defined similarly among databases were merged. For variables that represented a similar data construct, but were not defined and/or measured identically, a new variable, with a definition that combined the representations of the data construct, was created. For example, among the databases, amenorrhea was defined as the absence of 3 or more consecutive menstrual cycles in the past year and reporting a frequency of less than 4 menstrual cycles in the past year. In these situations, a new variable combining both definitions was used. Categorical variables that were collected and defined similarly but coded differently among databases were re-coded to ensure consistency. Only participants with complete data collection for all examined study variables were included in this manuscript (n=437). As such, participants with missing data for any one of the examined study variables were excluded from analyses (n=221).

Participants: This study sample included 437 adolescent and adult women (ages 13-35 yr), with 17 from the UCLA site, 272 from the SDSU site, 34 from the UT site, and 114 from the PSU site. Participants were either recreational- or competitive-level exercising women who were involved in a variety of sports and exercise training. Recreational exercising women (n=117) participated in ≥2 hours per week of “purposeful exercise” and met criteria for an exercising status as per ACSM Guidelines (2). Competitive exercising participants (n=320) were current members of a high-school or collegiate athletic team. Inclusion criteria for this study were: 1) age 13-35 yr, 2) no history of any chronic illness, 3) not taking any hormonal therapy in the past 12 months, and 4) not taking any medications that affect BMD. Written
consent from participants (if participant was 18yr or older) or participants and parents 
(SDSU study) was obtained prior to participation.

**Anthropometric data:** Height and weight, without shoes, was measured in each lab to the 
nearest 1.0 cm and 0.5 kg, respectively. BMI was calculated as a ratio of weight to height  
(kg·m⁻²).

**Bone density and body composition:** BMD (areal BMD, bone mineral content, and bone 
area) and body composition (percent body fat, fat mass, fat-free mass and lean body mass) 
(LBM)) were analyzed by a certified technician using DXA. Participants were scanned on 
either a GE Lunar (GE Lunar Corporation, Madison, WI) DPX-NT (n=272) Prodigy (n=34),  
iDXA (n=114), or a Hologic QDR 4500A (n=17, Hologic Inc., Bedford, MA) DXA scanner.  
For the SDSU study, the coefficients of variation (CV) for the BMD measurements were 
0.6% for the total hip, 1.2% for the lumbar spine (L1-L4), and 0.99% for the total body. For 
the UCLA study, the CVs were 1% for total hip, 1% for the lumbar spine (L1-L4), and 1%  
for the total body. For the UT study, the CVs were 0.47% for the total hip, 1.0% for the  
lumbar spine (L1-L4), 1.03% at the femoral neck, and 0.65% at the total body. For the PSU  
study, the CVs were 0.61% at the total hip, 0.93% at the lumbar spine (L1-L4), 0.97% at the  
femoral neck, and 0.66% at the total body. Since no cross calibration study was completed,  
data analyses for primary objectives used standardized scores only.

**Menstrual status:** Menstrual function was determined in all participants using a 
questionnaire. Our criteria for menstrual status considered the number of self-reported  
menstrual cycles in the past 12 months or menstrual cycle length prior to study enrollment.  
Categories of menstrual status included, 1) amenorrhea defined as the absence of menses for 
at least 3 consecutive months in the past year or <4 cycles in the past year, 2)
oligomenorrhea defined as a menstrual cycle length >35 days or between 4 and 9 cycles in the past year, or 3) eumenorrhea defined as a menstrual cycle length between 24-35 days or ≥10 cycles in the past year. Age of menarche (yr) was reported and late menarche was defined as onset of menses at age 15 yr or older (28).

**Eating attitudes and behaviors:** Dietary restraint was obtained using the Three Factor Eating Questionnaire (TFEQ) (32) (PSU and UT), the Eating Disorder Examination Questionnaire (EDE-Q) (25) (SDSU), or a pre-participation examination questionnaire corroborated with clinical interview with a physician (UCLA). The TFEQ is a 51-item questionnaire that measures three dimensions of human eating behavior: (1) dietary restraint, (2) disinhibition, and (3) hunger (32). A cut-off of ≥9 was used to identify an elevated value for the dietary restraint subscale. A TFEQ dietary restraint score of ≥9 was chosen as prior investigators have reported this cut-off as the median score for premenopausal women (37).

The EDE-Q measures four subscales: (1) weight concern, (2) shape concern, (3) eating concern, and (4) dietary restraint. Scores ranging from 0 to 6 on a Likert scale correspond to the number of days over the past 4 weeks the respondent experienced a particular attitude, feeling, or behavior. An EDEQ dietary restraint score of ≥3 was chosen to identify an elevated value (5,27). Since all participants in the current study were assessed for dietary restraint using the TFEQ, the EDE-Q, or an eating attitudes/behavior survey, elevated dietary restraint was defined/identified as either a score of ≥9 on the TFEQ, a score of ≥3 on the EDE-Q, or by a pre-participation examination questionnaire corroborated in a clinical interview with a physician. Pathogenic weight control behavior was defined as reporting at least one prior episode of self-induced vomiting or use of laxatives, diuretics, and/or diet pills (34).
**Sport/activity participation:** Sport/activity participation was obtained using a questionnaire wherein the participants reported the type, frequency (d·wk⁻¹), and duration per session (min·d⁻¹) in the past 6 months (PSU and UT) and number of years and/or months per year of the sport or activity in which they participated from age 10 yr to current year (UCLA and SDSU). Participants were categorized based on primary mode of sport/activity (lean vs. non-lean), using a classification system modified from Torstveit and Sundgot-Borgen (36). Lean sports/activities included endurance (cycling, middle and long-distance running (>800m), rowing, swimming, triathlon, cross-country skiing), aesthetic (figure skating, gymnastics, rhythmic gymnastics, dance, diving, cheerleading, acrobatics), weight class (boxing, weight-lifting, karate, judo, tae kwon do, wrestling, kickboxing, jujitsu, horseback riding- racing), anti-gravitation sports (indoor and outdoor rock climbing, high jump, long jump, pole vaulting, triple jump) and aerobic activities (aerobics, gym-related cardio such as elliptical and stair climber machines). Non-lean sports/activities included technical (bowling, curling, fencing, golf, horseback riding - dressage, sailing, snowboarding, water skiing), ball game (badminton, basketball, ice hockey, field hockey, soccer, lacrosse, softball, baseball, table tennis, squash, team handball, tennis, rugby, and volleyball), and power sports (alpine skiing, discus, hammer, hurdle, javelin, shot put, speed skating sprint, sprint (≤800 m) (36).

**Definitions of low BMD:** Participants were categorized as having low BMD if their values at the spine or total body in the adolescent population (aged 15 to 17 yr) (n=268) or the spine or total hip in the premenopausal female population (aged 18 to 35 yr) (n=169) were more than 1 or more than or equal to 2 SD below the age-matched, gender-specific reference data from the GE/Lunar and Hologic databases (Z-score < -1 or ≤ -2, respectively). The
International Society of Clinical Densitometry (ISCD) defines low BMD for chronological age as an age, gender, and body size-adjusted BMD Z-score $\leq -2$, whereas the American College of Sports Medicine (ACSM) 2007 Position Stand on the Triad (26) defines low BMD for a female athlete as a BMD Z-score $<-1$, particularly if accompanied by secondary clinical risk factors for fracture such as amenorrhea. We chose to examine both Z-score cut-offs (ACSM and ISCD criteria) (30) because exercising women typically should demonstrate BMD values approximately 10% higher vs. sedentary controls (26). As such, a BMD Z-score between -1 and -2 requires attention.

**Triad risk factors for low BMD:** Prior to the initiation of data analysis, we identified potential risk factors of low BMD associated with the Triad (i.e. factors associated with low EA/chronic energy deficiency and menstrual dysfunction). Definitions and/or cut-off points used for each risk factor were decided upon based on their recognition as criteria for an established clinical condition, or evidence linking the factor to the Triad and related clinical sequelae. The risk factors assessed included: low BMI (BMI<$18.5 \text{ kg} \cdot \text{m}^{-2}$); low BW (BW<$90\%$ ideal body weight (IBW)); elevated dietary restraint; pathological weight control behavior; lean sport/activity participation; late menarche ($\geq 15\text{yr}$); and current oligo/amenorrhea. The Center for Disease Control cites a BMI $<18.5 \text{ kg} \cdot \text{m}^{-2}$ as underweight. Torstveit and Sundgot-Borgen (34) also defined BMI $<18.5 \text{ kg} \cdot \text{m}^{-2}$ as an at-risk criterion of the Triad in large population of competitive female athletes by assuming that a very low BMI may indicate chronic energy deficiency. Additionally a BW less than 10% of ideal, using the IBW equation (18), has been used to define a moderate underweight status (29). Amenorrhea and oligomenorrhea are considered as the most severe clinical menstrual disturbances in the 2007 ACSM Position Stand on the Triad (26). Primary or secondary
amenorrhea is one of the required criteria in World Health Organization and Diagnostic and Statistical Manual of Mental Disorders IV definitions of anorexia nervosa and represents a well-documented factor associated with compromised bone mineral accretion and/or bone loss in exercising women (11). Pathological weight control behavior are unhealthy dieting strategies which may be used by female athletes to achieve a low body weight considered advantageous for performance or appearance-related reasons (34). Late menarche indicates a history of primary amenorrhea and has been associated with a negative effect on bone mineral accrual during adolescence (24). Investigators have previously reported associations among the following factors associated with the Triad and low BMD in exercising women: low BMI/BW, elevated dietary restraint (as measured by the EDEQ, TFEQ, or clinical interview), lean sport/activity participation, and menstrual dysfunction (5,6,30,33,37).

**Statistical analyses:** All statistical analyses were conducted using SPSS version 19.0 (SPSS, Inc., Chicago, IL). All hypothesis tests were two-sided and p<0.05 was considered significant. Data screening was conducted prior to analysis, involving outlier detection, evaluation of assumptions of normality, and regression diagnostics. Clinical characteristics (i.e., age, ethnicity, height, body mass, BMI, body composition, age of menarche, gynecological age) and percent of Triad risk factors were determined to describe the study sample. Descriptive statistics were reported to include means and standard deviations for continuous data and frequency and percent for categorical data between participants with BMD Z-Score < -1 and those participants with BMD Z-Score ≥ -1 based on ACSM criteria (26). Psychometric survey data was recoded and scored to determine subscale or total
scores. Group comparisons for descriptive purposes were performed using Independent Student’s t-tests for continuous data and chi-square analyses for categorical data. When variables were not normally distributed, the Mann Whitney test was used to determine group differences. Chi-square tests were performed to determine: (1) the association between Triad risk factors (individually and in combination) and low BMD (Z-Score < -1 and ≤ -2) in exercising women and (2) the association between the cumulative effect of Triad risk factors in exercising women (those who met criteria for multiple (any 2, 3, 4, or 5) Triad risk factors) and low BMD (Z-Score < -1 and ≤ -2). Crude odds ratios (OR) and 95% confidence intervals (95% CI) were determined to compare the risk for low BMD (Z-Score < -1 and ≤ -2) in participants with risk factors associated with the Triad (individually and in combination) compared to those participants that did not meet criteria for the evaluated individual or combined Triad risk factors. Adjusted OR with 95% CI were generated using multivariate logistic regression analyses to determine the strongest predictors of low BMD in exercising women (31). Items in the multivariate logistic regression model analyses included the Triad risk factors (current oligo/amenorrhea, late menarche, low BMI, elevated dietary restraint, and lean sport/activity participation) and relevant covariates known to impact BMD (age (yr) and LBM (kg)). Based on the tight correlation between BMI and BW, low BW was excluded from the multivariate regression analyses. Adjusted models were conducted for low BMD Z-Score < -1 and low BMD Z-Score ≤ -2. A sample size calculation based on data from previous publications (5,8,19,33) indicated that 375 women would provide adequate power (1-β = 0.80) to detect a significant: (1) association between Triad risk factors and low BMD (Z-Score < -1 and ≤ -2) in exercising women and (2)
association between the cumulative effect of Triad risk factors in exercising women and low BMD (Z-Score < -1 and ≤ -2).

Results

Participant characteristics: Descriptive characteristics of the total sample of 437 exercising women are presented in Table 1. Participants in this study were 18.0±3.5 yr, weighed 57.5±7.1 kg with 24.5±6.1 % body fat. The sample included a racial/ethnic distribution of 70.5% White/Caucasian, 9.6% Hispanic, 7.8% Asian, 7.1% African American, and 5.0% chose “Other”. In the total sample, 16.9% of the participants reported using oral contraceptives in their lifetime. The percent of Triad risk factors for the total sample is reported in Table 1. Of the total sample of participants, 42.8% presented with current oligo/amenorrhea, 71.6% participated in lean sport/activity, 13.3% demonstrated late menarche, 30.2% exhibited an elevated dietary restraint, 8.0% had engaged in pathological weight control behavior, and 13.7% and 11.4% had low BW and low BMI respectively.

Descriptive characteristics and percent of Triad risk factors in exercising women grouped by BMD status: Descriptive characteristics and percent of Triad risk factors in exercising women grouped by BMD status are presented in Table 1. Participants with BMD Z-Score < -1 were lighter, leaner, less gynecologically mature, and as expected, exhibited a lower BMD at the total body, lumbar spine, total hip, and femoral neck than participants with a BMD Z-Score ≥ -1. A greater percent of the participants with BMD Z-Score < -1 compared to participants with BMD Z-Score ≥ -1 participated in a lean sport/activity; demonstrated current oligo/amenorrhea and late menarche; and had low BW/BMI.
Table 1. Descriptive characteristics - total sample of adolescent and adult exercising women (n=437) and sample when grouped by BMD status.

<table>
<thead>
<tr>
<th>Descriptive characteristics</th>
<th>Total Sample (n=437)</th>
<th>BMD Z-Score ≥ -1.0 (n=311)</th>
<th>BMD Z-Score &lt; -1.0&lt;sup&gt;a&lt;/sup&gt; (n=126)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Descriptive characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(mean±SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>18.0±3.5</td>
<td>18.0±3.5</td>
<td>17.9±3.4</td>
<td>0.995&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.2±6.5</td>
<td>165.9±6.5</td>
<td>163.7±6.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.5±7.1</td>
<td>59.0±7.0</td>
<td>54.0±6.1</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg·m&lt;sup&gt;-2&lt;/sup&gt;)</td>
<td>21.1±2.3</td>
<td>21.5±2.3</td>
<td>20.1±2.0</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>24.5±6.1</td>
<td>25.1±5.9</td>
<td>23.2±6.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>14.3±4.8</td>
<td>15.0±4.8</td>
<td>12.7±4.3</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>40.2±4.4</td>
<td>41.0±4.4</td>
<td>38.2±3.9</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>43.3±4.5</td>
<td>44.2±4.5</td>
<td>41.3±4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at menarche (yr)</td>
<td>12.9±1.5</td>
<td>12.7±1.4</td>
<td>13.3±1.6</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gynecological age (yr)</td>
<td>5.0±3.7</td>
<td>5.3±3.6</td>
<td>4.5±3.7</td>
<td>0.020&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>BMD (g·cm&lt;sup&gt;-2&lt;/sup&gt;)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total body</td>
<td>1.14±0.09</td>
<td>1.17±0.08</td>
<td>1.06±0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>1.09±0.13</td>
<td>1.13±0.12</td>
<td>1.00±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total hip</td>
<td>1.09±0.12</td>
<td>1.13±0.12</td>
<td>0.99±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>1.13±0.13</td>
<td>1.19±0.11</td>
<td>1.00±0.07</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Triad risk factors (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current OA</td>
<td>42.8</td>
<td>39.2</td>
<td>51.6</td>
<td>0.018</td>
</tr>
<tr>
<td>Low BW (&lt;90% of IBW)</td>
<td>13.7</td>
<td>10.6</td>
<td>21.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Low BMI (&lt;18.5 kg·m&lt;sup&gt;-2&lt;/sup&gt;)</td>
<td>11.4</td>
<td>7.4</td>
<td>21.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LAOM (≥15yr)</td>
<td>13.3</td>
<td>8.4</td>
<td>25.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elevated dietary restraint</td>
<td>30.2</td>
<td>28.0</td>
<td>35.7</td>
<td>0.110</td>
</tr>
<tr>
<td>PWCB&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>0.982</td>
</tr>
<tr>
<td>Leanness sport/activity</td>
<td>71.6</td>
<td>65.8</td>
<td>85.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Low BMD defined as Z-Score<-1 according to ACSM Triad position stand criteria (26). Independent t-Tests were used to compare descriptive characteristics between groups. <sup>b</sup>Non-parametric comparisons using Mann-Whitney U Test, p<0.05. Chi-Square tests were used for group comparisons for percent of Triad-related risk factors, p<0.05. BMD = Bone mineral density; BMI = Body mass index; IBW = Ideal body weight; OA = Oligo/amenorrhea; LAOM = Late age at onset of menarche; PWCB = Pathogenic weight control behavior; Gynecological age = chronological age – menarcheal age.
**Risk for low BMD associated with individual Triad risk factors:** The percent of low BMD (Z-Score < -1 and ≤ -2) in exercising women with individual Triad risk factors are shown in Figure 1. Current oligo/amenorrhea ($X^2=5.6$, $p=0.018$), late menarche ($X^2=22.6$, $p<0.001$), low BMI ($X^2=17.5$, $p<0.001$), low BW ($X^2=8.9$, $p=0.003$) and lean sport/activity participation ($X^2=17.3$, $p<0.001$) were associated with low BMD (Z-Score < -1). Current oligo/amenorrhea ($X^2=4.1$, $p=0.043$), late menarche ($X^2=10.7$, $p=0.001$), and low BMI ($X^2=14.2$, $p<0.001$) were associated with low BMD (Z-Score ≤ -2). Of the individual risk factors, participants with late menarche, low BMI, and low BW demonstrated the highest percent of low BMD (Z-Score < -1), 55.2%, 54.0%, and 45.0% respectively. Participants with low BMI, late menarche, and low BW demonstrated the highest percent of low BMD (Z-Score ≤ -2), 16.0%, 13.8%, and 10.0%, respectively. Crude OR with 95% CI for low BMD (Z-Score < -1 and ≤ -2) in exercising women with individual Triad risk factors are presented in Table 2. Participants reporting late menarche (n=58) were four times more likely to present with low BMD (Z-Score < -1 and ≤ -2) compared to those reporting normal age at onset of menarche (OR, 3.7; 95% CI: 2.1, 6.6 and OR, 4.2; 95% CI: 1.7, 10.4, respectively). Participants who has low BW/BMI were more likely to present with low BMD (Z-Score < -1 and ≤ -2). Participants reporting low BMI (n=50) were three and five times more likely to present with low BMD (Z-Score < -1 and ≤ -2) compared to those reporting normal BMI (OR, 3.4; 95% CI: 1.9, 6.2 and OR: 5.1, 95% CI: 2.0, 12.8, respectively). Those participants reporting low BW (n=60) were two times more likely to present with low BMD (Z-Score < -1) compared to those with normal BW (OR: 2.3, 95% CI: 1.3, 4.0). Participants reporting current oligo/amenorrhea (n=187) were two and three times more likely to present with low BMD (Z-Score < -1 and ≤ -2) compared to those
reporting current eumenorrhea (OR, 1.7; 95% CI: 1.1, 2.5 and OR, 2.5; 95% CI: 1.0, 6.0, respectively). Lean sport/activity participants (n=313) were three times more likely to demonstrate low BMD (Z-Score < -1) compared to non-lean sport/activity participants (OR: 3.1, 95% CI: 1.8, 5.4).

**Figure 1.** The percent (%) of women with low bone mineral density (BMD) in adolescent and adult exercising women (n=437) with individual Triad risk factors (low body weight (BW<90% of ideal body weight) (n=60), low body mass index (BMI<18.5 kg·m⁻²) (n=50), current oligo/amenorrhea or OA (irregular interval of menses ≥36d or <9 cycles in past yr) (n=187), late menarche or LAOM (age at onset of menarche>15 yr) (n=58), elevated dietary restraint (DR) (n=132), and lean sport/activity participation (LS) (n=313)).
Table 2. Crude odds ratios (OR) with 95% confidence intervals (95% CI) for low BMD in adolescent and adult exercising women (n=437) with individual Female Athlete Triad risk factors.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>BMD Z-Score&lt;-1(^a) OR (95% CI)</th>
<th>BMD Z-Score≤-2(^b) OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low BW (&lt;90% IBW)</td>
<td>2.3 (1.3, 4.0)</td>
<td>2.5 (0.9, 6.7)</td>
</tr>
<tr>
<td>Low BMI (&lt;18.5)</td>
<td>3.4 (1.9, 6.2)</td>
<td>5.1 (2.0, 12.8)</td>
</tr>
<tr>
<td>Current oligo/amenorrhea(^c)</td>
<td>1.7 (1.1, 2.5)</td>
<td>2.5 (1.0, 6.0)</td>
</tr>
<tr>
<td>Late menarche (≥15yr)</td>
<td>3.7 (2.1, 6.6)</td>
<td>4.2 (1.7, 10.4)</td>
</tr>
<tr>
<td>Elevated dietary restraint(^d)</td>
<td>1.4 (0.9, 2.2)</td>
<td>0.7 (0.2, 1.9)</td>
</tr>
<tr>
<td>Lean sport/activity(^e)</td>
<td>3.1 (1.8, 5.4)</td>
<td>2.6 (0.8, 9.0)</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density; BW = Body weight (kg); IBW = Ideal body weight; BMI = Body mass index (kg·m\(^{-2}\))
\(^a\) = Low BMD (Z-Score<-1) as per ACSM criteria; \(^b\) = Low BMD (Z-Score≤-2) as per ISCD criteria; \(^c\) = Irregular intervals of menses≥36 d or <9 cycles in past yr; \(^d\) = Determined using TFEQ, EDEQ, or eating attitudes/behaviors survey corroborated by clinical interview; \(^e\) = Lean sports and activities included endurance, aesthetic weight class, and anti-gravitation type; whereas, non-lean sports/activities included technical, ball game, and power sports.

Risk for low BMD associated with combined Triad risk factors: Of the combined risk factors, participants with late menarche + low BMI, late menarche + low BW, and late menarche + elevated dietary restraint demonstrated the highest percent of low BMD (Z-Score < -1), 73.3%, 70.6%, and 64.0% respectively. Participants with late menarche + low BMI, late menarche + low BW, and current oligo/amenorrhea + late menarche demonstrated the highest percent of low BMD (Z-Score ≤ -2), 20.0%, 17.6%, and 17.4%, respectively. Crude OR with 95% CI for low BMD (Z-Score < -1 and ≤ -2) in exercising women with combined Triad risk factors are presented in Table 3. Varying combinations of risk factors indicative of current oligo/amenorrhea, late menarche, low BMI/BW, and lean sport/activity participation were strongly associated (p<0.05) with low BMD (Z-Score < -1 and ≤ -2). Elevated dietary restraint +
lean sport/activity participation and elevated dietary restraint + late menarche also represented combinations of Triad risk factors strongly associated (p<0.05) with low BMD (Z-Score < -1) (Table 3).

**Risk for low BMD based on number of Triad risk factors:** The percent of low BMD (Z-Score < -1 and ≤ -2) in exercising women who met criteria for 0 (n=59), 1 (n=159), 2 (n=120), 3 (n=58), or 4 (n=39) Triad risk factors (low BMI, late menarche, elevated dietary restraint, lean sport/activity participation, and current oligo/amenorrhea) are presented in Figure 2. The percent of low BMD (Z-Score < -1 and ≤ -2) in participants increased from 10.2 to 61.5% and 1.7 to 17.9% respectively as participants met criteria for 0, 1, 2, 3, or 4 Triad risk factors. Two participants met criteria for all 5 risk factors and BMD (Z-Score < -1) and zero participants met criteria for all 5 risk factors and BMD (Z-Score ≤ - 2).

**Triad risk factors as predictors for low BMD:** Adjusted OR with 95% CI for low BMD (Z-Score < -1 and ≤ -2) in exercising women associated with Triad risk factors were presented in Table 4. All risk factors (low BMI, late menarche, elevated dietary restraint, lean sport/activity participation, and current oligo/amenorrhea) were entered into the model in addition to relevant covariates (age (yr) and LBM (kg)). Multivariate logistic regression analyses revealed that late menarche, lean sport/activity participation, and LBM were the strongest predictors of low BMD (Z-Score < -1) when adjusting for BMI status, menstrual status, dietary restraint status and age. Late menarche and LBM were the strongest predictors of low BMD (Z-Score ≤ -2) when adjusting for sport/activity participation type, BMI status, menstrual status, dietary restraint status, and age.
Table 3. Percent (%) of and crude odds ratios (OR) with 95% confidence intervals (95% CI) for low BMD in adolescent and adult exercising women (n=437) with combined Female Athlete Triad risk factors.

<table>
<thead>
<tr>
<th>Factor Combination</th>
<th>Percent of BMD Z-Score&lt;-1 (%)</th>
<th>Percent of BMD Z-Score≤-2 (%)</th>
<th>BMD Z-Score&lt;-1 OR (95% CI)</th>
<th>BMD Z-Score≤-2 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligo/amenorrhea + Late menarche</td>
<td>60.9 (28/46)</td>
<td>17.4 (8/46)</td>
<td>4.7 (2.5, 8.8)</td>
<td>5.7 (2.2, 14.4)</td>
</tr>
<tr>
<td>Oligo/amenorrhea + Low BMI (&lt;18.5)</td>
<td>53.3 (16/30)</td>
<td>16.7 (5/30)</td>
<td>3.1 (1.5, 6.6)</td>
<td>4.6 (1.6, 13.5)</td>
</tr>
<tr>
<td>Oligo/amenorrhea + Low BW (&lt;90% IBW)</td>
<td>48.7 (19/39)</td>
<td>12.8 (5/39)</td>
<td>2.6 (1.3, 5.1)</td>
<td>3.3 (1.2, 9.5)</td>
</tr>
<tr>
<td>Late menarche + Low BMI (&lt;18.5)</td>
<td>73.3 (11/15)</td>
<td>20.0 (3/15)</td>
<td>7.3 (2.3, 23.5)</td>
<td>5.3 (1.4, 20.4)</td>
</tr>
<tr>
<td>Late menarche + Low BW (&lt;90% IBW)</td>
<td>70.6 (12/17)</td>
<td>17.6 (3/17)</td>
<td>6.4 (2.2, 18.7)</td>
<td>4.5 (1.2, 17.1)</td>
</tr>
<tr>
<td>Low BMI (&lt;18.5) + Low BW (&lt;90% IBW)</td>
<td>51.1 (24/47)</td>
<td>12.8 (6/47)</td>
<td>3.0 (1.6, 5.5)</td>
<td>3.4 (1.3, 9.2)</td>
</tr>
<tr>
<td>Oligo/amenorrhea + Lean sport/activity</td>
<td>40.3 (60/149)</td>
<td>8.7 (13/149)</td>
<td>2.3 (1.5, 3.5)</td>
<td>3.0 (1.2, 7.1)</td>
</tr>
<tr>
<td>Late menarche + Lean sport/activity</td>
<td>56.5 (26/46)</td>
<td>15.2 (7/46)</td>
<td>3.8 (2.0, 7.1)</td>
<td>4.5 (1.7, 11.7)</td>
</tr>
<tr>
<td>Low BMI (&lt;18.5) + Lean sport/activity</td>
<td>56.8 (25/44)</td>
<td>15.9 (7/44)</td>
<td>3.8 (2.0, 7.2)</td>
<td>4.8 (1.8, 12.4)</td>
</tr>
<tr>
<td>Oligo/amenorrhea + Elevated dietary restraint</td>
<td>33.3 (27/81)</td>
<td>6.2 (5/81)</td>
<td>1.3 (0.8, 2.2)</td>
<td>1.3 (0.5, 3.7)</td>
</tr>
<tr>
<td>Late menarche + Elevated dietary restraint</td>
<td>64.0 (16/25)</td>
<td>12.0 (3/25)</td>
<td>4.9 (2.1, 11.4)</td>
<td>2.8 (0.8, 10.3)</td>
</tr>
<tr>
<td>Low BW (&lt;90% IBW) + Lean sport/activity</td>
<td>49.1 (26/53)</td>
<td>11.3 (6/53)</td>
<td>2.7 (1.5, 4.9)</td>
<td>2.9 (1.1, 7.9)</td>
</tr>
<tr>
<td>Low BMI (&lt;18.5) + Elevated dietary restraint</td>
<td>50.0 (6/12)</td>
<td>8.3 (1/12)</td>
<td>2.5 (0.8, 8.0)</td>
<td>1.8 (0.2, 14.2)</td>
</tr>
<tr>
<td>Low BW (&lt;90% IBW) + Elevated dietary restraint</td>
<td>46.7 (7/15)</td>
<td>6.7 (1/15)</td>
<td>2.2 (0.8, 6.3)</td>
<td>1.4 (0.2, 10.9)</td>
</tr>
<tr>
<td>Elevated dietary restraint + Lean sport/activity</td>
<td>39.6 (38/96)</td>
<td>5.2 (5/96)</td>
<td>1.9 (1.2, 3.0)</td>
<td>1.1 (0.4, 2.9)</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density; BW = Body weight (kg); IBW = Ideal body weight; BMI = Body mass index (expressed in kg m⁻²).
Figure 2. The percent (%) of low bone mineral density (BMD) in exercising women (n=437) based on the number of Triad risk factors (low body mass index, late age at onset of menarche, elevated DR, lean sport/activity participation, current oligo/amenorrhea) present for each athlete.
Table 4. Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) for low bone mineral density (BMD Z-Score<-1 and ≤-2) in adolescent and adult exercising women (n=437) associated with Female Athlete Triad risk factors.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>BMD Z-Score&lt;-1 (n=126)</th>
<th>BMD Z-Score≤-2 (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AOR (95% CI)a</td>
<td>AOR (95% CI)b</td>
</tr>
<tr>
<td>Late menarchec</td>
<td>4.2 (2.1, 8.2)</td>
<td>3.4 (1.1, 10.4)</td>
</tr>
<tr>
<td>Lean sport/activityd</td>
<td>2.9 (1.6, 5.3)</td>
<td>-</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>0.8 (0.8, 0.9)</td>
<td>0.9 (0.8, 1.0)</td>
</tr>
</tbody>
</table>

*a = Final Model: $R^2 = 0.247$ (Nagelkerke), 0.173 (Cox & Snell) adjusting for body mass index (BMI) status (low BMI $\leq 18.5$ kg·m$^{-2}$; normal BMI = BMI $>18.5$ kg·m$^{-2}$), menstrual status (current oligo/amenorrhea; eumenorrhea), dietary restraint status (elevated dietary restraint; normal dietary restraint) and age (yr)

*b = Final Model: $R^2 = 0.186$ (Nagelkerke), 0.061 (Cox & Snell) adjusting for BMI status (low BMI $\leq 18.5$ kg·m$^{-2}$; normal BMI = BMI $>18.5$ kg·m$^{-2}$), menstrual status (current oligo/amenorrhea; eumenorrhea), dietary restraint status (elevated dietary restraint; normal dietary restraint), and sport/activity participation (lean sport/activity participation; non-lean sport/activity participation) and age (yr)

*c = Age at onset of menarche (late menarche $\geq 15$yr; normal age at onset of menarche $<15$yr)

*d = Lean sports/activities included endurance, aesthetic weight class, and anti-gravitation type; whereas, non-lean sports/activities included technical, ball game, and power sports

*e = Irregular intervals of menses $\geq 36$ d or $<9$ cycles in past year

*f = Determined using TFEQ, EDEQ, or eating attitudes/behaviors survey corroborated by clinical interview
Discussion

In this study, we examined the cumulative effect of Triad risk factors associated with low BMD in adolescent and adult exercising women. The Triad is well-recognized as an interrelated syndrome wherein several clinical sequelae (i.e., menstrual dysfunction, disordered eating, low EA), ranging in severity, may predominate and have a negative effect on BMD (5,6,11,27). To our knowledge, this is the first investigation that has reported a “dose-response” or cumulative effect of Triad risk factors on BMD. We demonstrated that exercising women presenting with multiple Triad risk factors were at a higher risk for low BMD than exercising women with no or individual Triad risk factors. In addition, we reported associations between combined Triad risk factors, particularly those related to menstrual dysfunction and low BMI/BW, and higher risk for low BMD. Furthermore, our findings suggest that as the number of Triad risk factors increase from one to four factors in exercising women, there is a cumulative increase in percent of participants with low BMD (Z-Score < -1 and Z-Score ≤ -2), ranging from 21 to 62% and 3 to 18%, respectively. Therefore, in our study, the exercising women with greater exposure to Triad risk factors were more likely to demonstrate low BMD. Findings from the current study also reinforce prior associations between Triad risk factors and low BMD such as: late menarche (6), current oligo/amenorrhea (11), low BMI/BW (14,39,40), participation in lean sport/activity (8,35) and elevated dietary restraint (5,37). As such, the results from the current study provide evidence of the cumulative effect of Triad risk factors on BMD in exercising women. In addition, these findings support the ACSM 2007 Position Stand (26), which advised that presentation of Triad-related disorders (i.e., hypoestrogenism, low EA, disordered eating behavior) translates to risk of lower BMD (23), particularly in those
exercising women with multiple persistent Triad conditions. Overall, our findings underscore the importance of early intervention in the presence of any one of the evaluated Triad risk factors in order to prevent an accumulation of risk for low BMD in exercising women.

Current oligo/amenorrhea and late menarche are risk factors indicative of chronic menstrual dysfunction with clinical implications for bone health. In our study, late menarche was a significant predictor of low BMD in the presence of other Triad risk factors, and notably, 55% and 14% of participants with late menarche reported low BMD (Z-Score < -1 and Z-Score ≤ -2, respectively). Adolescence is a critical time for bone mineral accretion, wherein approximately 35% of total body and lumbar spine bone mineral and 27% of femoral neck bone mineral is deposited (3). As such, normal age of menarche promotes skeletal benefits in exercising girls; whereas, late menarche may result in decreased estrogen exposure, which has the potential to exert a profound effect on the axial skeleton and in trabecular bone (4,11,24). Estrogen also plays a key role in maintaining BMD in adult women and importantly, oligo/amenorrhea associated with hypoestrogenism may represent a potential indicator of risk for bone loss (11). In this study, low BMD (Z-Score < -1 and Z-Score ≤ -2) was present in a significant proportion of exercising women with current oligo/amenorrhea, 35% and 8% respectively, and the risk for low BMD in these participants was higher than their regularly menstruating counterparts (10,11). In addition, the combination of these markers of menstrual dysfunction (current oligo/amenorrhea and late menarche) indicated a significantly higher risk for low BMD compared to a regularly menstruating woman with a normal age of menarche. Notably, self-report menstrual status can only identify menstrual disturbances clearly apparent to women as an absence of menses.
for greater than 3 months (amenorrhea), an irregularity in menstrual cycle length (oligomenorrhea), or late menarche (≥15 yr). Additionally, self-reported menstrual history alone does not detect subclinical menstrual disturbances (i.e., luteal phase defects and anovulation) (9). As such, objective measures of hormone analyses improve the accuracy of measuring the presence or absence of menstrual disturbances (subclinical and clinical). However, the implications for BMD in women with clinical menstrual disturbances (oligo/amenorrhea) are much more severe based on lower estrogen exposure compared to those with subclinical menstrual disturbances (11). Furthermore, objective measures of menstrual status are often not feasible in a clinical or field setting. Taken together, self-report measures of menstrual function provide an easy-to-obtain indication of risk for low BMD in exercising women.

Several investigators have reported associations among energy deficiency, menstrual disturbances, and low BMD in exercising women (10). Previous evidence supports the combined effect of estrogen and energy deficiency on bone turnover markers and/or BMD (10,40). De Souza et al. (10) reported that estrogen deficiency in the presence of an energy deficiency was associated with low BMD in exercising women. In the current study, combinations of menstrual dysfunction (late menarche or current oligo/amenorrhea) and low BMI/low BW were associated with a higher risk for low BMD in exercising women compared to those with normal menstrual function and normal BMI/BW. Specifically, the combination of low BMI and late menarche represented the most robust indicator of risk for low BMD such that participants with late menarche and low BMI were up to seven times more likely to demonstrate low BMD compared to those with normal age of menarche and normal BMI. Accordingly, the percent of low BMD (Z-Score < -1) in exercising women with different
combinations of low BMI, low BW, late menarche or current oligo/amenorrhea was notably high, ranging from 49 to 73%, indicative of clusters of Triad risk factors associated with the highest risk for low BMD.

Low BMI and low BW represented individual Triad risk factors of low BMD. We demonstrated that participants with low BMI/BW were between two to five times more likely to demonstrate low BMD. Low BMI and low BW were also significantly associated with low BMD in combination with other Triad risk factors, suggesting that there is a cumulative effect of low BMI/BW on BMD in exercising women along with late menarche, lean sport/activity participation, elevated dietary restraint, or current oligo/amenorrhea. Additionally, findings from case study reports in female athletes support the importance of nutritional and weight recovery (increase in BW by 20-34% greater than initial BW) to coincide with BMD recovery (increase in BMD by 17-25.5% greater than initial BMD) (14,39). From a methodological standpoint, the measurement of bone turnover markers, metabolic hormones, and DXA, or other imaging analyses of BMD, are often not feasible in field settings. Therefore, the assessment of BMI or BW may represent a non-invasive first-pass indicator of risk for low BMD, which can serve to assist health care providers in determining the need for further assessment and work up in athletes, especially when low BMI/BW is demonstrated in the presence of additional Triad-related risk factors for low BMD.

The Triad is most often observed in exercising women participating in lean sport/activity due to the emphasis on low BW, and possibly elevated dietary restraint which may lead to low EA or chronic energy deficiency (33). Beals et al. reported low BMD (defined as a Z-Score below -2.0) in 3.1% and 0% of lean and non-lean sport athletes, respectively (8). The prevalence of low BMD increases to 15.4% in lean sport athletes using
a less conservative criterion of Z-Score below -1.0 for low BMD compared to 0% in non-lean sport athletes (8). In addition, lean sport athletes exhibited 7% lower lumbar spine (L1-L4) BMD than the non-lean sport athletes (8). Similarly, we demonstrated that the lean sport/activity participants were more likely to present with low BMD compared to their non-lean sport/activity counterparts. The lower BMD in the lean sport/activity group observed in our study may be explained by a greater frequency of current oligo/amenorrhea in the lean sport/activity group vs. the non-lean sport/activity group (48% vs. 30%, respectively). Additionally, there may be a genetic predisposition of those exercising women with higher bone strength and greater LBM to favor participation in non-lean sport/activity; whereas those women with low BW and a lean figure (i.e., lower LBM) may self-select participation in lean sport/activity (35). Our findings showed that both lean sport/activity participation and LBM were identified as significant predictors of low BMD when adjusting for the other Triad risk factors and age. Accordingly, participants with low BMD (Z-Score < -1) demonstrated significantly lower LBM in comparison to women with normal BMD (Z-Score ≥ -1). Therefore, we suggest that lean sport/activity may have negative consequences on BMD independent of and in combination with other Triad risk factors. Furthermore, we demonstrated the robust effects of LBM on BMD in exercising women.

Associations between low BMD and disordered eating behavior (i.e., dietary restraint and pathological weight control behavior) have been established in previous reports in adolescent and adult exercising women (5,8,30). Findings from Barrack et al. (5) in adolescent female competitive cross-country runners demonstrate that elevated dietary restraint has a negative effect on BMD and bone mineral content, particularly at the lumbar spine. Vescovi et al. (37) provided evidence that high dietary restraint scores were
associated with reduced lumbar spine and total body BMD in exercising women
concomitant with a greater frequency of current oligo/amenorrhea. In contrast to these
previous reports, in the current study, elevated dietary restraint was not associated with low
BMD as an individual risk factor. However, when combined with other Triad risk factors,
such as lean sport/activity participation and late menarche, elevated dietary restraint was
associated with a higher percent of participants with low BMD. As such, elevated dietary
restraint may contribute to the accumulation of risk for low BMD in exercising women.
Notably, dietary restraint and pathological weight control behavior represented the only
indicators of eating behavior examined in this study. However, other disordered eating
behaviors such as a high drive for thinness or a self-reported eating disorder may also be
linked to low BMD by way of a chronic energy deficiency and/or hypoestrogenism (15).

Since the Triad is a complex syndrome to assess, the study of this interrelated
medical condition is limited by methodological and experimental challenges. The objective
of merging data obtained from several studies was to examine the cumulative effect of Triad
risk factors on BMD in a large sample of adolescent and adult exercising women, which is
notably one of the strengths of this study. The use of self-report or field measures in the
present study may represent a useful approach to identifying low BMD in exercising girls
and women in a field or clinical setting, wherein clinical or “gold-standard” methods are not
feasible. The advancement of a practical approach using easy-to-obtain and non-invasive
measures to assess risk for low BMD associated with the Triad is necessary, particularly for
coaches, athletic trainers and health practitioners. Furthermore, assessment of these risk
factors may be considered for validation and incorporation into pre-participation screening
in athletes at risk for low BMD. Importantly, these Triad risk factors may be effective at
identifying exercising women that require further assessment of BMD using DXA or other imaging analyses.

We acknowledge that our retrospective analysis involved certain limitations. Particularly, our sample includes exercising women of a wide range in age and growth/development. It is important to mention that there are distinct physiological and developmental differences between adolescent and adult exercising women. However, based on prior investigations in adolescent and high-school athletes (5,19,27), it is clear that the Triad is a clinically relevant issue in this younger population of exercising women, similar to their adult counterparts. Also, our analysis of BMD includes data from multiple DXA scanners and no cross calibration study was completed. Thus, data were compared using standardized scores only. Other risk factors that were considered but not included in this study due to inconsistencies or lack of measurement in any one of the included studies were: prior history of a clinical eating disorder, menstrual disturbances prior to the past year, cumulative menstrual history, subclinical menstrual disturbances, high training volume, high drive for thinness, and low EA. Despite our inability to assess these potential risk factors, we encourage future investigation of the role of each factor in contributing to this concept of cumulative risk for low BMD associated with the Triad in exercising girls and women. Lastly, it is difficult to interpret our findings with respect to the influence of sport/activity participation (lean vs. non-lean) on BMD since both groups included low, medium, and high-impact sport/activity. Therefore, classifying exercising women by sport/activity type defined as lean vs. non-lean may not appropriately capture the influence of mechanical loading properties on BMD (35). Future research should determine the degree of mechanical
loading or the peak strain score in these exercising women similar to prior investigations (17,22,35).

In conclusion, our retrospective study provides evidence of a cumulative effect of Triad risk factors on BMD in adolescent and adult exercising women. Additionally, we comprehensively evaluated the percent of exercising women with low BMD associated with Triad risk factors (individually and in combination). Future investigation on the utility of these Triad risk factors in a pre-participation screening protocol or a user-friendly algorithm to identify risk for low BMD and risk for bone stress injury and fracture in exercising women is necessary. Optimally, these risk factors may be utilized as indicators of risk for low BMD in exercising women in a clinical or field setting. The development of preventive and treatment strategies should focus on validating first-pass indicators of risk for low BMD for use by coaches, trainers, and physicians to flag at-risk exercising women for which additional assessment, using objective measures i.e., DXA or other imaging analyses, would be recommended to further evaluate BMD. Novel strategies for early detection of possible pathological bone loss, failure to reach peak bone mass, or bone stress injury may be paramount in determining clearance for or withdrawal from participation in sports/exercise and preventing detrimental bone loss across the lifespan. This study presents translational information on the cumulative effect of Triad risk factors on BMD in exercising women and emphasizes the importance of healthy, replete energy status combined with normal menstrual function and eating behavior in order to optimize osteogenic loading forces on BMD achieved during exercise.

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References


CHAPTER 7

CONCLUSIONS

The objectives of this dissertation were: (1) to examine the association between psychometric indicators of disordered eating behavior (high drive for thinness (DT) or high dietary cognitive restraint (DR)) and physiological adaptations to the Female Athlete Triad (Triad) clinical sequelae (particularly, chronic energy deficiency/low energy availability (EA), and menstrual disturbances) in exercising women, (2) to examine the effect of increased energy intake on psychometric indicators of eating behavior (DR, DT, body dissatisfaction (BD), and bulimia scores) during a randomized controlled trial (RCT) in women with exercise-associated menstrual disturbances (EAMD), and (3) to determine the risk for low bone mineral density (BMD) in association with individual and combined Triad risk factors. Overall, the major findings in these dissertation studies were: (1) psychometric indicators of disordered eating behavior (high DT and high DR) and Triad risk factors (current oligo/amenorrhea, late age at onset of menarche, lean sport/activity, low body mass index (BMI), low body weight (BW), and elevated DR) may translate valuable information on the risk for or presence of Triad conditions in recreational- and competitive-level exercising women and (2) an RCT of increased energy intake may have favorable outcomes for eating attitudes and behavior, weight gain and resting energy expenditure (REE) in exercising women with EAMD.

In Studies One and Two, we demonstrated the associations between psychometric indicators of disordered eating behavior (high DT and DR) and physiological adaptations
to Triad disorders (specifically, menstrual disturbances and chronic energy deficiency or low EA) in exercising women. We determined that the DT and DR subscales provide valuable information to coaches, athletic trainers, and health practitioners attempting to obtain an indication of energy and menstrual status in large groups of exercising women wherein direct assessment of energy and menstrual status is not feasible. In Study Three, we provided evidence that refeeding did not exacerbate DR or have an adverse effect on DT, BD, and bulimia symptoms during a 6 month RCT in women with EAMD. Therefore, increased energy intake combined with psychological and nutritional monitoring may be associated with favorable outcomes for eating attitudes and behavior, weight gain, and REE in women with EAMD. In Study Four, we elucidated that a cumulative number of Triad risk factors was associated with an increasing risk of low BMD, suggesting a dose-response relationship between the number of Triad risk factors and BMD in exercising women. As such, we exhibited that exercising women with multiple Triad risk factors presented with a higher risk for low BMD. These findings will guide the development of consensus statements establishing effective preventive and management strategies for low BMD in exercising women with the Triad clinical sequelae.

Overall, the findings from this dissertation have valuable implications for women’s health and exercise research, specifically focused on the Triad. A current priority in this research area is to improve the identification of risk for or presence of physiological adaptations to Triad disorders in clinical and field settings using easy-to-obtain and non-invasive proxy indicators. To this end, establishing effective clinical tools and field measures of the Triad is of notable importance since laboratory assessments are
often not feasible. In these dissertation studies, we provided relevant evidence for uptake by the academic community, health practitioners, and exercising women to inform knowledge translation initiatives focused on reducing the risk of the Triad and improving prevention, management, and treatment of Triad clinical conditions in exercising women.

A notable gap in this area of research is the lack of accurate prevalence estimates of the Triad. As such, in our systematic review (Chapter 2- Review of the Literature Part One), we summarized the studies that included data on the prevalence of individual and combined Triad conditions in exercising women. This review included 65 studies of 10,498 participants and confirmed that the Triad is a clinically relevant health problem in exercising women. Additionally, in this review, we informed knowledge users and the academic community on: (1) current prevalence estimates of the Triad, both subclinical and clinical outcomes, in exercising women and in lean-sport vs. non-lean sport athletes and (2) the methodological approaches/limitations to accurately investigating the prevalence of the Triad in a research setting. Thus, we conclude that the prevalence of individual and combined Triad conditions in exercising women is concerning and future investigation using objective measures, and self-report/field measures as necessary, is required to determine the prevalence of the Triad. Since objective measures may not always be feasible, self-report/field measures may be necessary in evaluating some components of the Triad to include: menstrual status using self-report menstrual history (if daily urinary analyses of reproductive hormones are not accessible); energy intake using 3- or 7-day diet logs; exercise energy expenditure using physical activity compendium (if heart rate monitors or another validated, objective technique is not obtainable); body composition using field techniques or surrogate measures (body
weight) (if dual energy x-ray absorptiometry (DXA) is not available to measure lean body mass (LBM)); and eating behavior using validated questionnaires and self-report (if a clinical interview is not feasible). It is noteworthy that to measure BMD, DXA or other appropriate imaging assessment must be utilized. Regardless, further prevalence research on the Triad conditions alone or in combination in exercising and athletic women would enable more accurate estimation of the magnitude of this problem. In addition, prevalence estimates are important for developing effective preventive measures, screening criteria, reliable field assessment tools, and management/treatment strategies for the Triad conditions.

Largely, physical activity and exercise may be associated with changes in metabolic and reproductive hormones in the presence of a chronic energy deficiency, menstrual dysfunction, and low BMD in women (see Chapter 2- Review of the Literature Part Two). In classic studies in animals and humans, investigators have illustrated that the etiology of clinical and subclinical EAMD is linked to a cascade of alternations in eating behavior, REE, and metabolic hormones indicative of a chronic energy deficiency. Alternatively, the restoration of an optimal metabolic and reproductive environment in exercising women is associated with favorable changes in eating behavior, REE, and the metabolic hormone profile. An interrelated network of hormonal responses is involved in the mechanisms associated with the induction and recovery of menstrual function in exercising women. Dietary strategies to restore menstrual function (i.e., by increasing energy intake) appear to be optimal approaches such that the focus of the treatment is on improvement of the full metabolic hormone profile (occurs within weeks) concomitant with recovery of menstrual function (occurs within months).
In **Study One**, we confirmed earlier findings from our lab that a high DT is associated with energy deficiency in exercising women when energy deficiency is defined using a ratio of lab-assessed compared to predicted REE (REE/pREE<0.90). We also found that exercising women with high DT demonstrated a greater frequency of severe menstrual disturbances (oligo/amenorrhea) compared to women with normal DT (74% vs. 38%, respectively) as measured using daily ovarian steroid metabolites. Therefore, a high DT score may provide valuable information to coaches, athletic trainers, and health practitioners attempting to get an indication of energy and menstrual status in large groups of exercising women where direct assessment is not feasible.

In **Study Two**, we provided evidence that exercising women with high DR exhibited lower EA and a greater frequency of menstrual disturbances (subclinical and clinical) compared to women with normal DR. Specifically, the women with high DR demonstrated a higher frequency of EAMD (amenorrhea, oligomenorrhea, anovulation or LPD) (75%) compared to the women with normal DR (51%). Interestingly, we did not observe a difference in the frequency of low EA, defined as <30 kcal·kg⁻¹LBM, in women categorized by DR score. This finding may be explained by the observation of only moderately low EA in the high DR group (mean EA of 35.0 kcal·kg⁻¹LBM). Notably, we observed menstrual disturbances in women with high DR despite demonstrating EA values above the proposed threshold. Therefore, low EA may simply not represent a useful marker of energy deficiency in free-living women categorized by DR score. Taken together, the threshold of EA consistent with energy deficiency sufficient to cause menstrual dysfunction may be higher in these women or vary inter-individually.
In Study Three, an RCT of increased energy intake (i.e., refeeding) did not exacerbate DR or have an adverse effect on DT, BD, and bulimia symptoms in women with EAMD. Specifically, the exercising women who increased energy intake demonstrated a DR score at IWk21 similar to the OV Control group and consistent with norms in premenopausal women. In addition, DT, BD, and bulimia scores remained within normal ranges for eating attitudes and behavior across the RCT (below the 75th percentile), such that the mean values for DT, BD, and bulimia scores were similar to the majority of college-aged women. Last, we demonstrated favorable associations between change in DR and BD score and change in body weight/percent body fat in the exercising women who increased energy intake from baseline to IWk21. Overall, an increased energy intake intervention combined with psychological and nutritional monitoring may be associated with favorable outcomes for eating attitudes and behavior, weight gain and REE in exercising women with EAMD.

In Study Four, we observed that a cumulative number of Triad risk factors were associated with an increasing risk of low BMD, suggesting a dose-response relationship between the number of Triad risk factors and BMD in exercising women. Specifically, as the number of Triad risk factors increased from one to four factors, there was an increase in percent of women with low BMD (Z-Score < -1 and Z-Score ≤ -2), ranging from 21-62% and 3-18%, respectively. In addition, we reported associations between combined Triad risk factors, particularly those related to menstrual dysfunction and low BMI/BW, and a higher risk for low BMD compared to women with no or individual Triad risk factors. Therefore, we conclude that the exercising women with multiple Triad risk factors are more likely to demonstrate low BMD.
FUTURE DIRECTIONS

In these dissertation studies, we identified several important future directions of this research focused on the Triad, specifically with respect to the physiological and psychological underpinnings of interrelationships among reproductive function, metabolism, and bone health in exercising women. Notable knowledge gaps in this research area include: (i) the prevalence of varying combinations of subclinical and clinical Triad outcomes, particularly in recreationally active women, (ii) the interrelationships among psychometric indicators of eating behavior (i.e., DR and DT scores), EA, and menstrual function with a focus on determining models of causality for chronic energy deficiency/low EA and menstrual disturbances in exercising women, (iii) the validation of first-pass indicators of risk for low BMD for use by coaches, trainers, and physicians to flag at-risk exercising women for additional assessment using DXA or other bone imaging analyses, (iv) the effect of an increased energy intake intervention on eating attitudes and behavior in exercising women with high scores on the DT, BD, DR, and bulimia subscales, and (v) consensus on optimal non-pharmacological strategies for improving disordered eating behavior and other Triad clinical sequelae in exercising women with EAMD.

The development and implementation of Triad preventive and treatment policies relies on establishing accurate research estimates of the prevalence of subclinical and clinical Triad sequelae in exercising women. Prospective longitudinal studies wherein investigators evaluate the incidence of the Triad in recreationally active women vs. sedentary controls are also needed. Furthermore, specific comparisons are necessary between: (1) those exercising women presenting with all three conditions of the Triad vs.
those with one or two conditions and (2) those women with subclinical Triad presentations vs. those with clinical Triad presentations. These comparisons would enhance our understanding of the development of and recovery from Triad subclinical and clinical conditions and identify potential risk factors for the most pathological presentations of Triad clinical conditions.

Since the work in Studies One and Two were cross-sectional, future prospective research is necessary to evaluate the interrelationships among psychometric indicators of eating behavior (i.e., DR and DT scores), energy status, and menstrual function with a focus on determining models of causality for low EA/chronic energy deficiency and menstrual disturbances in exercising women. Further analysis of the directionality of the associations among psychometric indicators of eating behavior (DR and DT), energy status, and menstrual function would improve our understanding of low EA/chronic energy deficiency and menstrual disturbances. Additional research on the role of EA in menstrual function is necessary to clarify low EA as a key factor associated with menstrual dysfunction in exercising women. In addition, investigators need to conduct studies focused on determining: (i) the effect of varying levels of EA on menstrual function in premenopausal women and (ii) the duration of ‘suboptimal’ EA that is required to induce menstrual disturbances (subclinical or clinical). Such investigation would clarify whether a dose-response relationship exists between magnitude of low EA and severity of menstrual dysfunction.

Future prospective investigation on the effect of an RCT of increased energy intake intervention on eating attitudes and behavior in exercising women with high scores on DT, BD, DR, and bulimia subscales is necessary. Such evidence would guide the
development of a treatment approach for exercising women presenting with disordered eating behavior combined with Triad clinical sequelae. Cluster analyses need to be performed to identify subgroups of women who have different psychological and eating behavior responses to an RCT of increased energy intake. Furthermore, consensus on optimal non-pharmacological strategies for negative eating attitudes and behavior is required to establish and implement effective preventive and treatment strategies in exercising women with EAMD.

Triad risk factors may be utilized as indicators of risk for low BMD and bone stress injury in exercising women in a clinical or field setting. Future investigation on the utility of these Triad risk factors in a pre-participation screening protocol or a user-friendly algorithm is necessary. To this end, the development of preventive and treatment strategies should focus on validating first-pass indicators of risk for low BMD for use by coaches, trainers, and physicians to flag at-risk exercising women for additional assessment using objective measures, i.e., DXA or other imaging analyses. Novel strategies for early detection of possible pathological bone loss, failure to reach peak bone mass, or bone stress injury may be paramount in determining clearance for or withdrawal from participation in sports and exercise and optimizing bone health across the life span.
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