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DIETARY STRATEGIES TO MAINTAIN LOW BODY WEIGHT INCLUDE CONSUMPTION OF LOW ENERGY DENSE FOODS IN WOMEN WITH MENSTRUAL DISTURBANCES

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ABSTRACT

Previous research has demonstrated that women with exercise associated menstrual cycle disturbances (EAMD) including oligomenorrhea or amenorrhea, display restrictive eating patterns. Energy density is defined as the number of kilocalories (kcal) per gram of food or beverage consumed. Strategies to manipulate energy density to achieve increased satiety by incorporation of low energy dense foods into the diet have been recommended for weight loss in obese individuals. The purpose of this study was to determine whether this same strategy to maintain low caloric intake by consuming foods low in energy density is particularly prominent in exercising women with EAMD. A secondary purpose was to identify what specific food groups are consumed to maintain a diet low in energy density. Volunteers in a cross-sectional study were retrospectively characterized by menstrual status into two groups: (1) EAMD (n=12), including women with amenorrhea, and (2) Ovulatory Controls (OV) (n=13). Two 3-day diet records were collected one month apart and analyzed to calculate energy density and other dietary parameters. Measures of aerobic fitness and body composition were obtained. EAMD and OV were similar with respect to age, age of menarche, gynecological age, BMI, VO_2 max, and exercise minutes per week. EAMD had a lower percent body fat (20. 9 ± 1.2%) compared to the OV women (25.7± 1.3%) (p=0.013). EAMD also had a lower total fat mass than OV women (11.6 ± 0.7 vs. 15.3 ± 0.9 kg, respectively; (p=0.005)). Energy intake was lower in EAMD compared to OV women (1663 ± 165 vs. 2187 ± 140 kcals, respectively; (p=0.024)). Diets in EAMD and OV were similar when macronutrient content was compared. Energy density was significantly lower in the EAMD women (0.71 ± 0.06 kcals/gram) compared to the OV women (1.02 ± 0.09) when beverages were included in the calculation (p=0.012), but there was no statistically significant difference in energy density between EAMD (1.07 ± 0.06) and OV women (1.24 ± 0.08) when non-caloric beverages were excluded from the calculation (p=0.098). Vegetable consumption was significantly higher in EAMD compared to OV women (29.6 ± 3.5 vs. 19.3 ± 3.0 servings, respectively; p=0.047), as was condiment consumption (9.2 ± 1.1 vs. 4.4 ± 1.0 servings, respectively; p=0.007). Women with EAMD consume food with lower energy density and likely achieve this lower energy density due to the consumption of non-caloric beverages. These behaviors may represent a strategy to successfully restrict calories and maximize satiety. This study is supported by the U.S. Department of Defense, Army Medical

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CHAPTER 1
LITERATURE REVIEW

Female Athlete Triad

The American College of Sports Medicine (ACSM) defines the Female Athlete Triad as a spectrum of interrelated conditions that range from healthy to unhealthy [1]. At one end of the spectrum, healthy status is considered to be comprised of optimal energy availability, eumenorrhea, and optimal bone health. On the other end of the spectrum, unhealthy status is considered to be comprised of states of low energy availability (with or without an eating disorder), functional hypothalamic amenorrhea, and osteoporosis. In between these two extremes are subclinical, intermediate conditions, such as reduced energy availability, subclinical menstrual disorders, and low bone mineral density. Female athletes move up and down the spectrums at different rates, depending on their own diet and exercise habits [1].

Very few studies, though increasing in number, have been able to determine the prevalence of all three components of the Female Athlete Triad in exercising women [2, 3]. One study showed that women participating in aesthetic sports, such as diving, gymnastics, or dancing, have a higher rate of disordered eating and menstrual disorders than women in non-aesthetic sports [3]. On the other hand, Beals and Hill (2002) found no significant difference in disordered eating or self-reported menstrual irregularities in athletes participating in lean-build sports (diving, swimming, cross-country, and track) versus non-lean build sports (field hockey, tennis, softball, field events) [2]. Nichols et. al (2006) studied female high school athletes and found that 18.2% had disordered eating, 23.5% had menstrual irregularities (amenorrhea or oligomenorrhea), and 21.8% had low bone mineral density for their age according to the World Health Organization. Furthermore, they found that 5.9% of the high school female athletes they studied met the criteria for at least two components of the triad, while 1.2% met the criteria for all three components [4]. Despite the fact that studies report differing prevalence rates, greater education around the female athlete triad is necessary, and research focusing on the etiology and appropriate treatment strategies is needed.

Functional Hypothalamic Amenorrhea

Amenorrhea is defined as the absence of a menstrual period for greater than six months [5-7]. Functional hypothalamic amenorrhea (FHA) is a type of amenorrhea that is due to decreased GnRH release from the hypothalamus, and an associated reduction in LH pulse frequency. This leads to reduced ovarian support and a suppression of folliculogenesis [5, 8-14]. Women with FHA do not have any type of endocrine or central nervous system disease [13]. Berga (1997) defined functional hypothalamic amenorrhea as having a "psychophysiological and behavioral cause" [14]. Unlike women with normal, ovulatory menstrual cycles, women with FHA do not experience a midcycle surge in LH or cyclic changes in estrogen [5, 15]. Women with FHA exhibit decreased estrogen [5, 6, 9, 15-18], progesterone [5, 15, 19, 20], prolactin [9], total triiodothyronine (TT_3) [10, 13, 17, 21], insulin-like growth factor-1 (IGF-1) [10], insulin [7-10], and plasma glucose levels [8-10]. They also exhibit elevated blood concentrations of ghrelin [15, 16, 21], cortisol [5, 9, 12], and growth hormone [10, 19]. The latter hormonal changes indicate a suppressed metabolic state, indicative of a chronic energy deficiency [8, 22, 23].

There are a number of theories as to what causes functional hypothalamic amenorrhea. Some believe that there is a critical amount of body fat that a woman must have to menstruate, and studies have found an association between low body fat and menstrual irregularities [12, 16, 24]. Frisch and McArthur (1974) claimed that women age sixteen or older must have at least twenty-two percent body fat in order to maintain or restore menses [25]. On the other hand, many studies have found similar body fat percentages among both amenorrheic and eumenorrheic female athletes [18, 20, 26, 27]. Another theory is that low body weight causes menstrual dysfunction. While multiple studies have shown lower body weights in amenorrheic subjects compared to eumenorrheic subjects [16, 24, 26, 28, 29], others have shown no significant difference in weight [20, 27]. Frisch and McArthur (1974) stated that a weight loss of ten to fifteen percent of body weight can cause cessation of menses in a normally menstruating female, while a gain of ten to fifteen percent of body weight can restore menses in a female with amenorrhea [25].

A third theory is that stress plays a role in causing functional hypothalamic amenorrhea. Some studies have shown increased hypothalamic-pituitary-adrenal (HPA) activation and higher cortisol levels in women with FHA [4]. Williams, Berga, and Cameron (2007) found that there was an increased rate of hypothalamic amenorrhea when a combination of both psychogenic and metabolic stress was induced in exercising monkeys [30].

One highly supported theory is that low energy availability can cause FHA. Energy availability is defined as energy intake minus energy expenditure during exercise divided by kilograms of fat free mass [23]. Loucks and Thuma (2003) found that LH pulse frequency was reduced by 16% and LH pulse amplitude was increased by 21% when energy availability was restricted to 20 kcal/kg lean body mass/day. LH pulse frequency was decreased by 39%, and LH pulse amplitude was increased by 109% when energy availability was restricted further to 10 kcal/kg lean body mass/day [10]. Manore, Kam, and Loucks (2007) recommended that female athletes should aim for 45 kilocalories per kilogram fat free mass per day [31]. An energy deficit, which can lead to decreased LH pulsatility and menstrual disturbances, can be due to decreased caloric intake [18, 20, 24], increased energy expenditure [32-35], or a combination of both [36]. Williams et. al (2001) showed that exercise associated amenorrhea is caused by an energy deficit, and not simply the act of exercising, by causing resumption of menses in exercising, amenorrheic monkeys by simply supplementing their diets with ~400 additional kilocalories per day without decreasing exercise [37]. In a study conducted by Couzinet et. al (1999), three women with FHA increased their caloric intake for four months. All three women regained menses after two months and had increases in plasma E2 levels and LH pulse amplitude [12]. Because energy availability plays such a prominent role in menstrual function, studies explaining how female athletes regulate their food intake are important.

Despite exhibiting physiological signs of chronic energy deficiency, many amenorrheic female athletes maintain their weight (16). One reason for this could be the lower resting energy expenditure per fat free mass (REE/FFM) that is typical in amenorrheic athletes. A lower resting energy expenditure can be considered an energy conservation mechanism [21, 38]. Myerson et. al (1991) was the first to report a significantly lower resting metabolic rate in amenorrheic runners when compared to eumennorheic runners [17]. Sterling et. al (2009) conducted a study examining amenorrheic versus eumenorrheic female adolescents. She found that the percent expected REE (as determined by the Harris-Benedict equation) was lower in the amenorrheic compared to the eumenorrheic subjects [29]. The results of these studies suggest that amenorrheic females use fewer kilocalories while at rest, thus enabling them to maintain weight despite either reduced caloric intake or increased exercise.

Another theory is that stress plays a role in causing functional hypothalamic amenorrhea. Some studies have shown increased hypothalamic-pituitary-adrenal (HPA) activation and higher cortisol levels in women with FHA [6]. Williams, Berga, and Cameron (2007) found that there was an increased rate of hypothalamic amenorrhea when a combination of both psychogenic and metabolic stress was induced in exercising monkeys [30]. While further research is necessary to explore the role of psychosocial stress in the etiology of exercise-associated amenorrhea, it is possible that the stress of maintaining a lower body weight and low body fat contributes to the suppression of reproductive function in exercising women.

Female Athletes and Nutrition

Nutrition is very important for all females, especially female athletes. Manore (1999) recommended female athletes consume at least 2200 to 2500 kilocalories per day, with endurance athletes that compete in events such as marathons and triathlons consuming upwards of 4000 kilocalories per day, to maintain weight [39]. Carbohydrate intake should equal at least 5 grams per kilogram of body weight so that glycogen levels can remain adequate, even after heavy exercise. It is also recommended that female athletes consume 1.2-1.4 grams of protein per kilogram of body weight, a higher amount than the typical 0.8 grams of protein per kilogram of body weight recommended for the general population. One interesting recommendation that is made is that the female athlete should be in a positive energy balance (i.e. the kilocalories consumed during the day should exceed the kilocalories expended during the day) before partaking in exercise [39].

There are multiple studies that have examined dietary intake in amenorrheic and eumenorrheic female athletes, and there is much variability in the findings of these studies. While a few claim that amenorrheic and eumenorrheic athletes have comparable fat intakes [38], many studies have found that amenorrheic athletes consume lower fat diets than their eumenorrheic counterparts [8, 12, 18, 20, 40]. In fact, Laughlin and Yen (1996) found that the percentage of kilocalories from fat in the amenorrheic athletes was half of that in the eumenorrheic athletes [8]. Sterling et. al (2009) reported a higher RQ in amenorrheic subjects compared to eumenorrheic subjects, which suggests that the amenorrheic subjects consumed a lower fat diet [29]. Some studies have found that the percentage of kilocalories from carbohydrates was significantly higher in amenorrheic than eumenorrheic athletes [8, 16], others have found lower carbohydrate intakes in the amenorrheic athletes [12, 18], and yet others have found similar carbohydrate intakes between the two groups [38]. Some studies have shown that amenorrheic women consume fewer kilocalories than their eumenorrheic counterparts [12, 18, 20], others have shown that amenorrheic athletes consume more kilocalories than eumenorrheic athletes [16], while yet other studies show no significant difference in kilocalorie intake between the two groups of athletes [38, 40]. As can be seen, much debate still exists about the macronutrient composition and total caloric intake of the diets of amenorrheic women.

Micronutrient composition is also a key factor that is studied in amenorrheic and eumenorrheic female athletes. The four key minerals that many female athletes lack, regardless of menstrual status, are calcium, iron, magnesium, and zinc, despite their high requirements for these nutrients [39]. There is much variability in study results regarding nutrient intake in eumenorrheic and amenorrheic athletes. Kaiserauer et. al found that the diets of both eumenorrheic and amenorrheic athletes were low in folic acid, iron, magnesium, and zinc while only amenorrheic athletes' diets were low in thiamin, riboflavin, calcium, and pantothenic acid [20]. Nelson et. al (1986) found that fifty-five percent of amenorrheic athletes and thirty-five percent of eumenorrheic athletes had a dietary calcium intake that was below the RDA of 800 milligrams per day for calcium [18]. It has been found that a higher percentage of amenorrheic athletes are vegetarians. One study found that while 11% of eumenorrheic athletes were vegetarians, 25% of amenorrheic athletes were vegetarians. None of the amenorrheic subjects consumed red meat [20]. As can be seen, both eumenorrheic and amenorrheic female athletes are at risk for micronutrient deficiencies, but they are more common in the amenorrheic population. Clearly, more research is necessary to determine the extent to which micronutrient deficiencies impact the health of these athletes, and to determine behavioral strategies that might be implemented to prevent these deficiencies.

Female Athletes and Disordered Eating

While not all female athletes have eating disorders that meet DSM-IV criteria [41], many have disordered eating, regardless of total kilocalories consumed [2, 41]. Disordered eating, as defined by Torstvejt et. al is “a spectrum of attitudes and behaviors such as a preoccupation with body weight and shape, food restriction, and dieting as well as bingeing, vomiting, and the abuse of diuretics, laxatives and diet pills” [42]. Hopkinson and Lock (2004) reported that female athletes experienced more disordered eating than men, and female recreational athletes experienced disordered eating more frequently than female varsity athletes [43]. Davis and Cowles (1991) found that physically active women had higher levels of body dissatisfaction and body focus than physically active men [44]. Nichols et. al (2006) found that 18.2% of the high school female athletes they studied met the criteria for disordered eating according to the Eating Disorder Eating Questionnaire (EDE-Q) [4]. These disordered eating patterns can eventually lead to clinical eating disorders [42] .

Disordered eating and eating disorders occur significantly more frequently in female athletes with menstrual disturbances, such as FHA, than in regularly menstruating female athletes [26]. Multiple studies have discovered that patients with FHA have statistically higher EAT-26 scores, which measures dieting behavior, bulimic thoughts, preoccupation with food, and self-control with regards to eating [16, 17, 19, 28]. Women with FHA also have been found to score higher on the Eating Disorder Inventory (EDI) [9]. Beals and Manore (2002) reported that 15.2% of female athletes had scores that suggested “at-risk” behavior for an eating disorder on the EAT-26, while 32.4% of female athletes scored in the “at-risk” category on the Body Dissatisfaction subscale of the EDI [3]. Marcus et. al (2001) reported that women with FHA scored significantly higher on the drive for thinness, bulimia, ineffectiveness, and interoceptive awareness subscales of the EDI [45]. Cobb et. al (2003) found that 65% of female subjects scoring high on the EDI had either oligomenorrhea or amenorrhea, while only 25% of the subjects that had a normal score on the EDI had menstrual irregularities [40]. Scheid et. al (2009) also found that exercising amenorrheic women scored higher on the drive for thinness scale than both exercising ovulatory women and sedentary ovulatory women [38]. Amenorrheic athletes have higher dietary restraint than eumenorrheic athletes, as measured by either the Three Factor Eating Questionnaire [38]. However, while many female athletes do suffer from some form of disordered eating, some simply do not eat enough kilocalories to compensate for their high energy expenditure during exercise [39].

Dietary Energy Density

Dietary energy density has become a popular topic of discussion in the weight loss community, due to the work of Dr. Barbara Rolls at The Pennsylvania State University and her development of the *Volumetrics* eating plan [46]. Dietary energy density has been broadly defined as kilocalories per gram of food [47]. Energy density of food is influenced by both moisture and macronutrient content of a food. Macronutrients have defined energy densities of four kilocalories per gram of carbohydrate or protein and nine kilocalories per gram of fat. Oil has the highest energy density in our food supply (9.082 kcals/gram), while water has the lowest energy density (0 kcals/gram) [47, 48]. Moisture can cause foods to have a lower energy density, because water adds to a food's weight without adding energy [47, 49, 50]. Together, water and fat are responsible for ninety-nine percent of the variance in energy density, while carbohydrate, fiber, and protein are responsible for the remaining one percent [50]. Examples of low energy dense foods include fruits, vegetables, soup, and beverages, while examples of high energy dense foods include fats, oils, and spreads such as butter, mayonnaise, and peanut butter [50]. Studies have shown that children and young men in the Army prefer higher energy dense foods. On the other hand, raspberries and other low energy dense foods were preferred amongst a sample of 159 college-aged women [49].

There is debate as to whether or not beverages should be included in the calculation of energy density. Beverages, due to their high water content, usually have low energy densities [47]. However, it is unclear as to whether or not liquids are more or less satiating than solid foods. Some studies have shown higher satiety scores after consuming liquids compared to solids [51], while others have shown the opposite effect [50, 52]. DiMeglio and Mattes (2000) found that subjects who were given a daily load of 450 kilocalories of solid carbohydrates in the form of jelly beans (4 kcals/gram) compensated for these extra kilocalories by consuming fewer kilocalories throughout the rest of the day, so that calorie consumption was not different from their baseline caloric intake. However, subjects who were given a daily load of 450 kilocalories of liquid carbohydrates in the form of non-diet soda (0.39 kcal/ml-0.55 kcal/ml), did not fully compensate for the additional calories in the rest of their daily diet by not decreasing caloric intake through the rest of the day, thus consuming more kilocalories per day than baseline [53]. On the contrary, Pliner (1973) found that obese individuals compensated for preload calories more accurately when given a preload of liquid calories than a preload of solid calories. Obese subjects were given a 600 kcal preload of solid food (white cake with strawberry jam and white icing), a 200 kcal preload of solid food (angel food cake with diet strawberry jam and white icing), a 600 kcal preload of liquid, or a 200 kcal preload of liquid (both skim milk-based formulas). When given the solid food as a preload, obese subjects ate statistically similar amounts of sandwich quarters for lunch afterwards regardless of whether they received the 600 kcal or 200 kcal preload. When given the liquid preload, the subjects ate significantly fewer sandwich quarters if given the 600 kcal preload versus the 200 kcal preload. Normal weight subjects compensated for the preload kcals regardless of whether they received a liquid or solid preload [51]. Other studies have also shown that solid preloads decrease appetite more than liquid preloads [54, 55]. Further studies are needed to determine whether or not to include beverages in energy density continues, because while beverages are low in energy density, it is still unknown if they are as satiating as solid foods.

Many studies have examined the effect of energy density on satiety [56-58]. In one study, lean men between the ages of 22-42 years old consumed one of three preload milk-based beverages: a 300 ml, 450 ml, or 600 ml drink [56]. All three of these drinks contained the same energy and macronutrient composition; the only difference was the amount of water added to each of the beverages. The men that consumed the 600 ml preload, which had the most water added to it, ate significantly less food for lunch than the men that consumed the 300 ml and 450 ml preload, presumably because they were more satiated [56] Drewnowski (1998) also reported that foods with lower energy density are more satiating than foods with higher energy density. However, this investigator also reported that those foods with lower energy density were less palatable than foods with higher energy density [49].

These findings have prompted researchers and dietitians to promote the consumption of low energy dense foods as one strategy in weight loss efforts. The theory is that eating lower energy dense foods will allow a person to eat a greater volume of food and fewer kilocalories, helping to create the feeling of satiety and fullness without excess kilocalories [59, 60]. Fruits and vegetables are also less energy dense because of their high water content [59]. Ello-Martin et. al (2007) found that obese women lost significantly more weight and experienced less hunger by reducing fat intake while increasing fruit and vegetable intake compared to women who only reduced fat intake [59]. Other studies have discovered that eating a low energy dense soup or salad before a meal can help reduce food intake during that meal [58, 61, 62]. Rolls et. al (1999) showed that adding water to a preload of chicken and rice casserole, thus creating a chicken and rice soup, increased satiety and decreased food intake during the next meal [61].

Dietary fiber's relationship with energy density and weight loss has also been studied. Perrigue and colleagues (2009) administered an equal volume preload of either a high energy dense or low energy dense yogurt drink with or without added inulin, a naturally occurring soluble fiber. The high-energy-density/high-fiber yogurt drink, high-energy-density/low-fiber yogurt drink, and the low-energy-density/high-fiber yogurt drink similarly increased fullness significantly more than the low-energy-density/low-fiber yogurt drink and orange juice. The subjects then were fed a lunch meal that contained 1,738 kilocalories and told they could consume as much or as little as they liked. The subjects that consumed the low energy dense yogurt with added fiber or either of the high energy dense yogurts consumed the same amount of kilocalories at lunch. Since the same amount of kilocalories were consumed at lunch, those subjects consuming the low energy dense yogurt with added fiber consumed less kilocalories overall because they consumed fewer kilocalories during the preload. [63].

To date, no studies have examined whether diets of eumenorrheic and amenorrheic athletes differ in energy density. Because amenorrheic exercising women have been shown to consciously restrict food intake, it is possible that these women naturally consume a diet that is lower in energy density as a method to reduce kilocalorie consumption without reducing food volume.

CHAPTER 2
INTRODUCTION

Female athletes are at risk for developing the female athlete triad, which consists of three interrelated conditions: low energy availability (with or without an eating disorder), amenorrhea, and osteoporosis [1]. Recently, the American College of Sports Medicine has recognized that the female athlete triad is represented by a spectrum of interrelated conditions ranging from healthy to unhealthy, including intermediate conditions such as reduced energy availability, subclinical menstrual disorders, and low bone mineral density [1].

The most severe form of menstrual disturbance described by the Female Athlete Triad is functional hypothalamic amenorrhea (FHA), which is the cessation of menses caused by a reduction in luteinizing hormone (LH) pulse frequency from the anterior pituitary gland due to decreased release of GnRH from the hypothalamus [5, 8-13]. Women with FHA do not experience the normal midcycle surge of LH or cyclic changes in estrogen that regularly ovulating women experience each month [5, 15]. While there are many theories as to what may cause functional hypothalamic amenorrhea, the most highly supported theory currently is that low energy availability, another component of the female athlete triad, is involved in the etiology of amenorrhea in exercising women [12, 37].

Low energy availability is defined by Loucks (1993) as the difference between energy intake and energy expenditure during exercise divided by total fat free mass [23]. Since adequate energy availability is necessary for maintaining proper menstrual function, it is critical for exercising women to consume enough kilocalories to account for energy expenditure. An increase in caloric intake could help amenorrheic exercising women to resume menses. In fact, Williams et. al (2001) found that supplementing the diets of exercising, amenorrheic monkeys with four hundred additional kilocalories caused the resumption of menses without decreasing exercise [37]. Couzinet et. al (1999) also found that after increasing their caloric intake for only two months, three women with amenorrhea were able to regain menses [12]. All of these studies indicate that caloric intake is one target of intervention in exercising women with functional hypothalamic amenorrhea.

Eating patterns such as food restriction, dieting due to high concern for body shape, binging, and purging that are not severe enough to be classified as eating disorders according to the DSM-IV criteria are considered forms of disordered eating [41, 42]. Women with functional hypothalamic amenorrhea are more likely to have disordered eating patterns than regularly menstruating women [26]. In fact, studies have shown that women with amenorrhea have higher scores on the Eating Attitudes Test (EAT-26) and the Eating Disorder Inventory (EDI) [9, 16, 17, 19, 28, 40]. Drive for thinness, as measured by the EDI, is typically found to be higher in amenorrheic women compared to eumenorrheic women [38, 45]. Cognitive restraint, a subscale of the Three Factor Eating Questionnaire, is also found to be elevated in amenorrheic women [38]. The increased prevalence of disordered eating patterns in amenorrheic women suggests that these women may have a preoccupation with weight and body image, which may lead to dieting and food restriction in an attempt to maintain a low body weight.

Dietary energy density, broadly defined as kilocalories per gram of food [47], has become a popular strategy for weight loss [59, 60]. By lowering dietary energy density, a larger volume of food can be consumed, thus leading to satiety, without increasing kilocalories [60]. Water decreases energy density, because it adds weight without adding kilocalories [47, 49, 50]. Fat, on the other hand, increases energy density, because it has nine kilocalories per gram, the highest energy density of all macromolecules [47, 48]. Therefore, moisture and fat account for ninety-nine percent of the variance in energy density between foods and beverages [50]. Nutrition experts are promoting the consumption of fruits, vegetables, and soups, all which have a high water content and low energy density, while at the same time reducing foods high in fat, which have high energy densities [59, 61]. Beverages also have low energy density, because of their high water content [47], but there is much debate as to whether or not beverages are as satiating as solid food, despite their low energy density [50-52]. While foods low in energy density are thought to be useful for weight loss, it is possible that incorporation of these foods may be part of a strategy to keep weight low in normal weight individuals.

The purpose of the present study was to determine if women with exercise associated menstrual disturbances (EAMD) consume a lower energy dense diet than eumenorrheic exercising women as a strategy to increase satiety while not increasing caloric intake. Furthermore, this study investigated what specific strategies were used to decrease energy density, such as a high intake of fruits and vegetables, low fat intake, or high intake of non-caloric beverages. We hypothesize that EAMD women will consume a diet that is lower in energy density when compared to exercising women with ovulatory menstrual cycles, and that strategies to achieve lower energy density foods will include increasing fruit and vegetable intake, lowering fat intake, and consuming non-caloric beverages.

CHAPTER 3
MATERIALS AND METHODS

Experimental Design

This cross-sectional study was part of a larger twelve month randomized control trial that was originally designed to study the effect of increased caloric intake on menstrual function and bone health in regularly exercising young women at the University of Toronto and The Pennsylvania State University.

Subjects were recruited through newspaper advertisements, fliers, and classroom announcements. All study procedures were approved by the Human Ethics Board at the University of Toronto and the Biomedical Institutional Review Board at The Pennsylvania State University. Informed consent was obtained from all subjects in both Toronto and Pennsylvania.

Inclusion criteria were as follows: 1) between the ages of 18 and 35 yrs; 2) BMI between 16-25 (kg/m^2); 3) less than 30% body fat; 4) no history of any chronic illness, including history of hyperprolactinemia (high levels of prolactin), thyroid, or metabolic disease; 5) gynecological age ≥ 5 years; 6) weight stable (± 2kg) for the past 6 months; 7) non-smoking; 8) no medications that would alter metabolic hormone concentrations; 9) not taking any form of hormonal therapy for the past 6 months; 10) no menses within the last 3 months <u>or</u> 6 or fewer menses within the last 12 months (EAMD) <u>or</u> regular menses within the last 6 months (OV) 11) no history of polycystic ovarian syndrome (PCOS) or a free androgen index (FAI) ≥ 3 with other diagnostic symptoms of PCOS; 11) ≥3 hours/week of aerobic exercise for EAMD women and ≥2 hour/week of aerobic exercise for OV women. Subjects were excluded from the study if they 1) had a chronic disease, such as thyroid disease, diabetes, or hyperprolactinemia (high levels of prolactin), 2) were sedentary with less than 2 hr/week of aerobic or resistance training, 3) smoked, 4) were pregnant or planning a pregnancy during the course of the study, 5) consumed medications or dietary supplements that were incompatible with measuring hormones or metabolism, 6) were currently diagnosed with a clinical eating disorder or other clinical psychiatric disorder, 7) had other contraindications that would preclude participation in the study, or 8) had a bone density that is greater than a z score of 3.0 (i.e. extremely high bone density).

In the current study, 25 female volunteers were retrospectively characterized by menstrual status as determined at Baseline into two groups: (1) Exercise associated Menstrual Disturbances, or EAMD, including only women with amenorrhea (n=12) and (2) Ovulatory Controls, or OV (n=13). Women with oligomenorrhea or PCOS were excluded. Women were categorized into the OV group if they experienced menses at regular intervals of 26-35 days with ovulation present. Women were categorized into the EAMD group if they had not experienced menstrual bleeding during the past three months and had a FAI < 3, which was calculated as (total testosterone (nmol/L) / serum hormone binding globulin (nmol/L))*100 [64].

Screening Procedures

During an initial visit, study details and participation requirements were explained, and written informed consent was obtained. Once consent was obtained, height and weight were measured, and subjects filled out the following questionnaires: 1) Health, Exercise and Nutrition Survey that includes information on demographics, medical history, exercise history, menstrual and bone health history, and nutrition history 2) Three Factor Eating Questionnaire [65], 3) Calcium Intake Inventory [66], 4) Beck Depression Inventory [67] , 5) Eating Disorder Inventory (EDI-2) [68], 6) Perceived Stress Scale [69], 7) Importance of Change Scale (Olmsted, 2005 unpublished), 8) Brief Resilient Coping Scale [70] 9) Profile of Moods Scale

[71], 10) Dsyfunctional Attitude Scale (DAS) [72, 73], and 11) Daily Stress Inventory (DSI) [74]. A physical exam is performed on all subjects by an on-site clinician to determine overall health and check for physical symptoms of PCOS. Additionally, a fasting blood draw was analyzed for a complete blood count and endocrine panel, including human chorionic gonadotropin (HCG), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, prolactin, thyroid stimulating hormone (TSH), thyroxine (T_4), free testosterone, and dihydroepiandrosterone sulfate (DHEA-S). An on-site psychologist visited with subjects to review scores from the previously completed questionnaires, as well to determine if subjects were suffering from depression or clinical eating disorders. Subjects will also meet with an on-site dietitian after completing a 3-day diet log (2 weekdays and 1 weekend day) to discuss eating patterns and preferences. Calcium and vitamin D supplements will also be prescribed in addition to dietary intake in order to reach 1000-1300 mg/day of calcium and 400 IU of vitamin D. calcium and Vitamin D supplements. Finally, bone mineral density (g/cm^2) of the total left and right hip, lumbar spine L1-L4, and total body was measured by a GE iDXA machine (General Electric Lunar Corporation, Madison, WI) by an on-site certified technician.

Baseline Procedures

Baseline lasted four weeks for both EAMD and OV subjects. EAMD subjects began baseline on the first Monday after screening was completed. OV subjects began baseline on Day 1 of their next menses. Body weight was measured to the nearest 0.1 kg weekly for all of baseline. Menstrual logs were used to record menstrual bleeding and other menstrual symptoms, such as cramping, spotting, and discharge. Exercise logs were completed each week, and subjects recorded the duration and self-measured heart rate measurement of each purposeful exercise session, were recorded each week. First morning urinary voids were collected for 28 arbitrary days in EAMD subjects and from the first day of menstrual bleeding until the first day of their next menstrual cycle in OV subjects. During week 3, resting metabolic rate was measured by indirect calorimetry using a Sensormedics Vmax metabolic cart (Yorba Linda, CA). Subjects reported to the lab first thing in the morning having fasted for twelve hours and refrained from exercise, alcohol, and caffeine for twenty-four hours. After a forty-five minute rest period, a ventilated hood was placed on the subjects, and resting metabolic rate was measured for forty-five minutes. Additionally during week 3, subjects wore activity monitors (RT3 Triaxial Research Tracker, Stayhealthy, Inc., Monrovia, CA) on their left hip to measure daily energy expenditure while keeping a log of daily activities. When engaging in purposeful physical activity, subjects wore a Polar S610 heart rate monitor (Polar Electro Oy, Kempele, Finland) to measure heart rate and energy expenditure during exercise. Each subject recorded a 3-day diet log on two weekdays and one weekend day during week 3 to measure energy intake. A fasting blood sample was collected during week 3 to measure bone turnover markers, including procollagen type 1 amino-terminal propeptide (P1NP) and osteocalcin, and metabolic hormones, including leptin, T_3, IGF-1, and ghrelin. A 24 hour urine collection was performed to measure bone turnover markers, particularly carboxyl-terminal telopeptide (uCTx), and stress hormones, such as cortisol. Aerobic capacity was measured by a VO_2 max test performed during week 3 of baseline.

Anthropometrics

Height and weight were both measured during an initial screening period. Height was measured to the nearest 1.0 cm without shoes. Total body weight was measured by a digital scale in the laboratory to the nearest 0.01 kg in a t-shirt and gym shorts. Body mass index (BMI) was calculated as a ratio of weight to height (kg/m^2). Body composition, including percent body fat, fat mass (FM), and total fat free mass (FFM), and bone mineral density was analyzed during screening by a trained medical technologist via dual-energy x-ray absorptiometry (DXA). The majority of subjects were scanned on either a GE Lunar Prodigy DXA scanner (n=14, General Electric Lunar Corporation, Madison, WI, enCORE 2002 software, version 6.50.069) or a GE Lunar iDXA scanner (n=7, General Electric Lunar Corporation, Madison, WI, enCORE 2008 software version 12.10.113). Remaining subjects were scanned on a Hologic QDR4500 DXA scanner (n=4, Hologic Inc., Bedford, MA). A cross calibration equation was used to remove any differences between measurements of the GE Lunar Prodigy DXA scanner and the GE Lunar iDXA scanner, and a cross calibration equation is currently being created to remove any differences in measurements between the GE Lunar iDXA and Hologic scanner.

Psychological Questionnaires

Results from four questionnaires were used in the current study. The Three Factor Eating Questionnaire (TFEQ) [65], the Eating Disorder Inventory (EDI-II) [68], the Beck Depression Inventory [67], and the Profile of Moods Scale (POMS) [71] were given to all subjects during the screening period and scored by a trained research assistant.

The Three Factor Eating Questionnaire (TFEQ) is a 51-item questionnaire that measures three subscales of disordered eating etiology: cognitive restraint, disinhibition and hunger. The TFEQ has been shown to have good reliability, validity, and internal consistency [65].

The Eating Disorder Inventory (EDI-II) is a 64-item questionnaire that measures 8 subscales of attitudes and behaviors related to disordered eating or preoccupation with weight. These 8 subscales are drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation, and social insecurity. All 8 subscales have established reliability and validity [68].

The Beck Depression Inventory is a 21-item questionnaire that assesses severity of current depressive symptoms. This questionnaire has established good internal consistency and reliability [67].

The Profile of Moods Scale (POMS) is a 65-item questionnaire that tests for friendship, tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, confusion-bewilderment, and total mood disturbance. This questionnaire has been shown to have good internal consistency, reliability and validity [71]. The current study focused on tension/anxiety in order to assess if certain eating patterns were causing increased tension and anxiety in subjects.

Aerobic Capacity

During the baseline cycle, subjects performed a VO_2 max test. VO_2 peak was determined using a progressive treadmill test to volitional exhaustion using open-circuit spirometry. After a two to five minute warm-up period of walking comfortably on the treadmill, volunteers selected a comfortable running speed at 0.0% grade. The grade of the treadmill increased 2.0% every two minutes for the first eight minutes of the test, after which the grade increased 1.0% for each subsequent minute. Expired air was collected continuously through a Hans Rudolph valve and corrugated plastic tubing connected to a flow transducer that measured inspired air volumes. Expired air samples were measured using an on-line MedGraphics Modular VO_2 System (St Paul, MN). Criteria that will be used to determine if VO_2 max is achieved was the attainment of three of the four criteria: 1) the attainment of age-predicted maximal heart rate (220 – age in years); 2) a RER $\geq$ 1.1; 3) a plateau (i.e., a rise < 2.50 $ml \cdot kg^{-1} \cdot min^{-1}$) in oxygen consumption despite an increase in exercise workload; and 4) the attainment of a RPE score $\geq$ 18. Any three or four of these criteria will be used to terminate a test, in addition to the volunteers' indication to terminate the test at anytime.

Dietary Intake

Dietary intake was assessed from 3-day nutritional logs during both screening and baseline. Subjects were asked to record all food and beverage consumption on 2 weekdays and 1 weekend day. Subjects were also asked to record time and location of every eating episode. On-site registered dietitians met with the subjects to train them to record dietary intake accurately. 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). Average kilocalorie intake, macronutrient composition, and weight of the food and beverages consumed from both screening and baseline diet records were averaged together and recorded for this study.

Energy Availability

Energy availability (EA) is defined as dietary energy intake (EI) minus energy expended during exercise (EE) divided by kilograms of fat free mass (FFM) (EA = (EI –EE)/FFM) [23]. Measures of dietary energy intake were calculated from 3-day diet logs. Both screening and baseline diet logs were averaged together to calculate dietary energy intake. During one week of baseline, calories expended during each exercise session were measured using the OwnCal feature on the Polar S610 heart rate monitor (Polar Electro Oy, Kempele, Finland). For physical activities in which participants were unable to wear the Polar S610 heart rate monitor, i.e. water activities, the Ainsworth et al. (2000) compendium of physical activities was used to determine the appropriate metabolic equivalent (MET) level for the exercise performed [66]. The MET level was then multiplied by the duration (min) of the exercise session and resting energy expenditure (kcal/min). This product provided the total calories expended during the exercise session. Fat free mass was measured by dual-energy x-ray absorptiometry (DXA) during the screening period (GE Lunar Corporation, Madison, WI).

Energy Density Calculations

Energy density has been described as kilocalories per gram of food consumed [44]. In the current study, energy density is defined as kilocalories per gram of food or beverage consumed, excluding water. Energy density was calculated for each day using both the screening and baseline 3 day diet logs, equaling a total of six daily energy density calculations. These six energy density calculations were then averaged to determine the energy density reported in this study.

Two analyses were performed using data for energy density. The first analysis included all foods and all beverages, except for water. The second analysis included all food and beverages, except for water and other non-caloric beverages, including plain coffee, unsweetened tea, diet soda, and other diet drinks.

Food Group Variety

Food group variety was determined by calculating the number of servings of eleven different food groups over six days (two 3 day food logs). This value was then divided by six to calculate the average servings per day of all food groups. Serving sizes were based on the recommendations made by the *Dietary Guidelines for Americans 2005* when available. Certain foods, such as cookies and fruit drinks, are not included in the *Dietary Guidelines for Americans 2005*, so Food and Drug Administration (FDA) serving sizes were used. The eleven food groups were as follows: 1) fruits (including whole fruits and 100% fruit juices); 2) vegetables (including non-starchy vegetables, starchy vegetables, legumes, and vegetable juices); 3) grains (including breads, pastas, cereals, crackers, snack bars, snack chips, and popcorn); 4) meat and meat alternatives (including meat, poultry, fish, eggs, nuts, and meat alternatives); 5) dairy (including milk, cheese, yogurt, and cream); 6) fats (including butter, margarine, oils, and salad dressings); 7) condiments and miscellaneous (including rich sauces, gravies, condiments, pickled foods, and soup broth); 8) Sweets (containing sugar, baked goods, frozen desserts, puddings, honey, jelly, sweet sauces, candy, and other desserts); 9) caloric beverages (containing sweetened soft drinks, tea, coffee, fruit drinks, and water); 10) alcohol (containing beers, liquors, and wines); and 11) non-caloric beverages (containing unsweetened or artificially sweetened soft drinks, tea, coffee, and fruit drinks). Water was not included in the food group variety analysis. Daily servings of each food group were summed over two 3 day food logs, for a total of six days, in order to determine total servings. Servings were then adjusted for daily caloric intake.

Determination of Menstrual Status

Subject groupings were based on self-reported menstrual history, physical exam, blood test, and daily first morning urinary hormone measurements. Subjects were classified as ovulatory if they reported experiencing regular menstrual cycles of 26-32 days for the past six months. Subjects were classified as EAMD if they reported experiencing six or less menstrual cycles in the past 12 months. Subjects also underwent a physical exam and blood test to ensure that they were not experiencing menstrual disturbances due to organic causes, such as polycystic ovarian syndrome (PCOS) or hyperprolactinemia. First morning urinary voids were collected for one month during baseline. Ovulatory controls started collecting urine on the first day of menstrual bleeding and continued collecting until the first day of their next menstrual period. EAMD subjects collected urine for an arbitrary 28 days. Samples were aliquoted into six 2 ml polyethylene tubes and stored at -80 C until analyzed. Luteinizing hormone (LH), pregnanediol 3-glucuronide (PdG), and estrone 3-glucuronide (E1G) were measured in daily urinary collections to determine if the menstrual cycle during baseline was ovulatory or anovulatory, as well to assess the length and adequacy of the follicular and luteal phases. Analyses of these hormonal profiles has been described elsewhere [75]. Menstrual calendars were used to chart menstrual symptoms, such as cramps, bleeding, spotting, discharge, etc. for both Ovulatory Controls and EAMD.

Statistical Analysis

Before statistical analysis was run, all variables were tested for outliers. Outliers identified were not included in analysis. To determine whether or not EAMD and OV women were similar in regards to demographics, nutritional intake, and psychological parameters, independent t-tests were conducted with menstrual status (EAMD vs OV) as the grouping variable. A similar independent t-test was also conducted to determine differences in energy density between EAMD and OV women. In order to adjust for caloric intake differences between EAMD and OV women, an analysis of covariance (ANCOVA) was conducted on food group data with each individual food group as the dependent variable, menstrual status (EAMD vs OV) as the fixed factor, and kilocalorie consumption as the covariate. All data are reported as mean ± sem, and $p \leq 0.05$ was considered statistically significant. All data were analyzed with SPSS for Windows (version 18; Chicago, IL).

CHAPTER 4
RESULTS

Basic Demographics, Nutrition, and Psychological Characteristics of Subjects

Twenty-five subjects were included in this study. Nineteen identified themselves as Caucasian (76%), two were Aboriginal or Inuit (8%), two were Asian (8%), one woman identified herself as Latin American (4%), and one woman identified herself as an unlisted ethnicity (4%). Descriptive data for all EAMD and Ov subjects are shown in Table 1. No significant differences existed between the two groups in age, age of menarche, gynecological age, height, weight, BMI, VO_2 max, fat free mass, or exercise minutes per week. The percent body fat of the EAMD subjects was significantly lower than the OV subjects (p=0.013), as was the total fat mass (p=0.005).

Table 1. Basic demographic characteristics of EAMD and OV subjects

	OV (n=13)		**EAMD (n=12)**		
Variable	**Mean ± sem**	**Range**	**Mean ± sem**	**Range**	**P-value**
Age (years)	24.0 ± 1.7	18-35	22.8 ± 1.0	18-30	0.542
Age of menarche (years)	12.8 ± 0.4	10-16	13.3 ± 0.4	11-16	0.363
Gynecological age (years)	11.2 ± 1.6	6-22	9.1 ± 1.0	4-17	0.295
Height (cm)	167.6 ± 1.4	162-178.5	165.7 ± 1.8	156.5-176.1	0.392
Weight (kg)	59.6 ± 0.8	55.0-64.5	56.3 ± 1.4	50.6-65.2	0.053
BMI (kg/m^2)	21.2 ± 0.3	19.2-22.9	20.5 ± 0.4	18.8-23.1	0.138
VO_2 max (ml/kg/min)	48.8 ± 3.1	36.6-67.6	50.6 ± 2.4	43.1-65.0	0.655
Percent body fat	25.7 ± 1.3	18.8-31.8	20.9 ± 1.2	10.7-28.0	0.013[a]
Fat mass (kg)	15.3 ± 0.9	10.3-20.4	11.6 ± 0.7	5.9-14.4	0.005[a]
Fat free mass (kg)	44.7 ± 0.7	39.7-48.2	45.1 ± 1.5	36.5-54.8	0.682
Exercise minutes (min/week)	358± 70	58-874	438± 59	190-820	0.402

[a]p<0.05 Independent samples t-tests EAMD vs Ov

Nutrition data are presented in Table 2. There were no statistically significant differences between total gram intake, total carbohydrate intake, percent carbohydrate, total fat, percent fat, total protein, or percent protein between EAMD and OV subjects. Total kilocalorie consumption was significantly lower in EAMD compared to OV subjects (p=0.024).

Table 2. Nutritional intake characteristics of EAMD and OV subjects

	OV (n=13)		**EAMD (n=12)**		
Variable	**Mean ± sem**	**Range**	**Mean ± sem**	**Range**	**P-value**
Total kilocalories (kcal/day)	2187 ± 140	1284-2852	1662 ± 165	725-2567	0.023[a]
Total grams (g/day)	2325 ± 225	998-3840	2761 ± 383	1013-6177	0.328
Total carbohydrate (g/day)	309 ± 29	147-480	246 ± 23	110-372	0.108
Percent carbohydrate (% kcals)	54.6 ± 2.3	39.2-65.4	58.5 ± 2.7	42.7-73.5	0.279
Total fat (g/day)	67 ± 4	38-99	53 ± 67	15-116	0.159
Percent fat (% kcals)	27.4 ± 2.0	20.1-43.9	23.0 ± 2.1	12.3-34.7	0.142
Total protein (g/day)	87 ± 5	52-113	78 ± 12	29-180	0.508
Percent protein (% kcals)	16.0 ± 0.8	9.9-21.1	15.5 ± 0.9	12.1-22.7	0.652

[a]p<0.05 Independent samples t-tests EAMD vs Ov

Data representing energy status are presented in Table 3. EAMD women had a lower kilocalorie consumption per kilogram of body weight than OV women (29.3±2.8 vs. 36.9 ± 2.5 kcals/kg; p=0.057) and a lower ratio of actual RMR to predicted RMR (0.86 ± 0.02 vs. 0.93 ± 0.03; p=0.053), but neither of these was able to reach statistical significance. However, EAMD women had a much lower energy availability than OV women (29.9 ± 3.6 vs. 43.2 ± 2.2 kcals/kg FFM; p=0.003).

Table 3. Energy status of EAMD and OV subjects

Variable	OV (n=13) Mean ± sem	Range	EAMD (n=12) Mean ± sem	Range	P-Value
Kilocalories per body weight (kcal/kg)	36.9 ± 2.5	19.9-49.4	29.3 ± 2.8	13.7-44.6	0.057
Actual RMR/Predicted RMR*	0.93 ± 0.03	0.81-1.11	0.86 ± 0.02	0.71-0.96	0.053
Energy availability (kcal/kg FFM)**	43.2 ± 2.2	27.9-56.3	29.9 ± 3.6	12.9-48.3	0.003[a]

*As predicted by the Harris Benedict equation
**Energy availability=(energy intake-energy expenditure during exercise)/kg FFM
[a]p<0.05 Independent samples t-tests EAMD vs Ov

Scores from the Beck Depression Inventory, Eating Disorder Inventory (EDI), Profile of Moods Scale (POMS), and Three Factor Eating Questionnaire (TFEQ) are shown in Table 4. Although scores were higher for all psychological measures in EAMD compared to OV women, the only statistically significant differences were the Drive for Thinness score from the EDI (p=0.019) and the Cognitive Restraint score from the TFEQ (p=0.009).

Table 4. Psychological scores of EAMD and OV subjects

Variable	OV (n=13) Mean ±sem	Range	EAMD (n=12) Mean ± sem	Range	P-Value
Drive for thinness*	0.6 ± 0.3	0-4	3.0 ± 0.9	0-8	0.019[a]
Beck depression inventory	3.3 ± 1.2	0-13	4.3 ± 0.9	0-10	0.546
Tension/anxiety**	12.5 ± 1.6	7-24	16.3 ± 2.6	5-32	0.199
Cognitive restraint***	6.8 ± 1.2	0-16	12.0 ± 1.3	6-21	0.009[a]
Disinhibition***	4.8 ± 0.9	1-13	6.5 ± 0.9	2-13	0.191
Hunger***	5.0 ± 0.9	0-11	6.3 ± 0.9	2-11	0.296

*As measured by the Eating Disorder Inventory
**As measured by the Profile of Moods
***As measured by the Three Factor Eating Questionnaire
[a]p<0.05 Independent samples t-tests EAMD vs Ov

Energy Density

Energy density data are presented in Figure 1. EAMD women had lower energy density both with (0.77 ± 0.06 vs. 1.06 ± 0.09) and without non-caloric beverages (1.07 ± 0.06 vs. 1.24 ± 0.08) , but statistical significance was reached only when beverages were included (p=0.018 vs. p=0.098).

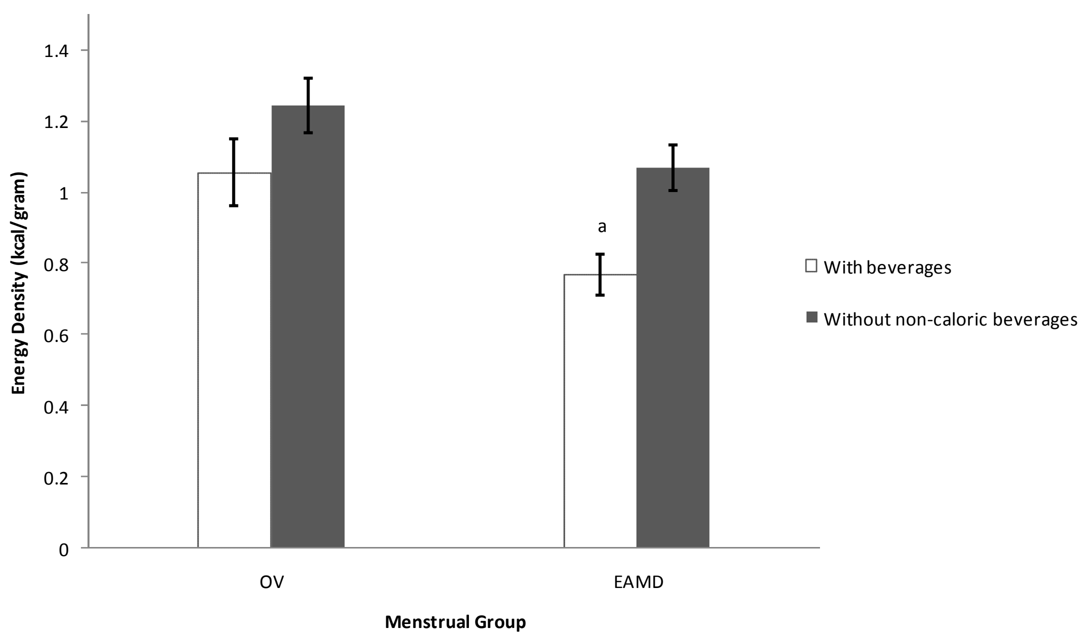

Figure 1: Bar graphs represent the mean (±SEM) of dietary energy density both with beverages (except water) and without non-caloric beverages. [a]P<0.05 OV vs. EAMD.

Food Group Variety

Figure 3 shows servings per day, adjusted for total caloric intake, of eleven different food groups. No significant differences were seen between intake of fruits, grains and cereals, meat and meat alternatives, dairy, fats and oils, sweets and sugar, caloric beverages, alcohol, or non-caloric beverages between EAMD and OV women. Vegetable intake per day was significantly higher in EAMD women compared to OV women (4.9 ± 0.6 vs. 3.2 ± 0.5 servings; p=0.047).

Condiments and miscellaneous intake per day was also higher in EAMD women compared to OV women (1.5 ± 0.2 vs. 0.7 ± 0.2 servings; p=0.007).

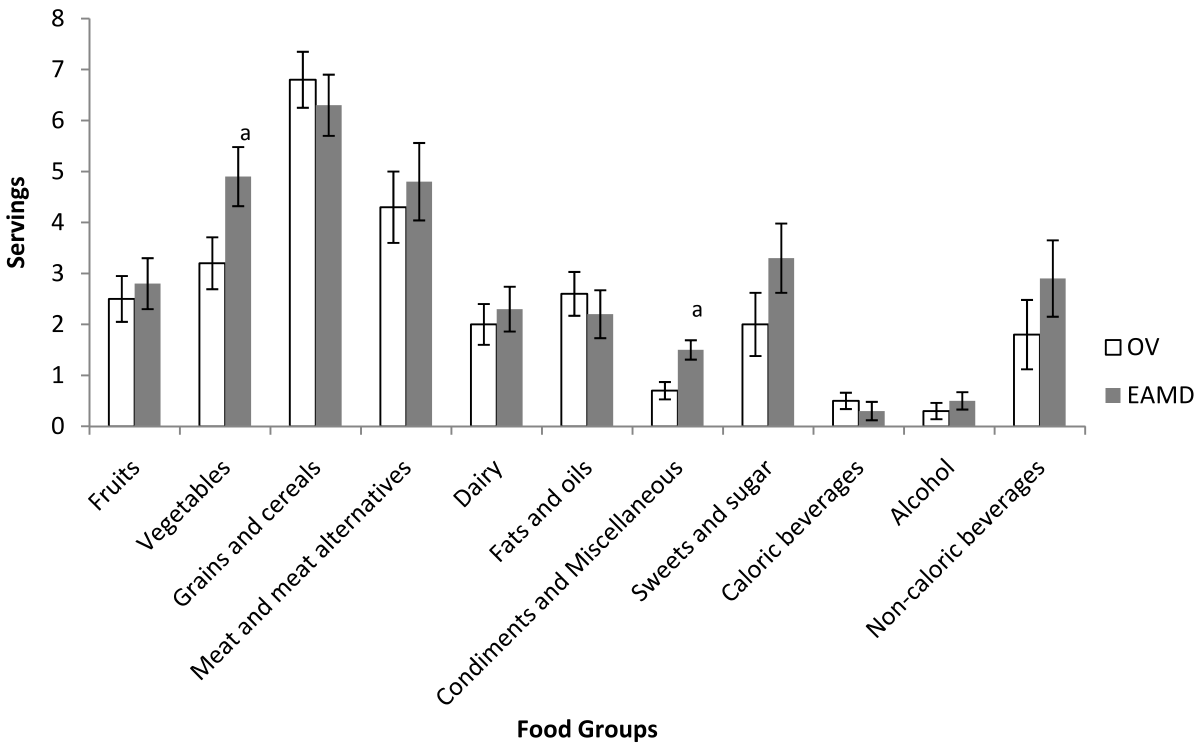

Figure 2: Bar graphs represent the total USDA servings per day (Mean ± SEM) of different food groups. [a]P<0.05 OV vs. EAMD.

CHAPTER 6
DISCUSSION

The current study is the first study to examine energy density in the diets of amenorrheic and ovulatory exercising young women to determine whether differences exist. The main finding of this study is that EAMD women consume a diet significantly lower in energy density when non-caloric beverages are included in the calculation, but not when non-caloric beverages are excluded. We also found that EAMD women consume significantly more vegetable and condiment servings than OV women when adjusted for caloric intake.

Energy density was calculated two different ways in both the EAMD and OV subjects. When calculated with all non-caloric beverages except water, energy density was significantly lower in EAMD compared to OV subjects. However, when calculated without non-caloric beverages, such as unsweetened or artificially sweetened tea, coffee, soft drinks, and fruit drinks, the difference in energy density was no longer statistically significant. Differences in energy density between the two groups when non-caloric beverages are considered can be explained by the significantly lower caloric intake and higher gram intake of the EAMD subjects. When non-caloric beverages are removed from the calculation, caloric intake does not change, but the difference in gram intake between the two groups is much less, thus explaining why energy density is no longer significantly different without non-caloric beverages.

Energy density can be altered by the moisture content and the fat content of food [47, 48]. Foods high in moisture, such as fruits, vegetables, soup, and beverages, are low in energy density, while foods high in fat are high in energy density [47, 49, 50]. We hypothesized that women with EAMD would achieve lower energy density by eating more fruits, vegetables, non-caloric beverages, while decreasing their intake of fats and oils. Our results show that EAMD women ate significantly more servings of vegetables than OV women. They also consumed more servings of fruits and non-caloric beverages, and less servings of fats and oils, though these values did not reach statistical significance. Although servings of fats and oils were lower in the EAMD compared to the OV women, percent of kilocalories from fat was similar between the two groups. Typically, the literature reports that fat consumption is lower in amenorrheic women [8, 12, 18, 20, 40]. This difference in our findings could be due to the increased consumption of non-caloric beverages as a method for controlling weight instead of the decreased consumption of dietary fat. In fact, studies conducted by de Castro report that subjects who consumed non-caloric beverages had a lower overall daily caloric intake than those who did not consume non-caloric beverages [76, 77].

An unexpected finding in this study is that EAMD women consumed significantly more condiments and miscellaneous foods than OV women. The difference in condiment intake is mostly due to an increased intake of low fat condiments and sauces, such as ketchup, mustard, soy sauce, barbeque sauce, and other related condiments. These foods add grams, but they are not high in kilocalories. Since these foods are typically used to add flavor, and not increase satiety, EAMD women may use them in place of other high fat options, such as mayonnaise or rich sauces, to improve the flavor of foods without drastically increasing kilocalories. Studies have correlated increased condiment intake with increased kilocalorie consumption [77] or obesity [78], yet these studies did not separate high fat condiments, such as mayonnaise or dressings, from lower fat condiments, such as ketchup, mustard, and soy sauce. These studies also associated condiment intake with intake of higher fat meats and fast foods. Our study considered condiments separately from any other foods, and the majority of condiments consumed were those lower in fat and kilocalories.

It has been well documented in the literature that amenorrheic women score higher on the EDI, particularly on the drive for thinness subscale, as well as on the cognitive restraint subscale of the TFEQ [9, 38, 40, 45]. We also found that our EAMD subjects had significantly higher drive for thinness and cognitive restraint scores. These higher scores may indicate that EAMD women, due to their desire to remain at a lower weight, choose to consume fewer kilocalories. Our study found that despite similar exercise levels, EAMD women consume significantly fewer kilocalories per day. We also considered energy availability in our EAMD and OV women, and we found significantly lower energy availability in EAMD women. All of these results suggest that despite actual caloric need, EAMD women are consuming fewer kilocalories. In order to achieve this low caloric intake without sacrificing satiety, women may consume foods low in energy density.

Our study was careful to only include EAMD subjects that were amenorrheic due to non-organic causes by screening out subjects that had polycystic ovarian syndrome (PCOS) or other hormonal irregularities, such as hyperprolactinemia or thyroid disease. Ovulatory women were identified using self-reported menstrual logs and daily urinary measures of LH, PdG, and E1G. Women with oligiomenorrhea were not included in the study. These strict criteria related to menstrual function helped to ensure that all subjects were actually amenorrheic or ovulatory.

Limitations to this study include the fact that dietary intake and nutrition data were collected using self-reported three day food records. Many studies have shown some inaccuracy when measuring dietary intakes using self-reported food records, particularly with underreporting [79, 80]. However, all subjects were trained thoroughly by on-site registered dietitians about how to accurately record food intake. Additionally, data about water intake were not consistently recorded by all subjects, so water was not included in any calculation of energy density. Rolls et. al (1999) reported that water given before a meal did not decrease food intake [61], so it may not be necessary to include water in energy density calculations when relating energy density to satiety. The small sample size used in this study could also contribute to difficulty finding significant trends between groups.

This study did not consider specific food choices, or other dietary patterns such as timing of meals or overall quality of the diet. In the future, consideration of these different dietary patterns may provide further insight into the food choices of women with FHA. Variety within food groups was not considered either. McCrory et. al (1999) reported that variety within the sweets, snacks, condiments, entrees, and beverages groups was positively associated with body fatness, while variety within the vegetable group was negatively associated with body fatness in adult men and women [81]. These additional dietary patterns and food choices would offer more insight into the eating behaviors of amenorrheic women in comparison to regularly menstruating women.

This study shows that women with FHA consume a diet lower in energy density than regularly menstruating women when non-caloric beverages are included in the calculation, yet the energy density is similar between the two groups of women when non-caloric beverages are no longer included. Additionally, it shows that vegetable and condiment intake is increased in FHA women, but no other significant differences in food group consumption exist between the two groups. Knowledge of specific dietary patterns, such as the consumption of low energy dense foods, can assist physicians, dietitians, and other medical providers to ensure optimal menstrual health in exercising young women without needing to alter physical training.

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Pre-physician assistant student member

RELATED EXPERIENCE:

Women's Health and Exercise Lab, Noll Laboratory, University Park, PA
Research Assistant Fall 2008-current

- Study the effect of increased caloric intake on the menstrual function and bone health of amenorrheic exercising women
- Created thesis project comparing energy density in amenorrheic and eumenorrheic exercising women
- Helped develop new study comparing the thermic effect of food and gut peptide response in eumenorrheic and amenorrheic women after consumption of a liquid meal.
- Processed urine and blood samples, performed Resting Metabolic Rate tests, worked with subject

Penn State Orthopedics and Sports Medicine, State College, PA
Shadow October 2008-December 2008

- Shadow an orthopedic physician assistant weekly in a major orthopedics center
- Gained hands-on experience in working with patients, reading x-rays, analyzing MRIs, and completing paperwork in a medical field

Global Medical Brigades
Member/Volunteer Fall 2008

- Traveled to Honduras for one week in January 2009 to set up medical brigades and pass out medicine to residents in impoverished villages.
- Weighed, took blood pressure, and handed out medicines to patients
- Shadowed both the physician assistant and doctor that met with the patients
- Sorted medicines and explained them to patients

Forbes Regional Hospital, Monroeville, PA
Joslin Diabetes Center Volunteer Summer 2008

- Shadowed dieticians giving nutritional counseling and diabetes education to patients
- Filed documents, contacted doctors, compiled education packets, and entered data into computers
- Entered data into spreadsheet to use for major report on effectiveness of diabetes education

Patient Aid Summer 2005

- Conducted patient surveys about food quality in the hospital
- "Most Helpful Volunteer Award" for Reliability

LEADERSHIP AND EXTRACURRICULAR ACTIVITIES

Vice President 2009-2010 United Campus Ministries

- Planned and lead Friday night activities for a group of Christian students
- Organized the bi-annual Barn Dance

Team Captain 2007, 2009, 2010 Relay For Life

Family Relations Chair 2009-2010, Chair 2008 - 2009, Secretary 2007 - 2008, Dancer 2008, 2010 Schreyer Honors College Dance Marathon Team

- Acted as liaison between team members and an adopted Four Diamonds family.
- Led team of 12 people in planning fundraisers, canning trips, and team meetings
- Helped raise $7.8 million dollars for pediatric cancer in 2010, $7.2 million in 2009, $6.6 million in 2008

Professional Development Coordinator 2008-2009 Kappa Omicron Nu Honor Society

- Honor society for those within the top 25% of the College of Health and Human Development
- Organize, schedule, and meet with speakers for every general meeting.

Check-in Chairperson 2008, Alumni Roundtable Chairperson 2007, Student Mentor 2007, 2008, 2009 Schreyer Honors College Fall Orientation for New Students

Campus Campaign Manager 2007- 2008 Teach For America

- One of three student leaders; managed recruitment efforts for Teach For America
- Organized the most class announcements in the country
- Achieved a 218% increase in applicants from previous year - recruited most applicants of all schools with only one Recruitment Director
-

Secretary, 2008 Homecoming Recruitment Chairperson 2007 Schreyer Honors College Student Council

Golden Key International Honour Society - Honor society for top 15% of juniors and seniors