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## Within-Day Energy Balance, Body Mass Index, and Body Composition in College Students

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## APPROVAL

This thesis, Within-Day Energy Balance, Body Mass Index, and Body Composition in College Students, by Rebecca Whitney Leet, was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee members, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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Observational design seeks to evaluate within-day changes in energy balance in university students and the relationship to body composition measures such as % fat mass and fat free mass.

## ABSTRACT

### WITHIN-DAY ENERGY BALANCE, BODY MASS INDEX, AND BODY COMPOSITION IN COLLEGE STUDENTS

Rebecca Whitney Leet, Dan Benardot, Walter Thompson, Sarah Henes

**Background:** The customary mechanism for assessing weight change involves an assessment of the imbalance in the energy consumed vs. the energy expended. This energy balance ratio is commonly assessed in 24-hour periods, but this strategy fails to account for the timing of macronutrient intake and within-day fluctuations in energy balance, which have an influence on body composition and, ultimately, weight. Hourly fluctuations in energy balance provides information on the time spent in a catabolic state and time spent in an anabolic state, which is not possible with a 24-hour energy balance assessment. Measuring hourly energy balance to optimize absorption and storage of specific nutrients may be a practical strategy for obese individuals to improve body composition. **Purpose:** The purpose of this study was to observe current dietary habits and assess hourly energy balance of college students with different BMI categories (i.e., below and above a BMI of 30) to determine if there are differences between body composition and hours spent in different energy balance states. **Methods:** The subjects completed a four-day diet and physical activity record from which energy intake and energy expenditures from a relative intensity activity scale was predicted linked to MET values. After completing the record, subject weight, height, % body fat and fat free mass was assessed using a multi-frequency bioelectrical impedance segmental body composition analyzer. **Results:** Data were analyzed from a total of 17 college students (9 men and 8 women) ranging from 20-28 years old (mean age  $23 \pm 2.6$ ). Predicted energy intake averaged  $2237.3 \pm 749.3$  kcals/d and predicted energy expenditure averaged  $2941.7 \pm 552.7$  kcals/d. The average body fat % of the subjects was  $27.1 \pm 11.6\%$  and the

average BMI of the subjects  $28.8 \pm 5.8$ . Using an Independent Samples T-Test, eight subjects with a BMI  $\geq 30$  spent more time in hours high deficit ( $< -400$  kcals Energy Balance) when compared to nine subjects with a BMI  $< 30$ . Nine subjects with a BMI  $< 30$  spent more time in  $\pm 400$  kcal energy balance. Using a Spearman Rho correlation, body fat % was positively correlated to hours high deficit ( $p \leq 0.01$ ) and negatively correlated to hours in  $\pm 400$  kcal energy balance ( $p \leq 0.01$ ). Analysis of men and women found that body fat % of men was not significantly associated to any energy balance variables. While not significant, there was a trend toward a positive correlation between body fat % and hours high deficit ( $p=0.065$ ) and a negative correlation between body fat % and hours in  $\pm 400$  kcal energy balance ( $p=0.065$ ). In women, subjects who spent more time in high energy deficit ( $< -400$  kcals Energy Balance) had higher body fat % ( $p \leq 0.05$ ). Subjects who spent more hours in optimum energy balance ( $\pm 400$  kcal Energy Balance) had lower body fat % ( $p \leq 0.05$ ). **Conclusions:** These data suggest that spending long periods of time in an energy deficit is correlated with higher body fat % and higher BMI in college students. Particularly for women, it may be beneficial for body composition to avoid long periods of time in energy deficit and strive to remain in or near  $\pm 400$  kcal energy balance. College students may be especially prone to extreme deficits in energy balance because of unpredictable schedules and frequent meal skipping. Recommendations for avoiding large energy deficits throughout the day may be beneficial for this population.

**Within-Day Energy Balance, Body Mass Index, and Body Composition in College Students**

A Thesis Submitted to the Graduate Committee in the Division of Nutrition  
At Georgia State University in Partial Fulfillment of the Requirements for  
the Degree

MASTER OF SCIENCE  
IN  
HEALTH PROFESSIONS  
WITH AN EMPHASIS IN NUTRITION

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Coordinated Program in Dietetics  
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2014

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**Abbreviations**

BMI	Body Mass Index
BMR	Basal Metabolic Rate
EB	Energy Balance
FFA	Free Fatty Acids
G	Grams
GLP-1	Glucagon-Like Peptide-1
GIP	Gastric Inhibitory Polypeptide
HDL	High Density Lipoprotein-Cholesterol
Kcal	Kilocalorie
Kg	Kilograms
LDL	Low Density Lipoprotein-Cholesterol
REE	Resting Energy Expenditure
RQ	Respiratory Quotient
VAS	Visual Analog Scale

## **Chapter I**

### **Introduction**

Energy balance represents the relationship between energy intake and energy expenditure (Saltzman & Roberts, 1995). Traditionally, energy balance has been assessed in 24-hour units, with typical dieting protocols inducing a daily energy deficit through a reduction in energy consumption or an increase in energy expenditure. However, this strategy has been found to be ineffective, as the initial weight loss is commonly followed by compensatory mechanisms that results in a decrease in energy expenditure with an increase in fat mass and a decrease in fat-free mass (Hill et al., 2012). Hourly fluctuations in energy balance may provide more information on the relationship between the time spent in an energy deficit (i.e., a catabolic state) and time spent in an energy surplus (i.e., an anabolic state) during the day, which is not possible with a 24-hour energy balance assessment.

Several studies have found that large fluctuations of within-day energy balance may affect body composition by increasing fat mass and decreasing fat-free mass (Arciero et al., 2013; Deutz et al., 2000; Zheng et al., 2009). Many studies have also measured meal frequency as a reflection of within-day energy balance (Bertelsen et al., 1993; Cameron et al., 2010; Drummond et al., 1998). During a 10-year follow up study of 1162 white female adolescents and 1210 black female adolescents, fewer eating episodes were related to greater 10-year increases in BMI and waist circumference levels (Ritchie,

2012). During a randomized crossover trial, nine healthy, lean women consumed a regular meal pattern (6 occasions per day with regular intervals between meals) or an irregular eating pattern (between 3 to 9 meals per day varied each day) for 14 days with a 14-day wash out period between test meal periods. Body composition variables did not change through the intervention period for either group. Irregular eating patterns were associated with decreased energy expenditure compared to regular meal consumption (Farshchi et al., 2004).

Low meal frequency has been shown to strongly correlate to poor health conditions. Bachman et al. (2011) measured meal frequency using 24-hour telephone food recalls (2 weekdays and 1 weekend day) to measure the differences in overweight subjects, weight loss maintainer subjects, and normal weight subjects. Meal frequency was higher in weight loss maintainers and normal weight subjects compared to overweight and obese subjects. BMI was negatively correlated with snack consumption. The study suggested that three meals and two snacks per day was the most beneficial combination (Bachman et al., 2011).

Improvements in body composition have also been found through increased meal frequency. Arciero et al. (2013) conducted a study measuring the effect of increased meal frequency with moderate to high protein and the relationship to total and abdominal fat percentage. The nutritional intervention lasted 62 days with three phases: a 5-day baseline control; a 28-day balance phase; and a 28-day negative energy balance phase. All of the subjects were allocated into three intervention groups: traditional intake of three meals/day, high protein group (30% kcal/d) consumed in three meals/day, or high protein group (30% kcal/d) consumed in six meals/day. The study found that the six meal

per day, higher protein diet decreased total and abdominal body fat percentage, maintained lean body mass and increased thermogenesis (Arciero et al., 2013).

In athletes, increased meal frequency has been found to have a similar advantage in maintaining lean body mass by decreasing muscle protein breakdown (Iwao et al., 1996). Iwao et al. (1996) studied 12 members of a university boxing club to test the effects of food restriction carried out over two weeks with a meal frequency intervention. Subjects followed a low meal frequency (two meals per day) or a high meal frequency (six meals per day). Both meal frequencies when consumed in an energy deficit yielded decreased body weight, however the decrease in lean body mass was significantly less in the high meal frequency group.

Increased meal frequency is not necessarily a measure of real-time energy balance, as fluctuations in expenditure and/or the volume of food consumed may still result in energy balance states that result in undesirable body composition outcomes. These findings may be better understood through an analysis of the hourly changes in energy balance. While energy intake and energy expenditure are often reported to measure energy balance, energy storage will additionally influence body composition changes (Hill et al., 2012). Humans are capable of absorbing and utilizing a finite amount of nutrients at one period of time. Only 30-40 grams of protein, for instance, can effectively be used by the tissues following a single eating opportunity (Paddon-Jones & Rasmussen, 2009; Schutz, 2011). Protein consumed in excess of this amount will be denitrogenated and stored as fat or used as an energy substrate, rather than as one of the multiple uses of protein, which include sustaining or increasing lean tissue and manufacturing of enzymes and hormones (Schutz, 2011). In contrast, well-timed protein

distribution of approximately 30 grams per eating episode throughout the day has been found to increase muscle protein stores and increase fat free mass, a desirable condition for individuals carrying excess fat mass (Arciero et al., 2013).

Excess carbohydrate consumption at one sitting, for example during a large meal, may also cause a biological disadvantage from excess insulin production, resulting in higher fat storage (Woods et al., 2006). However, a variety of factors may influence the amount of insulin released, including eating frequency. Insulin exponentially increases with calories consumed (Woods et al., 2006). Therefore, after larger meals, there is a hyperinsulinemic response to the high amount of energy, and hyperinsulinemia will increase fat storage regardless of the nutrient composition of the consumed meal (Kanaley et al., 2013; McNay et al., 2013; Woods et al., 2006). Sporadic meal patterns that allow for hypoglycemia may also cause postprandial hyperinsulinemia after the next meal regardless of nutrient composition and size (McNay et al., 2013; Woods et al., 2006). Stable energy balance can be achieved by consuming consistent amount of foods at time periods that optimize nutrient utilization and minimize unnecessary storing, thereby improving body composition.

Measuring hourly energy balance to optimize absorption and storage of specific nutrients may provide a practical strategy for obese individuals to improve body composition. The purpose of this study is to observe current dietary habits and hourly energy balance of college students with different BMI categories, below and above a BMI of 30, to determine if there are differences between body composition and hours spent in different energy balance states.



**Hypothesis**

The subjects in this study with a BMI above 30 will spend more time in an energy surplus or deficit outside of the range of  $\pm 400$  kcal energy balance.

**Null Hypothesis**

There will not be a difference in BMI for subjects spending more time in an energy surplus or deficit outside the range of  $\pm 400$  kcal energy balance.

## **Chapter II**

### **Review of Literature**

#### **College Students**

Inconsistent schedules and the high stress environment of a college campus may play a role in the eating habits of university students (Childers et al., 2011). In a national survey conducted in 2005, 3 out of 10 students were overweight (BMI [25.0-29.9 kg/m<sup>2</sup>]) or obese (BMI  $\geq$  30.0 kg/m<sup>2</sup>). The American College Health Association-National College Health Assessment conducted a survey in 1998 assessing the health characteristics and behaviors of university students. The survey was completed at 71 universities throughout the U.S. and included 51,199 students. The survey found that only 7% of students reported eating the recommended amount of fruits and vegetables per day ( $\geq$ 5 per day). Six out of 10 students participated in fewer than three days per week of vigorous or moderate exercise ("American College Health Association National College Health Assessment [ACHA-NCHA] Spring 2005 Reference Group Data Report [Abridged]," 2006).

Similar research conducted in Saudi Arabia collected data from 357 male students through a self-reported questionnaire about eating habits. They found that 63.3% of students reported irregular meal consumption with 55.7% consuming only two meals per day. Vegetables and fruits were not frequently consumed and 46.8% of the students reported consuming fried food at least three times per week (Al-Rethaiaa et al., 2010). A convenience sample of 160 college students from a university in Greece and Scotland

found that 26% of students reported breakfast skipping every day and 33% reported consuming breakfast only 1-3 days per week (Spanos & Hankey, 2010).

College students have several barriers, which may inhibit healthy food choices including time constraint, access to unhealthful food, and high monetary costs associated with healthful behaviors (Greaney et al., 2009). These factors may put this population at an increased risk of preventable diseases such as diabetes and cardiovascular disease. This study utilized university students between the ages of 19-30 years at Georgia State University to assess whether stable energy balance is correlated with ideal body composition and therefore could serve as a potential strategy for improved body composition for this population.

### **Factors affecting Energy Balance**

Weight regulation is traditionally maintained by consuming equivalent energy intake as energy expended in a 24-hour period. To lose weight, it is suggested to consume less energy than expended and therefore remain in a negative energy balance. However, this traditional view fails to consider the fluctuating nature of energy balance throughout the 24-hour period (Hall et al., 2012). The body is in a constant state of negative and positive energy balance. The hormonal and biological cues associated with negative and positive energy balance will fluctuate just as frequently throughout the day and will influence body weight and composition (Guyenet & Schwartz, 2012). Many factors will influence these hormonal cues including composition of food consumed, amount of food consumed and the state of current energy balance. These hormonal and biological cues

will then regulate the utilization of these nutrients for storage or expenditure (Guyenet & Schwartz, 2012).

Other variables influenced by energy balance include digestive capacity, absorption and utilization. Absorption will be dependent on the components and amount of intake which are all dependent on the individual, how the food is prepared and intestinal factors (Hall et al., 2012). Once nutrients are absorbed, nutrient utilization will vary depending on energy needs and storage capacity. When the amount of ingested food exceeds the typical amount of nutrients that can be absorbed and utilized effectively at one period, the nutrients may be converted to a storage form.

Protein can only be utilized in certain amounts at one sitting. Most individuals can consume ~30 g of protein in one sitting to maximize muscle protein synthesis (Schutz, 2011). However, protein consumed in excess of this amount may be denitrogenated and converted to a different energy substrate or stored in the adipose tissue (Schutz, 2011). Data from NHANES has found that Americans typically consume only a modest amount of protein in the morning (13 g) and consume three times that amount at dinner (38 g) (Mamerow et al., 2014). More balanced distribution of protein throughout the day can maximize muscle synthesis and discourage muscle breakdown. Mamerow et al. (2014) studied protein and calorie distribution throughout the day and the effects on muscle synthesis. Eight participants (5 males and 3 females) between the ages of 25 and 55 years consumed two 7-day diets with an even distribution or a skewed distribution of energy and protein. In the skewed distribution, dinner contained the most energy and protein (1100 kcals and 63 g, respectively) whereas breakfast contained the least (537 kcals and 34 g protein). The even distribution contained between 727-848 kcals per meal and ~30 g

protein per meal. Both diets contained equal amounts of daily energy (2320-2400 kcals) and daily protein (94-90 g). They found that even consumption of protein throughout the day stimulated muscle protein synthesis 25% greater than skewed consumption of protein. More even protein distribution throughout the day may be achieved while simultaneously remaining in stable energy balance.

The physiological inability to utilize all daily nutrient needs at one sitting promotes a division of daily nutrient needs into meals and snacks. The standard western meal pattern consists of three meals per day, breakfast, lunch and dinner with any additional eating occasions considered to be snacks. In the energy balance system, meals and snacks will distribute consumed energy over a span of time and adjust the daily energy balance ratio throughout the day. The number of meals and snacks consumed has thus been implicated as a way to measure the effects of energy balance on weight and health indices through research.

An assumption can be made that consistent and frequent meals suggest a more stable energy balance while avoiding long periods of time in negative (< -400 kcal energy balance) or positive (>400 kcal energy balance) energy balance. Meal frequency can serve only as an indirect indicator of possible changes in energy balance.

### **Meal Frequency and Obesity Markers**

Several studies have measured the relationship between obesity and meal frequency through diet records and food recalls (Ma et al., 2003; Ritchie, 2012). Ritchie, (2012) measured the dietary patterns of 2,372 adolescent girls (1213 black girls and 1166 white girls) in the 10-year National Heart, Lung, and Blood Institute Growth and Health

Study. Girls were recruited in three clinical centers: Berkeley, CA, Cincinnati, OH, and Washington, DC. Adolescents were between the ages of 9-10 years at the time of enrollment. The subjects provided annual information on nutrition and physical activity patterns and anthropometric measurements were performed by examiners. To collect nutrition information 3-day food records (2 weekdays and 1 weekend day) were collected and analyzed every year for 10 years, except year 6 and 9. The number of meals, snacks, and eating episodes were comprised regardless of amount or type of food or beverage reported. A meal was defined as  $\geq 15\%$  of total calories, regardless of the time of day or composition of foods or beverages. The snacks were defined as all other eating episodes. Decreased meal frequency was associated with increased BMI and waist circumference in the 10-year span (Ritchie, 2012).

Ma et al. (2003) analyzed the diet of 499 participants in the Massachusetts area aged 20-70 years using repeated 24-hour food recalls. Participants were followed up every three months for a year with serum lipid samples and body weight collected along with a 24-hour food recall and physical activity record at every follow-up. Eating episodes consisted of a minimum of 50 kcals with a time interval of at least 15 minutes. Each of the eating episodes was then tallied for all recalls. Participants ate on average 3.92 times per day. The number of eating episodes was inversely associated with the risk of obesity. Participants who ate four or more episodes per day had a 45% decreased risk of obesity. Skipping breakfast was associated with a 4.5 times higher risk of obesity (Ma et al., 2003).

Bachman et al. (2011) measured meal frequency using 24-hour telephone food recalls (2 weekdays and 1 weekend day) to measure the differences in overweight

subjects, weight loss maintainer subjects and normal weight subjects. Eating occasions were defined as  $\geq 50$  kcals with 60 minutes separating eating occasions. Meals and snacks were participant defined. A total of 257 subjects were interviewed with 81 overweight subjects, 96 weight loss maintainers and 80 normal weight subjects. Meal frequency was higher in weight loss maintainers and normal weight subjects compared to overweight and obese subjects ( $2.0 \pm 1.0$  snacks/day and  $2.3 \pm 1.1$  snacks/day versus  $1.5 \pm 1.2$  snacks/day, respectively). BMI was negatively correlated with snack consumption. This study suggested that three meals and two snacks per day was the most beneficial combination (Bachman et al., 2011).

Decreased meal frequency may additionally have a negative effect on lipid markers. In a crossover design study comparing meal frequencies and lipoprotein and glucose indices, 17 subjects with fasting cholesterol concentration below the 50<sup>th</sup> percentile for New Zealanders of their age and sex were recruited and completed this study. Subjects did not have a history of hyperglycemia or any other serious medical conditions. The 4-week protocol consisted of two isoenergetic diets consumed in a three meal and a nine meal diet plan for two weeks each. The subjects were allotted a daily energy allowance based off of an initial 3-day diet record that was completed to represent subject normal intake. The three meal per day diet period divided energy through the day as follows: breakfast 25%, lunch 25%, dinner ~50% and a single small snack ( $\sim < 600\text{kJ}$ ) to be consumed as desired. During the nine meal diet plan, the three meal per day eating regimen was divided into three smaller meals distributed as follows: early morning 8.3%, breakfast 8.3%, midmorning 8.3%, lunch 8.3%, midafternoon 8.3%, late afternoon 8.3%, dinner 16.6%, midevening 16.6%, and late evening 16.6%. The nine meal diet plan

yielded lower total cholesterol and LDL-C with a 6.5% decrease and 8.1% decrease respectively (Arnold et al., 1993). However, a similar study conducted with 11 females measured a nibbling versus gorging diet for two weeks. The nibbling diet consisted of 12 meals per day and the gorging diet consisted of three meals per day. No significant differences existed in the fasting or postprandial plasma concentrations of triacylglycerol, glucose, immunoreactive insulin, GIP and GLP-1 levels. There were no differences between fasting total and LDL-C concentrations (Murphy et al., 1996).

### **Meal Skipping**

Meal skipping is frequently encountered in higher stress jobs and shift work (Atkinson et al., 2008). The unpredictable schedules of college students may additionally impact the ability to maintain a routine meal frequency. University students in Cheongju would frequently miss breakfast because of limited time (Lee & Yoon, 2014).

Meal skipping has been shown to singularly increase risk of poorer health outcomes. House et al. (2013) analyzed the relationship between eating frequency and dietary, metabolic, adiposity and physical activity measures in children. The sample included 185 Hispanic and African-American children and adolescents (8-18 years old) in the Southern California area with a BMI  $\geq$  85<sup>th</sup> percentile for age and gender. Exclusion criteria included participation in a physical activity, nutrition or weight reduction program or diabetes. Dietary intake was assessed from two or three 24-hour recalls. Meal skippers were classified as eating less than three times per 24 hours and normal/frequent eaters were classified as those who ate three or more meals per 24 hours. All eating occasions were based on consuming 50 kcals with at least 15 minutes from a



previous eating occasion. The study included 54 males and 131 females. The average number of eating occasions per day was  $3.8 \pm 1.0$ . Meal skippers ate significantly fewer calories, however they had 18% higher triglycerides and 26% higher visceral adipose tissue in comparison to normal frequency eaters (House et al., 2013).

Jaaskelainen et al. (2013) conducted an ongoing, population-based study covering births in the northernmost provinces of Finland between July 1, 1985 to June 30, 1986. The study followed the parents and children via postal questionnaires for 16 years. At the 16-year follow-up, the adolescents filled out a questionnaire regarding meal patterns categorizing meals as five meals per day including breakfast (regular meal pattern),  $\leq$  four meals per day including breakfast (semi-regular meal pattern), and  $\leq$  four meals per day not including breakfast (breakfast skippers). The regular meal pattern in comparison to the breakfast skippers was associated with reduced risk of overweight/obesity (61% in boys, 43% in girls), abdominal adiposity (73% in boys, 44% in girls) in all adolescents, and hypertriglyceridaemia and low HDL-C in boys (Jaaskelainen et al., 2013).

### **Meal Consistency and Timing**

Meal consistency from day to day has been implicated to play a role in obesity. Farshchi et al. (2004) investigated the impact of irregular meal frequency on circulating lipids, insulin, glucose and uric acid. Nine women aged 18-42 years with no self-reported hypercholesterolaemia, hyperglycemia or other serious medical condition participated in three phases of a randomized crossover trial. The subjects consumed a regular (6 meals/d) and irregular meal frequency (3-9 meals/d) for 14 days each. Irregular meal pattern produced lower insulin sensitivity and a greater insulin response to a test meal than a

regular meal pattern. Irregular meal frequency caused increased cholesterol and LDL-C compared to a regular meal pattern (Farshchi et al., 2004). All of these markers are suggestive of an increased risk of cardiovascular disease.

The association between timing of meals and obesity has been frequently tested. Consumption of breakfast has consistently produced an inverse relationship to obesity (Rampersaud et al., 2005). In a literature review conducted with the inclusion of 47 studies pertaining to breakfast consumption in adolescents, breakfast eaters were less likely to be overweight and obese. The amount of energy supplied by breakfast was lower in obese subjects (Rampersaud et al., 2005). However, other meals have not consistently been evidenced by an association with obesity. Wang et al. (2013) utilized data from The Energetic Study to assess the association of energy intake throughout the day with the risk of obesity in a subsample of participants. Subjects from nearby the Los Angeles area between the ages of 21-69 years were recruited for the study. Participants (n=249) completed eight Web-based 24-h dietary recalls and completed additional self-assessments including a physical activity questionnaire when visiting the clinic. Morning meals were categorized between 12:00AM-11:00AM, midday between 11:00AM-4:00PM, and evening between 4:00-12:00AM. The study found no relationship between participants eating >33% of energy at breakfast and obesity, an inverse relationship between participants eating >33% of energy at lunch and obesity and a positive relationship between participants eating >33% of energy at dinner and obesity (Wang et al., 2013). This suggests that the traditional American meal consumption of a larger meal at night may be negatively affecting BMI. Maintaining a consistent energy balance throughout the day may be a better strategy for obesity prevention.

## **Body Composition and Meal Frequency**

Another measure of the effects of meal frequency can be demonstrated through changes in body composition. Improvements in body composition have been found through increased meal frequency (Arciero et al., 2013). Arciero et al. (2013) conducted a study utilizing 28 subjects who participated in a 62-day nutritional intervention consisting of a six dietary treatments, a traditional three meal per day intake versus a six meal per day intake with either traditional (~15%) versus higher (~35%) protein intakes during 28 days of energy balance or deficit. All participants were inactive (<30 min, 2 days per week of structured physical activity), overweight or obese ( $BMI \geq 25$ ), and were weight stable for six months prior to the study. Subjects were quasi-randomly assigned to one of three groups to balance BMI, body weight and body fat % in each group. The three groups were three meals per day, three meals per day with higher protein, or six meals per day with higher protein. Initially all subjects consumed a control diet during day -4-0. The control phase diet consisted of 25% protein, 45% carbohydrate and 30% fat. Subjects then consumed an energy balanced diet during day 1-28. During day 29-56 subjects consumed a negative energy balanced diet. Meal timing varied with each participant. The evening meal needed to be consumed by 8:00PM and within two hours of going to bed. Subjects on the six meal per day plan were instructed to eat approximately every 2 ½ -3 hours during the day. During energy balance when consumed in six meals, a higher protein intake (~35%) decreased total and abdominal body fat percentage and increased lean body mass. With an energy deficit and higher protein intake, six meal per day

decreased total and abdominal body fat and increased thermogenesis (Arciero et al., 2013).

Body composition is particularly important for athletes. Weight loss that includes lean body mass would be undesirable as it may translate into decreased athletic performance. Iwao et al. (1996) conducted a study in university boxers during a period of energy restriction to induce weight loss. The subjects consumed two meals per day or six meals per day from a commercially available liquid food. The diet was followed for 14 days. Prior to food restriction, no differences in lean body mass were noted between groups. After food restriction, the two meals per day athletes had significantly lower lean body mass than the six meals per day athletes. In athletes, increased meal frequency has been found to have a similar advantage in maintaining lean body mass by decreasing muscle protein breakdown (Iwao et al., 1996).

### **Inconsistencies in Meal Frequency Research**

Though a highly tested area of research, meal frequency studies often fail to thoroughly account for energy expenditure and the changes throughout the day in relation to energy intake. Without accounting for changes in energy expenditure per subject, these studies may inaccurately provide a rationale for increased or decreased meal frequency without thoroughly assessing the benefits or detriments of meal frequency.

Opponents to meal frequency have suggested that increasing meal frequency may increase hunger because of the frequent food encounters. Ohkawara et al. (2013) conducted a randomized, crossover trial comparing a three-meal-per-day pattern to a six-meal-per-day pattern in 15 healthy adults (BMI 19-25, free from acute or chronic

diseases). They measured changes in 24-hour fat oxidation and perceived hunger. The subjects consumed each diet for four days through a controlled outpatient diet, with the food received from the research site. The distribution of macronutrients in each diet consisting of 30% kcal from fat, 55% kcals from carbohydrate and 15% kcals from protein. No difference was found between 24-hour energy expenditure, RQ and fat oxidation. Higher hunger ratings were reported when subjects consumed six meals per day versus three meals per day. However, insulin AUC response was lower during the six meal consumption (Ohkawara et al., 2013). Though the study suggests lower insulin levels may explain the increase in hunger, lower insulin levels may also lead to decreased lipogenesis and may thereby improve body composition in the long run (Czech et al., 2013). Additionally, FFA concentrations fell following consumption of the first meal and remained below fasting levels throughout the remainder of the day for subjects consuming six meals per day (Ohkawara et al., 2013). Elevated FFA are associated with obesity and may increase insulin resistance by reducing insulin stimulated glucose uptake, therefore lower FFA may be a positive effect of increased meal frequency (Boden, 2008).

Though many studies have found decreased intake *ab libitum* with increased meal frequency, there is research to suggest that increased meal frequency does not decrease satiety and will increase daily energy consumption. Leidy et al. (2011) conducted a study to compare energy restriction with higher protein versus normal protein consumed during three eating occasions or six eating occasions. They found no difference in hunger between the two meal frequency groups in regards to hunger, fullness, desire to eat or preoccupation with thoughts of food. In the high protein group, they found six eating

occasions led to greater evening and late night hunger (Leidy et al., 2011). Other studies have found similar results where meal frequency has not led to significant differences in adiposity indices, appetite measurements or gut peptides (Cameron et al., 2010). This variation may be related to the amount of food consumption in the study atmosphere as compared to typical food consumption outside of the study setting. Additionally, the length of the study may not have been sufficient to notice changes from the research.

### **Hormonal Regulation of Energy Balance**

The start and end of a meal or snack are influenced by a variety of interactions between genetic, social, learned, environment, circadian and humoral cues (Guyenet & Schwartz, 2012). Hunger and satiety will influence the amount of food consumed and may be altered related to energy balance status. Hunger is better understood through hormonal changes that occur throughout the day.

After long periods without energy consumption and negative energy balance, hypoglycemia and hunger will inevitably ensue. This condition directly influences meal choice and energy consumption. Hypoglycemia induces a state of energy craving where the body will seek energy in any form. Schultes et al. (2005) found that induced hypoglycemia interfered with memory tasks. In the study, performance and memory of “food related” stimuli were higher than “non-food related” stimuli after a hypoglycemic level of 2.6 mmol/l (Schultes et al., 2005). This suggests that hypoglycemia induces a state of food craving and cognitive disruption to focus on alternative tasks with the sole objective to seek food.

Hypoglycemia may additionally lead to dietary unrestraint and poor choices to satiate extreme hunger. Dewan et al. (2004) studied the relationship of insulin induced hypoglycemia and regulation of food intake. Sixteen healthy men at normal BMI, aged  $29 \pm 11$  years old participated in the study. After an overnight fast and a bolus of insulin or saline, participants consumed a meal ad libitum and recorded feelings of hunger and fullness using VAS. The foods provided were categorized into high fat and low fat foods. Participants partook in the protocol twice and the results were averaged. Caloric intake was 17% greater following insulin compared to saline ( $1701.1 \pm 895.3$  kcal vs  $1427.7 \pm 815$  kcal). Total fat intake was higher after insulin (Dewan et al., 2004). This study finds uninhibited eating will be heightened in extreme hypoglycemia and negative energy balance.

Extreme hunger levels were found in other studies to lead to poor dietary choices and often excessive energy intake. Meal frequency is directly related to hunger levels. Several studies have supported the theory that increased meal frequency leads to lower hunger levels (Allirot et al., 2013; Allirot et al., 2014). These studies assessed various markers to prove this theory. Allirot et al. (2014) studied the effects of a spread out meal on appetite, metabolism and food intake in the subsequent ad libitum meal. Seventeen obese men participated in this randomized cross-over design study. The subjects were given a 678 kcal breakfast either in one 20 minute long eating episode or four 10 minute long eating episodes. The subjects were then offered ad libitum lunch four hours later. They found that an isocaloric spread out breakfast-decreased appetite when measuring electronic visual analog scores (VAS). Additionally, the spread out breakfast was positively associated with decreased ghrelin levels. This study found a lower intake in the

number of grams however the energy intake was not decreased. When this protocol was tested in nineteen lean, normal weight men, significantly less energy was consumed at lunch after an isocalorically spread out breakfast when compared to a single eating episode (Allirot et al., 2013). Similarly, seven obese men consumed either a single pre-load meal or a pre-load meal spread out over a five hour period. They were required to consume the entire pre-load meal and then the test meal was supplied ad libitum. When given a single pre-load meal, significantly more energy was consumed in the ad libitum test meal. While the effects on hunger levels remained the same when measured through VAS, the amount of food ingested was higher in the single meal versus the multiple meals (Speechly et al., 1999).

The hypoglycemic state will be indicative of negative energy balance. After a long period in negative energy balance, the subsequent meal may be high energy. This high-energy meal will increase blood glucose and exponentially increase insulin release. A high insulinemic state has been found to induce a lipogenic state and increase fat storage (Kanaley et al., 2013; McNay et al., 2013; Woods et al., 2006). Though daily energy balance may remain stable with calories in equivalent to calories out, lipogenic hormones will be stimulated after ingestion of a large bolus meal. These lipogenic hormones may increase fat storage and therefore fat mass after long periods in negative energy balance and consequent high-energy meals.

Increased meal frequency and stable energy balance has been recommended as an alternative treatment to maintain optimal blood glucose (80-120mg/dL) and insulin levels in diabetes. Bertelsen et al. (1993) included 12 non-insulin dependent diabetic subjects in a study to measure the effects of increased meal frequency. They received either six



isocaloric small meals in 80-minute intervals or two large isocaloric meals in a 240 minute interval. The incremental blood glucose area was lower in response to frequent, small meals. Free fatty acid levels remained low in the subjects consuming frequent, small meals, however rose in response to a few large meals. The incremental insulin response was lowest in response to frequent meals. The study concluded that frequent meal consumption acutely reduced blood glucose, lowered average insulin and free fatty acid levels in non-insulin dependent diabetic subjects (Bertelsen et al., 1993). The metabolic response can be indicative of a metabolic protective effect for non-diabetic individuals consuming small, frequent meals.

### **Summary**

Though the 24-hour measure of energy balance has become the standard, within-day changes in energy balance may more significantly influence body composition. This study seeks to further examine within-day energy balance by measuring the continuously changing energy balance ratio throughout the day. The traditional 24-hour view of energy balance may provide an inefficient representation of energy balance because of the hormonal changes, glycemic level, absorptive changes, and storage capacity. Assessing within-day energy balance may provide a more accurate view of the frequently changing energy balance ratio in relation to body composition.

## **Chapter III**

### **Methods**

#### **Inclusion Criteria**

Individuals were eligible to participate if they were between the ages of 19-30 years and had a BMI  $\geq 18.5$ . Exclusion criteria include metabolic conditions such as dyslipidemia, diabetes and cardiovascular disease per subject report. Additional exclusion criteria include affiliation with prior nutrition counseling at the Recreation Center as well as affiliation with a Georgia State University course instructed by Dr. Dan Benardot. Participants agreed to complete four days of diet and physical activity record and participate in a body composition analysis. Procedures were approved by Georgia State University's Institutional Review Board.

#### **Subjects**

The majority of recruitment for the study was completed at the Georgia State University Recreation Center through word of mouth. Consent was obtained from the Recreation Center prior to subject recruitment and data collection. The sample of the study was 17 university students between the ages of 19-30 years. Participation in the study was voluntary and each participant signed a written consent form. The data collection was partially conducted at the Georgia State University Recreation Center and at the laboratory of Dr. Dan Benardot.

## **Food Record**

The subjects completed a four-day food and physical activity record. Hourly energy intake and expenditure were recorded. Intake was predicted based on hourly consumption of food and beverages. Expenditure was assessed based on hourly physical activity. Subjects reported physical activity based on a 1-7 scale rating the difficulty of the activity and the time spent at that level of exertion. Energy balance was predicted in hourly units. Subjects' energy balance was predicted based on their weight, height, time and amount of energy intake, and physical activity level reported in the food and physical activity record. Energy balance was predicted through hourly intake and expenditure to establish the specific time and length of time a subject was in positive or negative energy balance. The subjects were educated on how to record their food intake and serving sizes to ensure validity of the food record. They were also instructed to consume their regular diet.

## **Study Protocol**

Each subject received a brief education session about how to thoroughly write a food log and estimate appropriate serving size. The subjects were given the diet and physical activity log to be completed. The subjects completed the four-day diet records (3 weekdays and 1 weekend day) with no requirement that they track consecutive days. The subjects reported to the laboratory to turn in their records. At the research site, weight, height and body water weight was collected using a Multi Frequency Segmental Body Composition Analyzer. The scale assessed the percent body fat and fat free mass. At the

research site, additional questions were asked regarding their logs to clarify any incomplete components of the food and physical activity logs. The food and physical activity logs were assessed using NutriTiming<sup>®</sup>.

### **Data Analysis**

Intake was analyzed using standardized items most similar to the log or searching the nutrient information of the given item online. If portion sizes were not provided, they were estimated using the suggested serving size. Physical activity was assessed using a scale from 1.0-7.0 as a representative value for activity factor per unit time of activity. Harris-Benedict (REE) is multiplied by these factors to assess the energy expenditure throughout the day ("Recommended Dietary Allowances," 1989).

Four days of data entry were assessed and the averages were derived from all four days. Variables evaluated included energy intake, energy expenditure, ending energy balance, kcals per kg, kcals per kg active, hours optimum, hours in high surplus, hours in high deficit, hours anabolic, hours catabolic, the highest surplus and the highest deficit. Energy intake was assessed from the average kcal intake of the four-day diet records. Hours in high deficit included the number of hours spent < -400 kcals energy balance. Hours high surplus included the number of hours spent above > 400 kcals energy balance. The highest surplus indicates the highest average daily peak in energy balance for the four days. The highest deficit indicates the lowest average drop in energy balance for the four days.

### **Statistical analysis**

The data were analyzed using SPSS (version 20.0, SPSS Inc. Chicago, IL) data software. A Spearman Rho Correlation test was used to assess the correlation between energy balance and body composition variables. An independent group T-Test was used to compare subjects with a BMI  $\geq 30$  and subjects with a BMI  $< 30$  to within day energy balance variables. A regression analysis assessed energy balance variables and body composition. Energy balance variables include minutes spent in negative energy balance and positive energy balance, energy surplus, and energy deficit, calorie level of energy surplus, and calorie level of energy deficit. Body composition variables include percent body fat and percent lean mass.

## Chapter IV

### Results

#### Participant Characteristics

Data were included on a total of 17 subjects (9 males/8 females). The age range of the subjects was 20-28 years with a mean age of  $23 \pm 2.6$  years. The mean weight of the subjects was  $85 \pm 19.8$  kg and the mean height was  $170 \pm 8.5$  cm. The mean body fat % of the participants was  $27.08 \pm 11.5$  %. The mean BMI of the participants was  $29 \pm 5.8$ .

Anthropometric variables for men and women were compared through an independent samples T-Test. The mean height (cm), weight (kg), BMI and body fat % were compared for men versus women. No significant differences existed between groups for BMI and weight (kg). Height (cm) and body fat % were significantly different ( $p=0.007$  and  $p=0.004$  respectively). These findings are expected given that men typically have higher fat free mass and are typically taller than women.

**Table 1.** Descriptive Data

	<b>Men (N=9)</b>	<b>Women (N=8)</b>	<b>Total (N=17)</b>
<b>Age</b>	$24 \pm 2.9$	$22 \pm 2.0$	$23 \pm 2.6$
<b>Ht (cm)</b>	$175 \pm 7.2$	$165 \pm 6.3$	$170 \pm 8.5$
<b>Wt (kg)</b>	$91 \pm 21.0$	$77 \pm 16.6$	$85 \pm 19.8$
<b>BMI</b>	$29 \pm 6.1$	$28 \pm 5.6$	$29 \pm 5.8$
<b>% Body Fat</b>	$20 \pm 8.6$	$35 \pm 9.3$	$27 \pm 11.6$
<b>Fat Mass (kg)</b>	$19 \pm 13.0$	$28 \pm 12.5$	$24 \pm 13.1$
<b>Fat Free Mass (kg)</b>	$72 \pm 11.5$	$49 \pm 5.5$	$61 \pm 14.7$

Energy balance descriptive data are provided in Table 2. The average number of kcal consumed per day was  $2237.3 \pm 749$ . The mean kcals/kg total was  $27.4 \pm 9.4$ . The subjects consumed a balanced array of nutrients consuming 48.8% of kcals from carbohydrates, 18.6% of kcals from protein and 32.4% of kcals from fat.

Participants spent more time in negative energy balance (hours catabolic) at  $21 \pm 3$  hours then in a positive energy balance (hours anabolic) at  $2 \pm 3$  hours. None of the participants spent time in high surplus ( $>400$  kcals energy balance). Subjects spent on average  $10.7 \pm 6.9$  hours per day in high deficit ( $< -400$ kcals energy balance). This shows that subjects spent the majority of their day in negative energy balance.

**Table 2.** Energy Balance Descriptive Statistics (N=17)

<b>Energy Balance Variables</b>	<b>Mean <math>\pm</math> SD in Men and Women</b>	<b>Mean <math>\pm</math> SD in Men</b>	<b>Mean <math>\pm</math> SD in Women</b>
24-Hr Energy Diff	-704.4 $\pm$ 548.1	-667.3 $\pm$ 617.8	-746.1 $\pm$ 496.8
Ending Energy Balance	-598.1 $\pm$ 572.4	-524.0 $\pm$ 642.8	-681.5 $\pm$ 511.4
Kcal/kg Total	27.4 $\pm$ 9.4	30.6 $\pm$ 10.2	23.8 $\pm$ 7.5
Kcal/kg Active	17.9 $\pm$ 12.3	19.8 $\pm$ 16.2	15.8 $\pm$ 6.0
Kcal Intake	2237.3 $\pm$ 749.3	2667.0 $\pm$ 769.6	1753.9 $\pm$ 319.4
Hours $\pm$ 400 kcal Energy Balance	13.4 $\pm$ 7.0	14.2 $\pm$ 7.2	12.4 $\pm$ 7.0
Hours High Surplus	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Hours High Deficit	10.7 $\pm$ 6.9	9.7 $\pm$ 7.2	11.6 $\pm$ 6.8
Hours Anabolic	2.4 $\pm$ 3.0	3.0 $\pm$ 3.2	1.8 $\pm$ 3.0
Hours Catabolic	21.6 $\pm$ 3.0	21.0 $\pm$ 3.2	22.3 $\pm$ 3.0
Highest Energy Balance Surplus	134.4 $\pm$ 118.3	193.2 $\pm$ 109.2	68.4 $\pm$ 94.7
Highest Energy Balance Deficit	-919.2 $\pm$ 424.3	-937.5 $\pm$ 467.7	-898.6 $\pm$ 400.7
% Kcal from Carbohydrate	48.8 $\pm$ 5.0	47.5 $\pm$ 4.7	50.4 $\pm$ 5.2
% Kcal from Protein	18.6 $\pm$ 5.3	20.4 $\pm$ 5.9	16.9 $\pm$ 3.9
% Kcal from Fat	32.4 $\pm$ 4.1	32.1 $\pm$ 5.1	32.8 $\pm$ 2.9

## BMI

BMI was correlated with several energy balance variables for all subjects. BMI was positively correlated with hours high deficit ( $r = .684$ ,  $p = 0.002$ ). BMI was negatively associated with hours optimum ( $r = -.684$ ,  $p = 0.002$ ). In women, BMI was negatively correlated with kcal/kg total ( $r = -.810$ ,  $p = 0.015$ ).

In an independent samples T-test, measuring the difference between energy balance variables, e.g. hours  $\pm 400$  kcal energy balance and hours high deficit and subjects with a  $BMI \geq 30$  and  $< 30$  depicted in Table 3. There was a statistically significant difference between subjects with a  $BMI \geq 30$  and  $< 30$ . Subjects with a  $BMI \geq 30$  had significantly more hours in a high deficit (mean 14.9 hours) compared to subjects with a  $BMI < 30$  ( $p = 0.01$ ). Subjects with a  $BMI < 30$  spent significantly more time in  $\pm 400$  kcal energy balance than subjects with a  $BMI \geq 30$  ( $p = 0.01$ ). These data shows that subjects with a  $BMI \geq 30$  spend significant more time in high negative energy balance ( $< -400$  kcals energy balance) suggesting that this may be an undesirable state for individuals seeking weight loss.

**Table 3.** Mann-Whitney U Test Comparing  $BMI \geq 30$  to  $BMI < 30$  in Men and Women

	BMI	N	Mean	Std. Deviation	Sig. (2-tailed)
<b>Hours High Deficit</b>	$\geq 30$	8	14.87	4.70	0.012
	$< 30$	9	6.90	6.59	
<b>Hours <math>\pm 400</math> kcal energy balance</b>	$\geq 30$	8	9.13	4.70	0.012
	$< 30$	9	17.11	6.60	



### **Body Fat Percent and Energy Balance Variables**

Body fat % of all participants was compared to the highest energy surplus, highest energy deficit and kcal intake. Body fat was negatively correlated with the amount of time in  $\pm 400$  kcal energy balance ( $r = -.802$ ,  $p = 0.017$ ). Participants with the lowest body fat spent significantly more time in  $\pm 400$  kcal energy balance compared to participants with higher body fat %. Body fat % was positively correlated with hours in high deficit ( $r = .802$ ,  $p = 0.017$ ). Participants with higher body fat % spent more hours in high deficit ( $< -400$  kcals energy balance). Highest energy surplus and deficit were the lowest and highest point of energy balance for each participant along the four-day average record. Body fat% was negatively associated with highest energy balance surplus ( $p = 0.01$ ), highest energy balance deficit ( $p = 0.05$ ) and kcal intake ( $p = 0.01$ ). Those with the highest body fat had the lowest energy balance surplus, energy balance deficit and kcal intake. These data show that contrary to typical recommendations, higher kcal consumption was not correlated with higher body fat %. These data also show that typical energy balance variables may influence body fat % as those in the highest energy balance surplus and highest energy balance deficit had higher body fat %, suggesting that extreme drops or peaks in energy balance may be undesirable and more steady energy balance may be more desirable for body composition.

To further examine the differences within the subjects, men and women were separated. Body fat %, BMI, kcal intake, kcal/kg total, and hours high deficit were correlated using a spearman rho correlation test. No statistically significant results were found between men ( $n = 9$ ) except body fat % was positively correlated with BMI ( $r = .803$ ,  $p = 0.009$ ). This suggest that higher BMI is a correlated with higher body fat %,

demonstrating that higher BMI in this population would be undesirable. Body fat % was also negatively correlated with kcal/kg total ( $r = -.703$ ,  $p = 0.035$ ). This suggests that those consuming more kcals/kg had lower body fat %, an unexpected result given typical recommendations that weight loss can be induced through decreased kcals.

The same test was run in women ( $n = 8$ ). Body fat % was negatively correlated with kcal/kg total ( $r = -.738$ ,  $p = 0.037$ ). Just as in men, those with a higher body fat consumed less kcals/kg. The number of hours high deficit was negatively correlated with kcal/kg total ( $r = -.778$ ,  $p = 0.023$ ) and positively correlated with body fat % ( $r = .802$ ,  $p = 0.017$ ). Therefore in women, those spending more time in high deficit ( $< -400$  energy balance) had a higher body fat %. This suggests that spending time in high negative energy balance may not be desirable for body composition. The fat free mass to height ratio was positively associated with hours high deficit ( $r = .719$ ,  $p = 0.045$ ) and negatively associated with time in  $\pm 400$  kcal energy balance ( $r = -.719$ ,  $p = 0.045$ ). Those with a higher fat free mass to height ratio (e.g. less fat free mass compared to their height) spent more hours in high deficit ( $< -400$  kcals energy balance) and less time in  $\pm 400$  kcal energy balance.

**Table 4.** Spearman Correlation of Body Fat % and Energy Balance

	<b>Hours <math>\pm 400</math> kcal energy balance</b>	<b>Hours High Deficit</b>	<b>Hours Anabolic</b>	<b>Hours Catabolic</b>
<b>Body Fat %</b>	-.644 ( $p = 0.005$ )	.644 ( $p = 0.005$ )	-.591 ( $p = 0.013$ )	.591 ( $p = 0.013$ )

In a regression analysis, the variables predictive of fat free mass to height ratio were the hours catabolic and the highest energy balance surplus for 60% of the sample ( $R=.609$ ,  $p=0.039$ ). Therefore, the larger amount of time spent in a catabolic state and the higher the energy balance surplus predict lower the fat free mass to height ratio. When predicting body fat %, the dependent variables that predict body fat % of 70% of the sample were hours catabolic, the highest energy balance surplus and the highest energy balance deficit ( $R=.696$ ,  $p=0.030$ ). This suggests that the larger amount of time spent in a catabolic state, the higher the energy balance surplus and the higher the energy balance deficit predicts the higher body fat %.

### **Macronutrient Composition**

Body fat % was correlated with carbohydrate/kg, protein/kg, fat/kg, and kcal/kg. Body fat % was negatively correlated with carbohydrate/kg ( $r= -.659$ ,  $p=0.002$ ), protein/kg ( $r= -.737$ ,  $p=0.00$ ), fat/kg ( $r= -.648$ ,  $p=0.004$ ) and kcal/kg ( $r= -.742$ ,  $p=0.004$ ). Body fat % was higher in subjects consuming the least amount of all macronutrients and kcals per kg. This indicates that all macronutrients including kcals per kg are less consumed in subjects with higher body fat % in this sample. This surprising finding may suggest that decreasing kcal, carbohydrate, protein and fat to a certain degree may negatively affect body composition and may not be a recommendation for body composition improvements in this population.

### **Daily Changes**

To test for within day variation between the samples, a one-way ANOVA tested for daily differences between hours in  $\pm 400$  kcal energy balance, hours high surplus, hours high deficit, hours anabolic and hours catabolic. No significant differences were found between each of the daily variables. This supports the assumption that the average of all four days will be a better indicator of changes in body composition compared to one day versus another. These findings support the use of four days of diet record (3 weekdays and 1 weekend) compared to solely utilizing one day.

## Chapter V

### Discussion

Body fat % was found to positively correlate with time in an energy deficit and negatively correlate with time in  $\pm 400$  kcal energy balance. Subjects with higher body fat % spent significantly more time in an energy deficit. Conversely, subjects spending more time in  $\pm 400$  kcal energy balance, avoiding low energy deficits had lower body fat %.

These findings are in accordance to previous literature in collegiate athletes (Deutz et al., 2000). In the study conducted by Deutz et al., 62 elite female athletes (31 artistic gymnasts, 11 rhythmic gymnasts, 14 long-distance runners and 6 middle distance runners) from either the United States National team or nationally/internationally ranked athletes were assessed for body composition and energy balance utilizing a Computerized Time-Line Energy Analysis. The number of hours in an energy deficit greater than 300 kcals was positively associated with body fat %. This finding is consistent with the current study, as hours in an energy deficit would not be recommended for body composition improvements. Deutz et al. (2000) found that athletes who spent the most time in an energy surplus had lower body fat %. Though our study could not establish a relationship between energy surpluses ( $>400$  kcal energy balance) as no subjects spent time in this state, the subjects who spent more time in an anabolic state had lower body fat %. The findings by Deutz et al. (2000) are consistent with our findings and may suggest that 24-hour energy balance assessment may be insufficient, as it does not consider the within-day variations in energy balance.

In our study, when examining males and females separately, the correlation between energy deficit and body fat % was significant only for females. Similarly, time in  $\pm 400$  kcal energy balance was correlated with body fat % for females only. These findings indicate that poorer body composition of females was most significantly correlated with large periods in negative energy balance, potentially mediated through long periods of time without energy intake or increased energy expenditure through physical activity without energy intake. This discrepancy between males and females may be due to the small sample size or may be due to the larger body fat % standard deviation in women when compared to men (9.3 and 8.6 respectively).

Contrasting evidence was found in a study conducted by Drummond et al. (1998). Forty two men and 37 women recorded 7 consecutive days of dietary intake and physical activity. Food diaries included the time of eating. A heart rate monitor assessed 48 hours of expenditure in the subjects. In men, there was a significant negative correlation between eating frequency and body weight. There was no significant relationship between female and body weight or body fat %. They suggest that men compensated for the extra eating occasions by reducing mean energy per eating episode or increasing energy expenditure whereas the women did not compensate (Drummond et al., 1998). This theory may be explained in the current study as the subjects remaining in energy balance throughout the day had the lowest body fat % compared to those with the highest deficits throughout the day. In the present study, subjects who did not compensate for increased energy expenditure throughout the day with increased energy intake had the highest body fat % whereas subjects who compensated for their energy expenditure and remained in energy balance had the lowest body fat %.

Body fat % was negatively correlated with kcals/kg, carbohydrate/kg, protein/kg and fat/kg. These findings suggest that those consuming the least amount of all nutrients had higher body fat %. These results may be explained by changes to the thermic effect of food with varying degrees of underfeeding. There is a clear downregulation of resting energy expenditure as calories consumed are decreased (Jebb et al., 1996; Luke & Schoeller, 1992). This may explain the correlation found in this study as those consuming the least amount of nutrients may have lower resting metabolic rate.

This study utilized both BMI and body composition as predictors of health. Higher measurements of both BMI and body fat % have been correlated to increased risk of cardiovascular disease (Javed et al., 2014). However, BMI solely utilizes a ratio of weight (kg) to height (m) without considering fat to fat free mass (Heinrich et al., 2008). Current research has found body composition may be more sensitive when categorizing obesity (Heinrich et al., 2008; Javed et al., 2014). Javed et al. (2014) conducted a meta-analysis of studies comparing BMI and body fat % measurements in adolescents aged 4-18 years. Thirty seven studies met the inclusion criteria and found a sensitivity of 73%, indicating that over a quarter of the children not labelled as obese by BMI might indeed have excess adiposity. This is especially important for children and young adults as excess fat mass at these stages of life may translate into future health risks and this could be prevented by early intervention (Javed et al., 2014). The current study found significant results when analyzing both BMI and body composition related to energy balance. However, analyzing both of these measurements is essential for health assessment.

Some of the strengths in this study the amount of time subjects tracked their food and physical activity. Four days (3 weekdays and 1 weekend day) provide sufficient information to ascertain an average intake and energy balance. Additionally, subjects were educated about tracking their diet and physical activity and were questioned after to ensure completion of the records.

Some bias may have occurred from recruitment as the subjects were primarily recruited in the Georgia State University Recreation Center. Therefore, they likely had an interest in improving their health. This may be a limitation when comparing to the general university population. Additionally, several limitations exist when subjects self-report their diets. Underreporting and lower consumption of energy while tracking may interfere with the results. Karelis et al. (2010) found that underreporting was more common in subjects with higher BMI, fat mass and visceral fat. As the subjects with the higher BMI and fat mass consistently reported consuming less energy, underreporting may be responsible for these results (Karelis et al., 2010). Goris and Westerterp (1999) found that when 27 lean women participated in a study to record their food intake, they changed their habitual food intake in the recorded week, probably unconsciously done and ate less than usual (Goris & Westerterp, 1999). This may have occurred in this study as many subjects consumed less than their needs for weight maintenance and were consistently in a catabolic state.

Future research might include longitudinal trials addressing within-day energy balance and the effects on body composition. Clinical weight loss trials could monitor within-day energy balance changes to ascertain the effects on body composition.



## **Conclusions**

This study found that individuals who spent more time in negative energy balance with high deficits throughout the day had higher BMI and body fat %. This may support recommendations for limiting large energy deficits throughout the day. The results from our study suggest that maintaining stable energy balance may be beneficial for body composition in college students.

## Appendix

### NutriTiming® Data Entry Form

**Instructions:** Completing this form will help us understand whether the amount of energy (calories) you consume comes close to matching the energy (calories) you expend. This form provides a way of entering your energy expended by using an 'Activity Factor', and your energy consumed by using a description of the foods and drinks you ate. The information is entered by hourly units, so you don't have to remember precisely the time you had an activity or ate some food. Rather, you are asked to enter when you had an activity, its intensity by using the activity factor scale, and how long you did it (example: I had a slow jog between 10 and 11 in the morning that lasted for 30 minutes). Use the NutriTiming Activity Factor Scale Descriptions to help you figure out the best factor to enter when describing an activity. When entering food, describe the food and the way it was prepared fully (example: chicken breast with no skin that was baked; or fried, battered chicken breast, etc.), and the amount you consumed (example: 1 apple; 1 ½ cups; 15 red grapes; 1 large banana, etc.). A factor of 1.5 is considered normal daytime activity, and we will assume a factor of 1.5 unless you indicate otherwise. A factor of 1 is equal to sleep, and a factor greater than 1.5 suggests you are doing something more vigorous than normal daytime activity. Please enter a full 24 hours of all your activities and all the foods/drinks you consume. Use the example below to help you understand how to enter the information.

NutriTiming Activity Factor Scale					
Factor	Description				
1	<b>Resting, Reclining:</b> Sleeping, reclining, relaxing				
1.5	<b>Rest +:</b> Normal, average sitting, standing daytime activity				
2.0	<b>Very Light:</b> More movement, mainly with upper body. Equivalent to tying shoes, typing, brushing teeth				
2.5	<b>Very Light +:</b> Working harder than 2.0				
3.0	<b>Light:</b> Movement with upper and lower body. Equivalent to household chores				
3.5	<b>Light +:</b> Working harder than 3.0; Heart rate faster, but can do this all day without difficulty				
4.0	<b>Moderate:</b> Walking briskly, etc. Heart rate faster, sweating lightly, etc but comfortable				
4.5	<b>Moderate +:</b> Working harder than 4.0. Heart rate noticeably faster, breathing faster				
5.0	<b>Vigorous:</b> Breathing faster and deeper, heart rate faster, must take occasional deep breath during sentence for conversation				
5.5	<b>Vigorous +:</b> Working harder than 5.0. Breathing faster and deeper, and must breath deeply more often to carry on conversation				
6.0	<b>Heavy:</b> You can still talk, but breathing is so hard and deep you would prefer not to. Sweating profusely. Heart rate very high				
6.5	<b>Heavy +:</b> Working harder than 6.0. You can barely talk but would prefer not to. This is as hard as you can go, but not for long				
7.0	<b>Exhaustive:</b> Can't continue this intensity long, as you are on the verge of collapse and are gasping for air. Heart rate is pounding				

  

Begin Hour	End Hour	Activity Factor	Activity Description	Food/Drink Description	Food/Drink Amount
****Begin Example****					
12am	7am	1.0	Sleep		
7am	8am	1.5	Nothing Special	Whole Wheat Waffles (froze-bellogg)	4
				Maple Syrup	2 tablespoons
				1% Milk	1 Cup
				Orange Juice (from concentrate)	1.5 Cups
				Coffee	2 Cups
				1% Milk for Coffee	2 tablespoons
10am	11am	3.0	Jog 30 minutes	Sauzade	16 Ounces
12noon	1pm	1.5	Nothing Special	medium size beef sandwich with white bread, mayonnaise, lettuce, and tomato.	1 Sandwich
				Coffee	2 Cups
				Artificial Coffee Creamer	2 Packets
				Apple Pie	1 Slice (small)
4pm	6pm	3.0	Walk 1 hour	Water	16 ounces
7pm	8pm	1.5	Nothing Special	Lasagna with ground beef and cheese	Large Plate
				Lettuce Salad with Tomatoes and Cucumbers	Medium size Salad
				Blue Cheese Salad Dressing	1 Tablespoon
				Red Wine	1 Medium Glass
10pm	11pm	1.5	Nothing Special	Popcorn (air popped, no butter)	100 Calorie Pack

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