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**The Relationships Between Real Time Energy Balance,
Hunger, and Body Composition**

By

Ashley Delk

A Thesis Presented in Partial Fulfillment of Requirements for the Degree
Master of Science in Health Sciences
The Byrdine F. Lewis School of Nursing and Health Professions
Department of Nutrition
Georgia State University

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Atlanta, Georgia
2014

ACCEPTANCE

This thesis, *The Relationships Between Real Time Energy Balance, Hunger, and Body Composition*, by Ashley Delk, 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 Masters 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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ABSTRACT

The Relationships Between Real Time Energy Balance, Hunger, and Body Composition

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Introduction: Previous research has indicated that hunger is associated with the quantity, volume, and macronutrient composition of food intake. Hunger has never been assessed from the viewpoint of real time energy balance, although there is limited research on hunger and eating frequency.

Purpose: The purpose of this study was to evaluate the relationships between real time energy balance, hunger, and body composition in college students between the ages of 18-30.

Methods: Participants were assessed for body composition using a Tanita scale and energy balance was determined on an hourly basis using NutriTiming® software. A hunger scale was used to assess participant hunger every hour.

Results: There is a statistically significant relationship for the entire population between energy balance average and body fat percent ($R = -0.376$; $P = 0.037$). Hours spent in energy deficient is positively associated with body fat percent ($R = 0.467$; $P = 0.008$), while hours spent in an optimal energy balance is negatively associated with body fat percent ($R = -0.465$; $P = 0.009$). Hours spent in an energy balance surplus (+400 kcals) were not significantly associated with body fat percent. However, hours spent in an anabolic state (>0 kcals) was negatively associated with body fat percent ($R = -.457$; $P = .010$). Conversely, hours spent in a catabolic state (<0 kcals) were positively associated with body fat percent ($R = .457$; $P = 0.10$). Using linear regression analysis with body fat percentage as the dependent variables and age, height, weight, gender, and hours in optimal energy balance, we determined that we could predict a large amount of variance in body fat percentage ($R = .931$; $P = <.001$). The only time during the day that there was a significant correlation between body fat percent and hunger was at 5pm ($R = -0.391$, $P = 0.029$).

Conclusions: These data suggest that that the more time spent in energy deficit is associated with a higher body fat percent. This should encourage college students between the age of 18-30 to avoid restrictive eating patterns and strive to maintain optimal energy balance in order to achieve a low body fat percent.

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LIST OF ABBREVIATIONS

24hrEBNet	24 Hour Net Energy Balance
AI	Adequate Intake
ANOVA	Analysis of Variance
AMDR	Acceptable Macronutrient Distribution Range
BF%	Body Fat Percent
BMI	Body Mass Index
Carb	Carbohydrate
CHO	Carbohydrate
cm	Centimeters
DRI	Dietary Reference Intake
EB	Energy Balance
HDL	High-density Lipoprotein
FFM	Fat Free Mass
g	Grams
g/kg	Grams per kilogram
hrOpt	Hours in Optimal Energy Balance
hrs	Hours
kcal	Kilocalories
kg	Kilograms
LBM	Lean Body Mass
LDL	Low-density Lipoprotein
m	Meters

n	Sample Size
p	Probability value
r	Correlation Coefficient
Satiety1Hrs	Hours Spent in Satiety Level 1
Satiety5Hrs	Hours Spent in Satiety Level 5
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
U.S.	United States
yr	Years

LIST OF DEFINITIONS

Anabolic	Metabolic state that occurs when the body has excess energy to use for cell growth and development. This is represented by an Energy Balance value of > 0 .
Catabolic	Metabolic state that occurs when the body has inadequate energy and therefore must breakdown tissue to provide energy. This is represented by and Energy Balance value of < 0 .
End of Day Energy Balance	Total calories consumed minus total calories expended in a 24-hour period.
Energy Balance	Calories consumed minus calories expended.
Energy Balance Average	Average of all 24 hourly energy balances calculated.
Energy Deficit	Energy balance of less than -400 kcal.
Energy Surplus	Energy balance of greater than +400 kcal.
Hourly Energy Balance	Energy balance at a given hour.
Macronutrient	Substances (carbohydrates, fats, and proteins) required in large amounts to sustain life.
Nutritiming™	Nutrition diet analysis software that can be used to assess energy balance as well as macro and micro nutrient intakes.
Optimal Energy Balance	Energy balance between -400 to +400 kcal.
Percent Body Fat	The ratio of body fat to total body weight.
Within Day Energy Balance	Energy balance that is assessed in real time throughout the day rather than in 24-hour units.

CHAPTER 1

INTRODUCTION

Previous research has indicated that hunger is associated with the quantity, volume, and macronutrient composition of food intake. Hunger has never been assessed from the viewpoint of real time energy balance, although there is limited research on hunger and eating frequency.

Eating frequency represents the number of eating opportunities a person has each day. A nationwide food consumption survey indicated the average number of times people ate per day in the United States between 1987-1988 was 3.47 (Longnecker et al. 1997). Research has indicated that only about half of high school and collegiate athletes eat a minimum of three meals a day (Savoca et al. 2011). There have been numerous research studies that have assessed how eating frequency affects body weight in healthy adults. A study conducted by Ma et al. (2003) found that those who ate four or more times a day were 45% less likely to be obese than those who ate three or less times each day. It also showed that those who skipped breakfast more than 75% of the time were 4.5 times more likely to be obese. Lastly, it indicated that the last time people ate in relation to going to sleep had no impact on the risk of obesity (Ma et al. 2003).

Past research suggests that energy restriction results in a human adaptive response in which the resting metabolic rate (RMR) is reduced (Hall et al. 2004). This process is associated with an increase in fat storage (Deutz et al. 2000). Few studies have assessed how eating frequency impacts food choice and therefore body composition. The studies

on food frequency and body composition generally indicate that higher food frequency results in a lower risk of obesity, lower fat mass, and higher lean mass (Ma et al. 2003; Carlson et al. 2007; Palmer et al. 2009; Westerterp-Plantenga et al. 2002). The mixed results found in these studies may be due to the fact that food frequency is being analyzed instead of hourly energy balance. Although it may be easier to stay in a reasonably good hourly energy balance with smaller more frequent meals, a greater number of meals per day does not necessarily mean a person is achieving proper energy balance throughout the day.

Energy balance is important because it determines whether people are matching their intake and expenditure. When there is too much time between eating episodes, blood glucose levels drop and hunger results. There are no studies on hourly energy balance and blood glucose levels; however, there are several studies on food frequency and blood glucose levels. The basis of research on food frequency and blood glucose levels suggests that low blood glucose may be responsible for the cycle of energy intake (Westerterp-Plantenga et al. 2002; Dewan et al. 2004). Data from a study conducted by Westerterp-Plantenga et al. (2002) indicates that meal frequency is positively correlated with the number of blood glucose declines. “High intake of simple carbohydrates may drive meal frequency through blood glucose dynamics, inducing a viscous circle of blood glucose dynamics driving energy intake” (Westerterp-Plantenga et al. 2002). The macronutrient composition of a meal, because of how it affects blood glucose, will determine the timing of the next meal (Westerterp-Plantenga et al. 2002, Holmstrup et al. 2010). It is known that low blood glucose, or hypoglycemia, is associated with several

symptoms including: headache, loss of concentration and fatigue (Benardot 2007).

Hypoglycemia impacts cognition and results in higher hunger ratings. However, the full impact of hypoglycemia on selecting food is unknown (Schultes et al. 2005).

Many studies on food frequency and blood glucose indicate that a higher food frequency raises blood glucose levels, reduces drastic changes in insulin levels, and helps prevent hypoglycemia (La Bounty et al. 2011). Prevention of hypoglycemia results in increased satiety (Schultes et al. 2005). An increase in eating episodes throughout the day has shown to decrease hunger levels and thus improve control of appetite (La Bounty et al. 2011). A study conducted by Speechly & Buffenstein (1999) suggests that when the delivery of nutrients is evenly distributed throughout the day there is better appetite control, potentially mediated by the insulin response (Speechly & Buffenstein, 1999).

Other studies looking at meal frequency and hunger resulted in opposing findings. These studies found that an increase in meal frequency resulted in increased hunger ratings (Ohkawara et al. 2013). Ohkawara et al. (2013) determined that when participants were given 6 equally sized meals per day they were more hungry than when they were given an isocaloric diet of 3 equally sized meals (Ohkawara et al. 2013). However, this study did not assess current energy balance and, therefore, the size of the meals did not necessarily reflect the participants' current needs. Perhaps if the meal's energy content satisfied the participants' current energy needs the resulting hunger levels would have been altered. The impact of real time energy balance on hunger ratings has not yet been studied.

Hypotheses

The purpose of this study is to examine how real time energy balance is associated with hunger levels and body composition.

H1: Negative real time energy balance is associated with higher hunger scores.

H1⁰: Negative real time energy balance is not associated with higher hunger scores.

H2: Negative real time energy balance is associated with higher body fat percent.

H2⁰: Negative real time energy balance is not associated with higher body fat percent.

CHAPTER II

LITERATURE REVIEW

Eating frequency represents the number of eating opportunities a person has each day. There have been numerous research studies that have assessed how eating frequency affects body weight in healthy adults. A few of these studies assessed how eating frequency impacts body composition. Researchers often use eating frequency to assess how evenly spread out energy intake is throughout the day. However, looking at eating frequency has yielded conflicting study conclusions. We propose that a better variable to use may be real time energy balance, because it determines whether or not people are matching their intake and expenditure throughout the day. When there is too much time between eating blood glucose levels may drop to below normal levels and hunger results. However, when energy expenditure proceeds at a faster rate (as with physical activity) eating episodes must be even closer together to sustain energy balance and to prevent a significant drop in blood glucose. A drop in blood glucose results in other hormones being released, such as cortisol. A positive correlation has been seen between cortisol levels and body fat percent. The following is a review of the current body of knowledge on the associations between real time hourly energy balance, body composition, and hunger. More specifically, this review will discuss the current literature on food frequency and BMI, food frequency and body composition, energy balance and body composition, food frequency and blood glucose, food frequency and hunger, energy substrates and body composition, and energy substrates and hunger.

Real Time Energy Balance

Traditionally, energy balance is measured in 24-hour units from the ratio of energy intake and expenditure. As it became evident that meal timing was also important for metabolic and health outcomes, researchers tried to capture this variable by analyzing meal patterns or meal frequency. The problem is that meal frequency still did not accurately capture the real time balance between energy intake and energy expenditure and therefore left researchers with conflicting data. The following further explains these different methods of capturing energy balance.

Eating frequency represents the number of eating opportunities a person has each day. A nationwide food consumption survey conducted between 1987 and 1988 revealed that the average number of times adults ate per day in the United States was 3.47 times. In this study 3,182 people selected from random American households completed 3-day diet records. Eating occasions were considered in 15-minute increments. When any eating episode that was composed of more than 70 calories was considered an eating occasion, the average number of eating occasions was 3.12 times. Further, when any eating episode that was composed of more than 150 calories was considered an eating occasion, the average number of eating occasions was 2.80 times.

Savoca et al. (2011) conducted a study in which they analyzed the meal patterns, food choices, and activity schedules of 106 African American males in their late teens and early twenties. Interviewers utilized a structured meal pattern timeline interview. They categorized the men into one of five groups: high school students (HS), high school athletes (HA); college students (CS); collage athletes (CA); and non-students (NS). This allowed for the comparison of meal patterns between athletes and non-athletes. A meal

pattern timeline interview of daily eating habits was utilized in order to be able to analyze times of food consumption and types of foods consumed. This study found that athletes and non-athletes have significantly different eating patterns. Athletes' eating patterns were determined by their training schedules and, therefore, this group was more likely to eat meals on a consistent schedule. Athletes are more likely than non-athletes to eat breakfast. Most non-athletes do not eat three meals a day and only about half of athletes eat three meals a day. All participants ate between 2-3 snacks per day. The study also showed a positive correlation between eating dinner with others, eating regular meals, and fruit and vegetable consumption. This study provided valuable insight into the current differences in eating habits between athletes and non-athletes.

In 2012, the American Society for Nutrition published a consensus statement about energy balance. Research has indicated that energy restriction results in a human adaptive response in which the resting metabolic rate (RMR) is reduced. The concept that a 3500-calorie reduction, through decrease intake and/or increased expenditure, will result in 1 lb. loss of body weight is often wrongly applied. Many falsely believe it will result in a linear change in body weight. It has been acknowledged that weight change slows down because of energy expenditure changes that result from adaptive thermogenesis. New dynamic energy balance models are recommended over older simplified equations to predict weight changes (Hall et al. 2012).

Food Frequency and BMI

The relationship between food frequency and BMI is often analyzed (Howarth et al. 2007; Ma 2003). Howarth et al. (2007) examined the relationships between eating

patterns as well as dietary composition and BMI in younger (ages 20- 59 years, n=1792) and older (ages 60-90 years, n = 893) adults. The participants completed the *Continuing Survey of Food Intakes* between 1994 and 1996. Any subject reporting a dietary intake below or above a level that was considered physiologically plausible, <78% or >122% of predicted energy requirement, was excluded. They chose to divide participants at age 60 because previous research indicates that typically BMI increases with age until age 60 and then it begins to decline (Roberts & Williamson 2002). This study found that older adults consumed more meals, but fewer snacks than younger adults. Participants of both age groups skipped lunch more often than breakfast. Both groups eat the greatest proportion of their daily calories at dinner. In younger adults, lower fiber intake and a higher percent of calories from fat were both associated with being overweight or obese. Younger adults who ate more than 6 times per day had significantly higher BMIs than those who ate 6 times or less. Older adults who ate more than 3 times per day had significantly higher BMIs than those who ate three or less times each day. Therefore, increased eating frequency may contribute to a high BMI (Howarth et al. 2007). It is important to recognize that this study used self-reported height and weight and did not collect body composition data. Therefore, there is no way to discern if the increase in BMI for the 6 meal per day group was related to increase muscle mass, increase fat mass, or both. Unfortunately, most studies analyze the impact of food frequency on BMI, which fails to take into account body composition (Mattes et al. 2013).

A literature review conducted by Palmer et al. (2009) analyzed 10 small weight loss studies to determine the impact of eating frequency on weight and health. Most of the studies were short and had small sample sizes. This review found conflicting evidence

and suggested that perhaps there is no association with eating frequency and weight. Larger long-term studies would be beneficial; however, this lack of conclusive evidence could potentially be related to the fact that eating frequency does not actually measure the variable in question, which is how well people are meet their current energy needs (Palmer et al. 2009).

Food Frequency and Body Composition

Recent studies have begun to assess food frequency and body composition, rather than just strictly BMI. Currently there are conflicting study conclusions on how food frequency affects body composition (La Bounty et al. 2011). Many of these studies are limited by their narrow samples, small participant numbers, and short durations. There are also many conflicting studies on the impact of snacking on energy balance and weight (Ma 2003; Bellisle 2014). Hawley & Burke (1997) examined how the timing and frequency of meals can be used to benefit athletes. This article helps establish the framework for why this topic is beneficial for not only the general population but also athletes. The main purpose of the article was to explain that athletes have higher energy expenditures than non-athletes and therefore require additional calories. This is why meal frequency is even more relevant for athletes or physically active people. They require additional calories but the question is whether these calories should be added to existing meals or should constitute additional meals. This article also explains the additional pre, during, and post exercise fueling needs of athletes. The pre-exercise goal is to optimize glycogen stores, avoid GI discomfort, and avoid dramatic increase in insulin that results in rebound hypoglycemia. The during-exercise goal is to provide adequate fluid and

carbohydrate to support activity. The post-exercise goal is to restore glycogen stores. These fueling needs alone constitute three additional meals on training and performance days. The article concluded that a high meal frequency that supports pre, during, and post exercise carbohydrate fueling goals helps increase glycogen availability and performance.

Carlson et al. (2007) published a study that analyzed the difference between one meal per day vs. three meals per day isocaloric diets on health indicators in 40-50 year olds. The BMI range of the participants was 18-25. To qualify for the study, participants had to typically consume 3 meals per day. The study had a randomized crossover design in which participants underwent two isocaloric 8-week diets with 11 weeks in between. On the three meals per day diet participants ate breakfast, lunch, and dinner every day. On the one meal per day diet participants ate all of their calories in one meal between 4:00pm and 8:00pm every day. The number of calories the participants consumed throughout the study fluctuated to help them maintain their initial body weight. Oral glucose tolerance tests were used throughout the study. Metabolic response to the diet was measured with an oral glucose tolerance test (OGTT) and by measuring levels of glucose, insulin, glucagon, leptin, ghrelin, adiponectin, resistin, and brain-derived neurotrophic factor (BDNF). The major finding of this study was that, when participants were on the 1 meal/day diet, they had a significant reduction of fat mass and significant increase in LDL and HDL. While on the one meal per day diet, morning glucose tolerance was impaired and plasma glucose levels were elevated longer. However, there was no significant difference in fasting plasma glucose levels between the two diets. Additionally, there was no significant difference in insulin response during the OGTT. Oral glucose insulin sensitivity (OGIS) and the 1st phase of β -cell function were

significantly lower for the one meal per day diet. There was no significant difference for glucagon, leptin, adiponectin, resistin, and BDNF between the diets. These results suggest that consuming one large meal at the end of the day has a negative impact on glucose tolerance the next morning. A limitation of this study was that an average energy intake for each diet was not listed. Therefore, it is unclear during which diet participants ate more calories to maintain the same body weight. Another limitation is that changes in body composition were not assessed throughout the study, and likely fluctuated despite the researchers' efforts to keep the participants' weight stable.

Food frequency is implicated in having an effect on energy expenditure.

Westerup-Platenga et al. (2003) published a study that examined the effect of habitual intake frequency on energy expenditure. There were 80 participants, ages 18 to 70 years, who did not have fixed time schedules during the week. Body composition was analyzed using a ^2H dilution. The groups were divided into four groups, first by age with 50 years as the cut point, and then by gender. Habitual meal frequency was assessed using 7-day food diaries. Only 56 participants were determined to have accurately reported their intake accurately, based on energy expenditure, and were included in the study. There was no relationship between eating frequency and energy expenditure variables in the women. However, in the older men meal frequency was positively related to resting energy expenditure and inversely related to activity induced energy expenditure. Meal frequency was positively related to BMI. In the younger men, meal frequency was inversely related to resting energy expenditure and positively related to activity-induced energy expenditure. Meal frequency was inversely related to BMI. It was also related to a higher RQ, which means they had a higher carbohydrate oxidation. The young men with

higher meal frequency had lower total energy intakes, lower percent fat intake, and higher percent carbohydrate intake. This study suggested that habitual meal frequency only affected energy expenditure in males, who had higher FFM than females (Westerup-Platenga et al. 2003).

Another study by Drummond et al. (1998) found that, in younger men, there was a negative correlation between eating frequency and BMI. Similar, to the findings of the study by Westerup-Platenga et al. (2003), they did not find a relationship between eating frequency and BMI in women. This study included 48 male participants and 47 female participants, between the ages of 20 and 55 years, with BMIs ranging from 18-30 m/kg². Participants kept 7-day food diaries and activity logs. For men there was a negative correlation between eating frequency and body weight. There was an inverse relationship between eating frequency and BMI. In men eating frequency was not related to total energy intake, but it was positively correlated with an increase in percent of the diet from carbohydrate. In women there was no relationship between eating frequency and weight or BMI. However, for women there was a positive correlation between eating frequency and total energy intake. There was also a positive correlation between eating frequency and intakes of carbohydrate and sugar. This study suggested that men, but not women, compensated for increase meal frequency with decreased caloric intake at each meal. Women who ate more frequently had higher energy intakes, but not higher BMIs. This study also indicated that those who ate more frequently consumed more high carbohydrate snacks and therefore had diets with a higher percentage of carbohydrates (Drummond et al. 1998).

Researchers have suggested that the risk for obesity is related to eating frequency (Ma et al. 2003). A study conducted by Ma et al. (2003) utilized data from the Seasonal Variation of Blood Cholesterol Study (1994-1998) to determine the relationship between eating patterns and obesity. This large study of 499 participants between the ages of 20-70 years used 3-day food recalls and body weight measurements spaced evenly over five testing times during a year to examine this concept. Data from the five testing days was averaged for each participant to provide an average intake and bodyweight that was used in the analysis. In order to analyze eating patterns, they had to establish a definition for what constituted an eating episode. They used a definition from Gibney and Wolever, which says that an eating episode is an event that involves 50 or more calories and is separated from another eating episode by at least 15 minutes. The data collected in this research study showed that on average people eat 3.92 times each day. This study found that those who ate four or more times a day were 45% less likely to be obese than those who ate three or less times each day. Additionally, those who skipped breakfast more than 75% of the time were 4.5 times more likely to be obese. Contrary to many common diet claims, this study found that the last time people ate in relation to going to sleep had no impact on their risk for obesity. Another interesting finding was that those in the 1st and 4th quartiles for energy intake, or those who consumed the least and most calories, were the most likely to be obese. Unfortunately, the article did not compare the number of eating episodes with total daily calorie intake. This means that average calorie intake cannot be compared to the number of times people eat each day.

Energy Balance and Body Composition

How well a person is meeting their energy needs is more accurately assessed with real time energy balance than with eating frequency. A study published by Deutz et al. (2000) looked at the relationship between energy balance and body composition. The participants in this study were elite female gymnasts and runners. There were 31 artistic gymnasts, 11 rhythmic gymnasts, 14 long distance runners, and 6 middle-distance runners. DEXA and skin folds were both used to analyze body composition. Dietary intake and energy expenditure were simultaneously measured using a computerized timeline of energy assessment (CTLEA). This method provided data of energy distribution in terms of macronutrient substrates, total energy intake as kilocalories from food, total energy expenditure, level of kilocalorie surplus and deficit throughout the 24-hour period. Energy balance was determined by several variables: greatest energy surplus, greatest energy deficit, the number of surpluses and deficits greater than 300 kcals, total energy intake, and total energy expenditure. A Pearson correlation coefficient was used to determine if there was a relationship between energy balance and body composition. An ANOVA was used to analyze between group differences regarding energy balance. A linear regression with body fat percent as the dependent variable and energy balance variables as independent variables was also run. This study found that energy balance deficits are positively associated with body fat percentage, while energy balance surpluses are negatively associated with body fat percentage. Because body fat percentage from DEXA and skinfolds were significantly different both were analyzed. Both body fat percentages were statistically significant in the association with energy balance. There were similar correlations seen with the largest daily energy deficits. Total

hours with deficit energy balance is positively associated with body fat percentage, and total hour with positive energy balance is negatively associated. Age, height, and weight are positively associated with body fat percentage. Using a stepwise regression analysis, energy balance, age, and athlete type were able to explain 30.9% of variance of body fat percentage (from DEXA).

It is important to consider that inadequate energy balance results in the loss of both fat and lean mass. The body does this as a compensation mechanism to reduce the energy needed by lean mass. This type of weight loss is undesirable for physically active people who strive to increase lean mass (Benardot 2007). In fact, the greater the energy deficit the greater the proportion of lean mass that will be lost (Louis-Sylvestre et al. 2003). However, high protein diets during calorie deficit dieting may help preserve lean body mass (La Bounty et al. 2011).

Food Frequency and Blood Glucose

Westerterp-Plantenga et al. (2006) examined the impact of normal meal pattern and manipulated meal frequency on blood glucose levels and energy intake. This study was based on many previous studies that demonstrated that appetite is better controlled when meals are spread evenly throughout the day. The purpose was to determine the impact of their regular meal pattern as well as a manipulated meal pattern on blood glucose pattern, macronutrient intake, and energy intake. The study used twenty healthy males between the ages of 18-31. The average BMI of the participants was 22.8. Upon arrival, participants drank either a high carbohydrate or high fat drink. Afterwards, they

were allowed to eat ad libitum throughout the day while being monitored (Westerterp-Plantenga et al. 2006).

Participants also gave a 3-day intake diary so that habitual food intake could be assessed. Meal frequency was defined as the number of eating times that were separated by at least 15 minutes. Manipulated meal frequency was defined as the intervention during which a high carbohydrate or high fat preload is given. Energy intake regulation, also assessed, was defined as the ability to minimize the difference in energy intake between the habitual meal pattern and manipulated meal pattern day. During the study, participants were given the pre-load, either isocaloric and isovolumetric high CHO or high fat, as a lemon flavored beverage. In this single-blind crossover design study, participants came in two times so that they could be given both preloads. After drinking the preload, participants were given typical Dutch lunch foods, differing in macronutrient composition, that they could eat ad libitum. Blood glucose was measured continuously throughout the trial period. Hunger was measured before and after the preload drink on a 100mm visual analog scale (VAS). It was also measured randomly throughout the day, in order to avoid giving subjects time cues. Appetite ratings were completed before and after each meal (Westerterp-Plantenga et al. 2006).

This study found that meal requests were related to drops in blood glucose levels and that meal frequency was greater on the carbohydrate preload day. The results of this study demonstrated that 24 of the 26 participants who experience blood glucose declines in the first 30 minutes of monitoring subsequently requested a meal. After the preload meal, 32 of the 40 participants' next meal request was associated with postprandial dynamic declines. When habitual meal frequency was analyzed it became apparent that

habitual meal frequency and manipulated meal frequency were closely related (Westerterp-Plantenga et al. 2006).

The study also showed a difference in intermeal time between the CHO (62 ± 17 min) and fat (121 ± 23 min) preloads. Manipulated meal frequency was higher on the CHO preload day than the fat preload day and was positively related to the number of blood glucose declines. There was no evidence of macronutrient compensation, difference in energy intake, or number of blood glucose declines between the two days. Using a stepwise regression they were able to explain 91% of the variance in meal frequency using the following variables: percent of diet from CHO, percent of diet from fat, number of blood glucose declines, baseline blood glucose level, sweetness perception of preload, and hunger depression after preload. The results also showed the higher the eating frequency, the higher the energy intake. However, the ability to closely match previous energy intake on preload days was inversely correlated with habitual meal frequency. Therefore, the more often the men typically ate the more likely they were to be able to match their typical energy intake. This demonstrates that people who snack may be better able to regulate energy intake (Westerterp-Plantenga et al. 2006).

The data also indicated that meal frequency was positively correlated with the number of blood glucose declines. “High intake of simple carbohydrates may drive meal frequency through blood glucose dynamics, inducing a vicious circle of blood glucose dynamics driving energy intake” (Westerterp-Plantenga et al. 2006). Therefore, this study demonstrated that the macronutrient composition of a meal, because of how it affects blood glucose, will determine the timing of the next meal.

Holmstrup et al. (2010) conducted a study to determine the impact of meal frequency and macronutrient composition on blood glucose and insulin levels. Eight healthy participants between the ages of 18 and 35 came for four separate trial dates. On the introductory visit, participants completed questionnaires on habitual dietary intake, meal frequency, general health, and physical activity levels. Body composition was also assessed using a BOD POD. During the subsequent three visits, the meals were determined in a single-blind randomized crossover method. Participants arrived at 7:00 am, after fasting all night, and had their blood drawn every 15 minutes for 12 hours. One of the days, participants had three high carbohydrate meals that consisted of a total of 6276 kJ, or 1500 kcals, with a macronutrient distribution of 15% PRO, 65% CHO, and 20% FAT. Another day, the participants had the same high carbohydrate diet, but it was broken into six smaller meals. On the other day, the participants were given six high protein meals that consisted of a total of 6276 kJ, or 1500 kcal with a macronutrient distribution of 45% PRO, 35% CHO, and 20% FAT. This study demonstrated that six frequent meals led to higher sustained blood glucose levels throughout the day. Participants had higher blood glucose concentrations after the 6 CHO meal condition, than both the 3 CHO and 6 protein meal patterns, when looking at 12-hr AUC. The 6 CHO meals resulted in blood glucose AUC being approximately 30% higher throughout the day than when 3 CHO meals were consumed. The 12-hr insulin AUC was lower in the 6 CHO condition than in the 3 CHO condition. During the high protein day, participants had lower blood glucose as well as lower insulin responses. This study implies that if controlling glucose levels is of concern, people who eat frequently should consume dietary protein. Future studies that examine the effects of various levels of

protein would be beneficial. These results suggest that people who are trying to lower their blood glucose levels would benefit from a higher protein diet that consisted of six meals per day. However, those people who need higher sustained blood glucose levels, such as athletes, would benefit from frequent high carbohydrate meals.

Chapelot et al. (2004) examined the influence of hormones and blood substrate profiles on a midafternoon snack. The study included 24 males between the ages of 19-25 years, all of whom had BMIs between 19-25 kg/m². This study excluded anyone who had experienced a body in body weight greater than 1 kg in the past 3 years. Subjects provided a 5-day food diary and were then divided into 3 meal per day eaters (people who did not consume any food between lunch and dinner) or 4 meal per day eat (the fourth meal had to be a gouter, or a typical French afternoon meal between 1600 and 1730). The 3 meals per day group (non gouter eaters, NGE) had 8 people, while the 4 meals per day group (gouter eaters, GE) had 16 people. The NGE group was split again into two groups based on whether they were offer the afternoon snack, non-gouter snack eaters (NGSE) and non-gouter and non-snack eaters (NGNSE). All participants were time blinded and given lunch and then told to request dinner. The GE was allowed to request a gouter between lunch a dinner. The mean time of the request was calculated and used to determine when half of the NGE would be offered an afternoon snack. Data were collected on the energy intake at the gouter for GE and NGSE, energy intake at dinner, period between lunch and gouter and between the gouter and dinner for GE, period from gouter to dinner for the NGSE, period from lunch to dinner for the NGNSE, hunger sensations throughout the study, as well as glucose, insulin, TAG, FA, and leptin concentrations from before lunch to after dinner. The results indicated that total energy

intake was higher in GE group that was provided a gouter and the NGSE group that was provided a snack than in the NGNSE group that was not given either. The GE group, but not the NGSE group, requested dinner later than the NGNSE group. The GE group experienced more hunger and a blood glucose and insulin decrease within 30 minutes of the gouter meal, while neither of the other two groups did. Both the GE and NGSE groups experienced blood glucose and insulin declines before dinner. A predictive equation was used for intermeal interval included the following variables: group factor, energy intake at lunch, leptin correlation at lunch ($R=.970$; $P<.001$). There were clear differences in the effects of the gouter on the GE versus the snack on the NGSE. The GE had higher blood glucose and insulin levels. In conclusion, the subjects who requested a gouter, were therefore given a meal based on their hunger. The group that consumed a snack was not eating because of hunger. When a snack was given to non-hungry people it did not delay the onset of hunger for the next meal (Chapelot et al. 2004).

Gold et al. (1995) analyzed the effect of hypoglycemia on mood state. The study used 24 healthy adults with an average age of 29.5. Participants were given insulin, 60 mU/m²/min, in conjunction with a 20% dextrose solution. Blood glucose levels were tested every 3 minutes and the rate of the glucose infusion was adjusted. In condition A, the target blood glucose was normal at 4.5 mmol/l for 2 hours. In condition B, a hypoglycemic blood glucose levels of 2.5 mmol/L was obtained. In condition B, a blood glucose of 4.5 mmol/l was maintained for 30 minutes. Next, hypoglycemia of 2.5 mmol/l was achieved over a ten-minute time period and maintained for one hour. Then, blood glucose was returned to 4.5 mmol/l and maintained for 30 minutes. At each point of blood glucose manipulation researchers evaluated the participant's moods using the

UWIST Mood Adjective Checklist. This study looked at three different aspects of mood (hedonic tone, tense arousal, and energetic arousal). The results indicated that hedonic tone was significantly lower during hypoglycemia. Conversely, tension was increased during hypoglycemia. The data also showed that energetic arousal was significantly lower during hypoglycemia, and remained lower during the recovery period. Based on these findings, future research could examine how these hypoglycemic induced mood states impact food choices.

Food Frequency and Hunger

Current research suggests that increasing meal frequency improves appetite control (La Bounty et al. 2011; Speechly & Buffenstein 1999). Speechly & Buffenstein (1999) conducted a study to determine the impact of meal frequency on appetite and subsequent food intake. There were 8 male participants with an average age of 22.9 years and mean BMI of 23.11 kg/m². The study was a crossover design in which participants received a single preload meal one day and 5 preload meals (the total was isocaloric to the single meal). One third of their energy needs, calculated by using the Harris-Benedict equation, was given as the single preload meal or divided into 5 equal meals and given every hour. Appetite, hunger, glucose, and insulin levels were all assessed hourly. Six visual analog scales that accounted for hunger, perceived amount of food that could be eaten, and urge to eat were used to assess appetite and hunger. After 5.5 hours, participants were allowed to eat cottage pie and orange juice ad libitum. Appetite, hunger, glucose, and insulin levels were all assessed 15, 45, and 75 minutes after lunch. After the single preload meal, participants consumed 26.6% more at lunch. There were no significant differences in

blood glucose levels between the two groups. There were no differences in hunger scores between the trials. There was a correlation between actual energy intake at lunch and the pre-lunch scores for assessment of how much they thought they could eat, urge to eat, and preoccupation with food for the 5 meal group. There was no correlation seen for the 1 preload meal group. This suggests that spreading energy intake into small meals throughout the day helps with appetite control and energy intake regulation (Speechly & Buffenstein 1999).

Verger et al. (1992) analyzed the impact of exercise on food intake. The participants were thirteen college-aged males and females. Participants in the study came in for five separate trial dates that were one week apart. During four of the trials, they exercised submaximally for 2 hours performing various athletic activities. Next, they were randomly assigned to eat 0, 30, 60, or 120 minutes after exercising. During one trial they were asked to rest for two hours and then eat 60 minutes later. Prior to beginning the meal, each participant rated his or her hunger. For each meal they were given a pre-weighed tray that consisted of hard-boiled eggs, ham, cheese, tabouleh and gelatin fruit. Throughout the entire trial they were allowed to drink water ad libitum. The data were analyzed using repeated measures ANOVA in terms of total calories, type of food, and macronutrients. This study found that at the 60-minute mark after exercise participants were significantly hungrier than at the 60-minute mark after rest. On average participants ate an additional 470 calories after exercise. The average energy expenditure of participants during exercise, using predictive equations, was approximately 500 kcals. The increase in calories eaten appeared to closely match the participants increased energy expenditure. Additionally, as time increased after exercise, hunger also increased.

Therefore, the later the meal was eaten the more food that was consumed. However, the increase in food consumed was not equal among the foods. Participants ate significantly more carbohydrates, taboule and gelatin fruit, the later they ate.

Ohkawara et al. (2013) analyzed the effect of consuming 3 meals per day versus 6 meals per day on 24-hour fat oxidation and hunger ratings. The study included 15 subjects, 7 males and 8 females, with BMI's less than 25 kg/m². Body composition and RMR were analyzed. The study had a crossover design so that all participants experienced both the 3 meal (3M) and 6 meal (6 M) per day isocaloric conditions. Each condition lasted 4 days with 1-2 weeks between conditions. During the first three-days, participants were treated as outpatients and were provided with meals to consume. On the fourth day of each trial period participants entered the calorimeter at 0800h and stayed until 0700h the next day. Hunger and satiety were assessed using VAS, and blood samples were drawn before and after each meal and upon waking on the second morning. The results demonstrated that there was no difference in 24-hour energy expenditure, energy balance, respiratory quotient, or fat oxidation for the 3 meal vs. 6 meal trials. There was also no difference between activities on the two trial days. Although the 6 meals a day resulted in more glucose peaks, there was no difference in glucose AUC for 3M vs. 6M. There were also more insulin peaks in the 6-meal day; however, insulin AUC was lower for 6M than 3M. FFA decreased after each meal and rose before the subsequent meal for 3M; however, FFA decreased after the first meal in the 6M trial and remained below baseline for the remainder of the day. Perceived hunger (41850 ± 2255 vs. 36612 ± 2556 mm.24 h, P = 0.03) and desire to eat 47061 ± 1791 vs. 41170 ± 2574

mm.24 h, $P = 0.03$) AUC were greater during the 6M than the 3M. Therefore, consuming 6M a day rather than 3M increased hunger and did not impact 24-hour fat oxidation.

Schultes et al. (2005) assessed the affect of hypoglycemia on cognitive functioning and hunger. The study used fifteen healthy male participants with an average age of 26 years. They participated in two trial dates and arrived after fasting for ten hours. One day, participants underwent a stepwise hypoglycemia clamp and the other a euglycemic clamp procedure. These were single blind and the order was balanced across the participants. During the baseline period, they received $1.5 \text{ mU min}^{-1}\text{kg}^{-1}$ and a 20% dextrose solution simultaneously. During the euglycemic clamp blood glucose was maintained at a target level of 5.2mmol/l. During the hypoglycemic clamp tests, four blood glucose levels of 4.1, 3.6, 3.1, and 2.6 mmol/L were achieved and each was maintained for 45 minutes. When each pre-determined blood glucose level was achieved, participants performed a memory task and Stroop task as well as a hunger scale rating. The main findings of this study demonstrated that cognition is impacted by hypoglycemia and that during hypoglycemia more cognition functioning is focused on food stimuli. This study also found that ratings for hunger were much higher during hypoglycemia. Even a slight change in blood glucose from normal, 1.1 mmol/l, was enough to result in a significant increase in hunger.

Dewan et al. (2004) conducted a study to determine how insulin-induced hypoglycemia affects food choice. Sixteen healthy males, with an average age of 29.8 years and BMI of 23.6 kg/m^2 , were given either saline or insulin in a double-blind crossover method. Insulin was given in 0.05 units per kg of body weight. Twenty minutes later they were allowed to eat breakfast from a buffet ad libitum. Blood glucose and

hunger, using a VAS, were assessed at baseline, 5, 10, 15, 20, 40, 60, 80, 100, 120 minutes after receiving the IV. They were also asked to rate the following to assess hunger: fullness, hunger, desire to eat, prospective consumption (how much could you eat right now?), thirst, and mood. Participants were allowed to leave after 2.5 hours but kept a food diary for the remainder of the day. They also completed the hunger scale every hour until 10:00 pm. Participants had similar appetite scores despite the treatment they received. However, participants consumed 17% more calories after being given insulin and had an increase in fullness five and fifteen minutes after the meal. There was a significant increase in high-fat food consumption after insulin. This study suggests that hypoglycemia may lead to increased fat, and therefore calorie, intake (Dewan et al. 2004). This relationship between hypoglycemia and subsequent food consumption should be studied further.

Energy Substrates

Research on how the macronutrient distribution in the diet affects body composition has been inconclusive. According to NHANES data from 2009-2010, males in the US ages 20-29 years consumed an average of 2626 kcals (± 79.4), 16% protein (± 0.3), 50% carbohydrate (± 0.5), and 31% fat (± 0.4). In addition, approximately 4% of their calories come from alcohol. Females aged 20-29 years on average consumed 1949 kcals (± 54.7), 15% protein (± 0.2), 52% carbohydrate (± 0.6), and 32% fat (± 0.5). In addition, approximately 2% of their calories come from alcohol.

These data also show the typical meal distribution. Males consumed 15% of their daily calories at breakfast, 25% at lunch, 34% at dinner, and 26% at snacks. In terms of

macronutrient distribution, they consumed 14% of their protein at breakfast, 30% at lunch, 42% at dinner, and 14% at snacks. They consumed 17% of their daily carbohydrates at breakfast, 23% at lunch, 30% at dinner, and 31% at snacks. They consumed 15 % of their daily fat at breakfast, 30% at lunch, 37% at dinner, and 18% at snacks. Females consumed 16% of their daily calories at breakfast, 23% at lunch, 35% at dinner, and 25% at snacks. They consumed 17% of their protein at breakfast, 26% at lunch, 43% at dinner, and 14% at snacks. They consumed 18% of their daily carbohydrates at breakfast, 23% at lunch, 30% at dinner, and 29% at snacks. They consumed 16% of their fat at breakfast, 25% at lunch, 38% at dinner, and 21% at snacks. Only 49% of males and 54% of females consumed all three meals. Half of the males consumed between 2-3 snacks per day. Just under half of the females consumed between 2-3 snacks per day. This study indicates that people not only backload their calories during the day, but also all the macronutrients (USDA 2012).

The Recommended Dietary Allowance (RDA) for protein is 0.8g/kg of body weight. This is the amount of protein that should be consumed daily in order to prevent deficiency. This recommendation is made for a 24-hour period and does not specify how the protein intake should be distributed (Otten et al. 2006). Studies have indicated that moderate consumption of protein throughout the day provides the maximal benefit for muscle growth. Symons et al. (2009) looked at the anabolic response to one moderate serving of 90% beef with 30g of protein and a large serving with 90g of protein. The study had 17 young adult participants with an average age of 34 years, and 17 older adult participants with an average age of 68 years. The study found that lean muscle synthesis increased by 50% in both age groups regardless of which size meal they consumed.

Therefore, large quantities of protein above 30 grams do not provide an additional benefit for building muscle (Symons et al. 2009).

It has been suggested that protein may be beneficial for weight management because it has been implicated in playing a role in increased satiety, increased thermogenesis, and maintenance of lean mass (Paddon-Jones et al. 2008). There has been debate on what level of protein intake not only prevents deficiency but also promotes muscle growth. There are also questions on the best method of protein distribution throughout the day. Mamerow et al. (2014) conducted a study to examine how different distributions of protein impact skeletal muscle synthesis. The study included 8 participants with an average age of 36.9 years (± 3.1). All participants had BMI's below 30 kg/m². The study consisted of two seven-day trial periods and had a crossover design with a 30-day washout period. Participants were fed isocaloric diets with 90g of protein. Diet 1 (EVEN) provided an even protein distribution of 30g of protein at breakfast, lunch, and dinner. Diet 2 (SKEW) provided 10g of protein at breakfast, 15g at lunch, and 65g at dinner. Participants had 24-h metabolic testing on day 1 and 7 of each trial. Carbohydrates remained stable, but fat was manipulated to provide the same total calories for each meal. It is important to note that the protein provided by both diets was approximately 50% more than what is recommended by the RDA. For the EVEN breakfast meal (30g of protein) the protein synthesis response was 30% higher than for the SKEW breakfast meal (10g of protein). On day 1 and day 7, the EVEN diet resulted in approximately 25% higher muscle protein synthesis than the SKEW diet. This suggests that even protein distribution is important to maximizing muscle synthesis (Mamerow et al. 2014).

Layman et al. (2009) conducted a study to examine the long-term effects of a moderate protein diet on weight loss and blood lipids. The study lasted one year and included 4 months of active weight loss and 8 months of weight maintenance. There were 58 men and 72 women, between the ages of 40-56 years, with BMIs greater than 26 kg/m² and body weights greater than 140 kg. Participants were assigned to one of two treatment groups. One group had a low carbohydrate diet consisting of approximately 40% carbohydrate, 30% protein, and 30% fat. Protein was calculated at 1.6 g/kg/d. The other group had a high carbohydrate diet that consisted of approximately 55% carbohydrate, 15% protein, and 30% fat. Protein was calculated at 0.8g/kg/d. The diets were isocaloric. Females were given 1700 kcals and males were given 1900 kcals per day. At baseline, 4 months, and 8 months participants had body weight, blood lipids, and DEXA measurements. Diet compliance was supported by not only food logs, but also with urinary urea measurements. Urea excretion by the high protein group was 489 ± 13 mmol/d at 4 months and 502 ± 27 mmol/d at 8 months, which was significantly higher than for the high carbohydrate group who had urea excretion levels of 252 ± 22 mmol/d at 4 months and 283 ± 15 mmol/d at 8 months. At 4 months there was a difference in blood lipid levels between the two groups. The high carbohydrate group had lower total cholesterol and LDL. The high protein group had higher HDL and lower triacylglycerol (TAG). At 8 months the LDL and TC returned to baseline levels in the high carbohydrate group. HDL remained higher in the high protein group. Both groups experience lower TAG and total cholesterol levels, with a greater reduction in both seen in the high protein group. There was no difference in weight loss between the groups at 4 months, however the high protein group lost 22% more body fat. At 8 months, the high protein group had

lost 23% more weight and 38% more fat mass than the high carbohydrate group (Layman et al. 2009).

Studies have also yielded inconclusive results on the impact of macronutrients on hunger. A review by Gerstein et al. (2004) suggests that hunger is the biological basis of why people seek food. It is one of the factors that determines when, what, and how much people eat. Satiety, or the feeling of fullness, is typically what causes an eating episode to end. Many studies have examined the affect of the different macronutrients on satiety. However, the fact the people tend to consume a mixture of macronutrients during an eating episode complicates the studies. Protein has been implicated as the macronutrient that provides the greatest satiety per calorie. Foods that are highly palatable and those that have a low energy density lead to the most satiety. This would be a difficult combination to find because people tend to rate high energy density foods as highly palatable (Gerstein et al. 2004).

Summary

Past studies provide an overview of the current findings on food frequency as it relates to blood glucose and hunger. The following provides the main conclusions from the articles. Athletes have greater caloric needs than non-athletes. It is believed that these additional calories should be distributed in a high meal frequency that supports pre, during, and post exercise CHO fueling goals. This helps increase glycogen availability and improves performance (Hawley et al. 1997). Research has indicated that athletes are

more likely than non-athletes due to consume regular meals due to the structure provided by their training schedules (Savoca et al. 2011).

When on a 1 meal/day diet vs. 3 meal/day diet participants experienced a significant reduction of fat mass, increase in LDL and HDL, and impaired glucose tolerance. However, conflicting research results remain on the topic of ideal food frequency. On average, people eat 3.92 times each day. Those who ate four or more times a day were 45% less likely to be obese than those who ate three or less times each day. Those who skipped breakfast more than 75% of the time were 4.5 times more likely to be obese (Ma, 2003). Exercise increases hunger and increases percent carbohydrate consumed (Verger et al. 1992).

The basis of research on food frequency and blood glucose levels suggests that low blood glucose may be responsible for the cycle of energy intake (Plantenga et al. 2006). Meal frequency and macronutrient composition impacts blood glucose and insulin levels. Higher frequency of meals results in higher sustained blood glucose levels. Higher protein diets result in lower blood glucose and insulin responses (Holmstrup et al. 2010). After being given insulin, food consumption increases, especially of high fat foods, despite unchanged hunger ratings (Dewan et al. 2004). Hypoglycemia also impacts cognition. In this study, ratings for hunger were much higher during hypoglycemia. Although the full impact of hypoglycemia on selecting food is unknown (Schultes et al. 2005).

Conclusion

The studies on food frequency and body composition generally find that higher food frequency results in a lower risk of obesity, lower fat mass, and higher lean mass. The studies on food frequency and hunger indicate that a higher food frequency decreases hunger and thus elicits better control of appetite. The studies on food frequency and blood glucose indicate that a higher food frequency maintains blood glucose levels to help prevent hypoglycemia, which results in improved satiety. Future research could examine how the number of hypoglycemic incidents each day correlates with total caloric intake. This would provide information for people on how many meals they should eat each day to control blood glucose, prevent hunger, and improve body composition. Further research should be done to determine how food frequency impacts blood glucose and thus food choice. There is also a need to further research the link between not just weight, but body composition and energy balance in a wide variety of populations.

CHAPTER III

METHODS

Recruitment Strategy

Using an IRB-approved protocol, subjects were notified in classes at Georgia State University about the opportunity to become involved in this study. Flyers were also distributed around campus to recruit participants for the study. No incentives were provided.

Inclusion Criteria

Participants were eligible to participate if they were healthy college students at Georgia State University and between ages of 18-30 years old. Participants agreed to complete a one day food log, a one day hunger scale, and a body composition assessment using a Tanita scale. The study was approved by the Institutional Review Board of Georgia State University, and all participants provided written informed consent in accordance with the Georgia State University guidelines for the protection of human subjects.

Participants

Thirty-six eligible participants responded as interested. Thirty-one subjects completed the full protocol. Students who were taking mood or appetite altering medications or who had metabolism altering conditions were excluded. The study included both male (n=10) and female (n=21) participants.

Data Acquisition Procedures

All assessments took place in the Laboratory for Elite Athlete Performance at Georgia State University. All data collection occurred over a 4 month period. All participants signed the informed consent form, after the details of the study were explained. Participants chose, based on preference, to receive either an electronic or hard copy version of the necessary study forms. All subjects met once with researcher, at least once, during which time all anthropometric data was collected. One researcher completed all assessments. Upon arrival, the participant's height was measured in inches using a standard sliding scale stadiometer. Body composition and weight were measured using a Tanita Scale (Arlington Heights, Illinois USA), a multi-current 8-mode bioelectrical impedance device. Weight was measured in pounds to the nearest tenth and later converted to kilograms. Bioelectrical impedance measures the conductance and impedance of an electrical signal that travels through the body and uses this information along with height, weight, age, and gender to predict body composition.

Hourly energy intake and expenditure for a single day was recorded by using NutriTiming® Data Entry Form (Appendix I). Time of each eating and exercising episode was included. Activity intensity was recorded using an activity factor scale from 1 (resting, reclining) to 7 (exhaustive). Participants were asked follow up questions if their 24-hour recall was lacking sufficient detail. Food items that were reported, but were not listed in the database, were added using nutrition labels. Every hour participants also recorded their level of hunger on a hunger scale (Appendix II). The scale used was a double scale, in which -2 represented very hungry and +2 represented very full. This design was used to match the concept of positive and negative energy balance. At the end

of the day, immediately before going to sleep, participants indicated the hours of the day during which they felt the most and least hungry. This scale has construct validity only. It has not been otherwise validated.

Data Analysis

Dietary intake and energy expenditure data were analyzed using NutriTiming® software (NutriTiming® Nutrient and Energy Analysis 2.1, Calorie and Pulse Technologies, 2009), a nutrient analysis based on U.S. Department of Agriculture National Nutrient Database for Standard Reference Release 26. This assessed end-of-day and within-day hourly energy balance. Energy balance was evaluated based on each participant's greatest caloric surplus, greatest caloric deficit, hours in energy surplus (>400 kcals), hours in energy deficit (<-400kcal), hours anabolic (energy balance >0), and hours catabolic (energy balance <0). Statistical analyses were performed using SPSS (version 20.0, SPSS, Inc., Chicago, IL). Frequencies and descriptive statistics were used to describe participant characteristics. Based on the small sample size (n=31), abnormal distributions of the data were assumed. Therefore, analyses were conducted using non-parametric statistical methods. Statistical significance was set at $P < 0.05$. The participants' real time energy balance, hunger levels, and body composition were assessed, using a regression analysis, to determine the degree to which participant characteristics, energy distribution (percent of kilocalories from carbohydrate, protein, and fat), total energy consumed from food, total energy expended, percent of energy requirement achieved over the 24-h period of analysis, starting energy balance, highest energy, lowest energy, and hours spent in an energy surplus and deficit state during this

24-h period explain differences in body composition and hunger. Relationships between these variables, using Pearson correlations, were also assessed. Using z-scores as the split points, t-tests were used to assess if there was significant differences in body composition and hunger scores, and body composition and within-day energy balance values.

CHAPTER IV

RESULTS

Subject Characteristics

Of the 36 eligible participants, 31 completed the entire study. The participants were college students between 18 and 30 years of age, with 10 male participants and 21 female participants. The mean age of study participants was 21.94 years (± 3.02). The median age was 22 years. Mean height and weight were found to be 165.67 cm (± 12.05) and 73.34 kg (± 16.10), respectively. The median height was 162.56 cm and the median weight was 71.09 kg. Mean body fat percentage was found to be 24.35 percent (± 11.43). Median body fat percent was 24.3%. The mean BMI was 26.90 (± 6.43) and median BMI was 24.43.

	Subject	Minimum	Maximum	Mean	Median	Std. Deviation
Age (yr)	All	18	30	21.94	22.00	3.02
	Male	19	30	22.60	22.00	3.20
	Female	18	28	21.62	22.00	2.96
Height (cm)	All	138.43	191.77	165.67	162.56	12.05
	Male	157.48	191.77	177.80	176.53	10.35
	Female	138.43	175.26	158.90	161.29	7.82
Weight (kg)	All	46.09	105.45	73.34	71.09	16.10
	Male	58.73	102.91	81.57	81.18	11.91
	Female	46.09	105.45	69.42	63.55	16.58
Body Fat (%)	All	4.30	47.90	24.35	24.30	11.43
	Male	4.30	22.20	13.50	12.80	5.64
	Female	9.20	47.90	29.51	28.60	9.73
BMI	All	17.44	45.34	26.90	24.43	6.43
	Male	22.03	33.99	25.88	24.22	4.01
	Female	17.44	45.34	27.37	24.50	7.35

We tested to determine if there were gender differences in body composition, energy balance, energy substrates, and hunger. Table 2 shows the differences in means for the body composition variables that were significantly different for males and females. No differences were seen for energy balance variables or energy substrates.

Table 2: Gender comparison of body composition variables.					
	Gender	N	Mean	Standard Deviation	P
Fat (%)	Male	10	13.50	5.64	<0.001
	Female	21	29.51	9.73	
FFM (kg)	Male	10	70.38	10.21	<0.001
	Female	21	47.56	5.35	
Weight (kg)	Male	10	81.57	11.91	0.029
	Female	21	69.42	16.58	
Height (cm)	Male	10	177.80	10.35	<0.001
	Female	21	159.90	7.82	
FFM per kg	Male	10	0.86	0.06	<0.001
	Female	21	0.71	0.10	
FFM to Height Ratio	Male	10	0.40	0.05	<0.001
	Female	21	0.30	0.04	

Energy Intake

Using a Spearman's correlation, we determined that calories per kg of body weight and calories per kg of body fat were negatively correlated with body fat % (Table 3). Calories per kg of body weight and calories per kg of body fat were positively correlated with FFM per kg. Additionally, calories per ht (cm) was positively correlated with FFM (kg) and FFM to height ratio.

		Calories per kg	Calories per kg BF	Calories per kg FFM	Calories per Ht (cm)
Body Fat (%)	R	-.531	-.888	-.134	-.263
	P	.002	.000	.472	.154
FFM (kg)	R	.120	.263	-.029	.406
	P	.521	.154	.877	.023
FFM per kg	R	.532	.889	.136	.263
	P	.002	.000	.465	.152
FFM to Height Ratio	R	.153	.262	-.004	.446
	P	.411	.154	.983	.012

Energy Balance

Over a 24-hour period, the average energy intake for all participants, with a relatively wide standard deviation, was estimated to be 2,326 kcal (± 824). This is less than the predicted average energy requirement of 2662 kcals (± 514). This resulted in the predicted average 24 Hour Net Energy Balance of -333 kcals (± 725). On average, participants spent 13 hours in optimal energy balance. Participants spent more time in an energy deficit >-400 kcals (7.97 hrs ± 5.9), than they did in an energy surplus $>+400$ kcals (2.58 hrs ± 4.9). Additionally, using an energy balance of 0 kcals as the cut point, participants spent more time catabolic than they did anabolic (16.71 ± 6.7 vs. 7.28 ± 6.7). The mean largest energy balance surplus during the day was +446 kcals (± 481). The mean largest energy balance deficit during the day, was -848 kcals (± 403). The average time during the day at which people experienced their highest energy balance was 1354, or 1:54pm. The average time during the day at which people experienced their lowest energy balance was 1523, or 3:23pm.

Table 4: Energy balance descriptive statistics (N=31).					
Variables	Min	Max	Mean	Median	Std. Deviation
Calories In	864	3814	2326	2292	824.28
Calories Out	1877	3785	2662	2546	514.12
Calories per kg	10.99	62.90	32.40	31.40	11.60
24 Hour Net Energy Balance	-1427	1382	-332.55	-390	724.87
Optimal Energy Balance (hr.)	1	24	13.45	13	5.33
Energy Surplus >400 (hr.)	0	23	2.58	0	4.85
Energy Deficit >-400 (hr.)	0	19	7.97	8	5.93
Hours Anabolic	0	24	7.29	5	6.71
Hours Catabolic	0	24	16.71	19	6.71
Largest Surplus	-152	1795	445.68	271	481.22
Largest Deficit	-1708	111	-847.71	-869	402.56
Time Highest Energy Balance	0000	2300	1390.32	1500	645.68
Time Lowest Energy Balance	0000	2300	1538.71	1700	580.04

Note: The zeros in the minimum column indicate that some subjects had zero hours spent in these values. The 0000 values in Time of Highest and Time of Lowest Energy Balance indicate 12am, as the time is indicated in military time.

Energy Substrates

On average, participants consumed a diet that consisted of 20% protein (± 5.9), 50% carbohydrate (± 10.5), and 30% fat (± 9.4). This fits within the Acceptable Macronutrient Distribution Range, AMDR, for 19-30 year olds, which states that a diet should be 10-35% protein, 45-65% carbohydrate, and 25-35% fat. The mean protein g/kg consumed was 1.6 g/kg (± 0.8). For comparison, the Adequate Intake (AI) level is set at 0.8g/kg/day for adults ages 19-30 years. The mean carbohydrate g/kg consumed was 4.1 g/kg (± 1.7), and the mean fat g/kg consumed was 1.07 g/kg (± 0.4).

	Min	Max	Mean	Median	Std. Deviation
Protein (g/kg)	0.56	4.82	1.61	1.47	0.84
Protein (%)	9.10	35.99	19.82	20.14	5.94
Carbohydrate (g/kg)	1.17	8.67	4.06	4.19	1.70
Carbohydrate (%)	21.91	71.24	49.80	50.14	10.51
Fat (g/kg)	0.45	2.19	1.07	0.98	0.44
Fat (%)	15.48	65.37	30.38	27.85	9.36

Hunger

Satiety, also referred to as ‘hunger average’, is a scale that measures from 1 (extremely hungry) to 5 (extremely full). The mean satiety average throughout the time subjects were awake during the 24-hour period was 2.9 (± 0.3) indicating that participants were slightly hungrier than they were full. On average, participants spent 5.4 hrs (± 3.0) satiated, 4.5 hrs (± 2.6) full, and 6.5 hrs (± 1.9) hungry. This also demonstrates that, on average, while they were awake, participants spent more time hungry than satiated.

	Min	Max	Mean	Median	Std. Deviation
Satiety	2.29	3.29	2.85	2.90	0.27
Hungry (hrs)	3.00	13.00	6.45	6.00	1.93
Full (hrs)	0.00	8.00	4.45	5.00	2.55
Satiated (hrs)	0.00	14.00	5.35	5.00	3.07
Extremely Hungry (hrs)	0.00	7.00	1.97	2.00	1.49
Extremely Full (hrs)	0.00	4.00	1.61	1.00	1.41

Note: The zeros in the minimum column indicate that some subjects had zero hours spent in these values.

Energy Balance and Body Composition.

Using a Spearman’s rho we determined that there is a statistically significant relationship for the entire population between energy balance average and body fat percent ($R = -0.376$; $P = 0.037$). Table 7 indicates that hours spent in energy deficit is positively

associated with body fat percent ($R=0.467$; $P=0.008$), while hours spent in an optimal energy balance is negatively associated with body fat percent ($R= -0.465$; $P=0.009$). Hours spent in an energy balance surplus (+400 kcals) were not significantly associated with body fat percent. However, hours spent in an anabolic state (>0 kcals) was negatively associated with body fat percent. The more hours spent anabolic, the lower the body fat percent. Conversely, hours spent in a catabolic state (<0 kcals) were positively associated with body fat percent. A catabolic state occurs when the body is in a negative energy balance and therefore must breakdown tissue to provide energy. This process can result in the breakdown of muscle. The more hours spent catabolic, the greater the body fat percent.

Table 7: Body fat and energy balance correlations (N=31).						
		Hr Anabolic	Hr Catabolic	HrDef	HrSurp	HrOpt
Body Fat (%)	R	-.457	.457	.467	-.061	-.464
	P	.010	.010	.008	.746	.009
R= Correlation; P = Probability						

We also determined that the number of protein eating opportunities, which was defined as consumption of ≥ 20 grams of protein separated by at least 1 hour, was negatively associated with body fat percent ($R=-.363$, $P=.045$). Protein eating opportunities was positively associated with FFM ($R= .379$, $P=.035$), FFM/kg ($R=.363$, $P= 0.45$), and FFM to height ratio ($R=.420$, $P=.019$) (Appendix III). We also determined that number of protein eating opportunities was positively associated with 24 Hour Energy Balance Net. The greater the number of protein eating opportunities over 20g, the higher the net energy balance. The 24-Hour Energy Balance Net is calculated from total caloric intake minus energy expenditure. Therefore, the greater the number of protein

eating opportunities, the higher the energy balance. It is important to note that the average 24-Hour Net Energy Balance was -333 kcals (± 725), suggesting that subjects with more protein eating opportunities did not necessarily have adequate or excessive energy intakes.

Regression Analysis

Using linear regression analysis with body fat percentage as the dependent variable and age, height, weight, gender, and hours in optimal energy balance as the independent variables, we determined that we could predict body fat percentage in the assessed population using the equation:

$$\text{Body Fat \%} = ((\text{Age in years} \times .305) + (\text{Height in cm} \times -.134) + (\text{Weight in kg} \times .419) + (\text{Gender} \times 18.256) + (\text{Hr Opt} \times -.535)) - 14.310.$$

$$(R = .931; R^2 = .866; \text{SEE} = 4.582; P = <.001) \text{ (Appendix IV)}.$$

It is important to note that net energy balance (end of day), which is a standard for measuring intake and predicting weight and body composition, did not explain a significant amount of variance in body fat percent, and therefore was not included in the equation.

Again using body fat percent as the dependent variable, a linear regression analysis was performed utilizing both hunger variables and energy balance variables. We determined that we could predict body fat percentage in the assessed population using the equation:

Body Fat % = ((Gender x 13.543) + (Height in cm x -.281) + (Weight in kg x .328) + (24hrEBNet + .011) + (Hr Opt x -.777) + (Hrcatabolic x .250) + (Opt Satiety x .905) + (Satiety1Hrs x 3.436) + (Protein % x -.253) + (Carbohydrate g/kg x -5.219) + (Fat % x -.674)) – 69.108.

(R= .983; R2 = .965; SEE = 2.67; P= <.001) (Appendix V).

The linear regression determined that hunger explained a significant amount of variance when predicting body fat percent. However, there were no statistically significant correlations between any hunger values and body composition.

Another a linear regression analysis was performed to predict body fat percent utilizing both hunger variables and energy balance variables. This equation also includes variables that assess time variables related to energy balance and hunger. We determined that we could predict body fat percentage in the assessed population using the equation:

Body Fat = ((Gender x -.762) + (Weight in kg x -.477) + (24hrEBNet x -.342) + (Protein g/kg x -.167) + (Hrcatabolic x -.292) + (Time of Least Hunger x -.104) + (Time of Highest Energy Balance x .153)) + 1.343.

(R= .949; R2=.901; SEE = 0.041; P <.001 AppendixVI)

The same variables were used to create an equation to predict fat free mass.

FFM = (((Gender x -.588) + (Weight in kg x .553) + (24hrEBNet x -.207) + (Protein g/kg x -.100) + (Hrcatabolic x -.215) + (Time of Least Hunger x -.028) + (Time of Highest Energy Balance x .093)) + 51.034.

(R= .963; R2=.927; SEE = 4.00; P <.001 Appendix VII)

The linear regressions demonstrate that time of the greatest energy balance surplus and time of greatest hunger are useful in predicting both body fat and fat free mass.

Energy Balance and Hunger

Hunger at 10am was significantly associated with energy balance at 10am and the energy balance average for 9am-11am ($R = -0.402$, $P = 0.034$ and $R = -0.473$, $P = 0.011$) (Appendix VIII). That is to say the more hungry people were, the higher their energy balance. Hunger at 1pm was significantly associated with energy balance at 1pm and 2pm ($R = -0.378$, $P = 0.36$ and $R = -0.387$, $P = 0.031$) (Appendix IX).

As shown in Appendix X, hunger at 6pm was positively associated with energy balance at 6pm ($R = 0.360$, $P = 0.047$). Hunger at 7pm was positively associated with energy balance at 6pm ($R = 0.631$, $P < 0.001$), 7pm ($R = 0.487$, $P = 0.005$), and 8pm ($R = 0.404$, $P = 0.024$). Early evening hunger average, 6pm-8pm, was positively associated with energy balance at 6pm ($R = 0.647$, $P < 0.001$), 7pm ($R = 0.606$, $P < 0.001$), 8pm ($R = 0.445$, $P = 0.012$), and the average energy balance from 6-8pm ($R = 0.546$, $P = 0.001$).

The only time during the day that there was a significant correlation between body fat percent and hunger was at 5pm ($R = -0.391$, $P = 0.029$). The more hungry participants were at 5pm the higher the body fat percent.

Hunger Average was significantly correlated with ratio of anabolic to catabolic ($R = .366$, $P = .047$). However, when assessing males, hunger average was associated with hours spent in optimal energy balance ($R = .758$, $P = .011$). The more hours they spent in optimal energy, balance between +400 and -400 kcals, the more satiated they felt. Also,

the more hours males spent in an energy deficit the more hunger they experienced ($R=-.707$, $P=.022$). For the females, the more hours they spent extremely full (satiety score 5), the more hours they spent in energy surplus ($R=.456$, $P=.038$).

Energy Substrates and Energy Balance

In females, 24 Hour Net Energy Balance was negatively associated with protein g/kg ($R= -.479$, $P=.028$), while it was positively associated with carbohydrate % ($R=.822$, $P<.001$), and fat g/kg ($R=.640$, $P=.002$). For males, 24 Hour Net Energy Balance was not significantly associated with energy substrates.

Participants were divided into groups based on Z-scores of their average energy balance. Those with a z-score less than -0.5 were group 1, those with a score of -0.5 to +0.5 were assigned to group 2, and those with a score greater than 0.5 were assigned to group 3. Using a nonparametric Kruskal-Wallis we found that there was a significant difference between groups for carbohydrate (g/kg) and fat (g/kg). Using a one-way ANOVA with the Bonferroni post-hoc test, there was a statistically significant difference between groups 1 and 2 and between groups 1 and 3 for carbohydrate g/kg (Appendix XI). The average carbohydrate g/kg intake was 2.5 g/kg for group 1, 4.2 g/kg for group 2, and 5.4 g/kg for group 3. Therefore, those with the highest average energy balance also had the highest g/kg intake of carbohydrate. Additionally, there was a statistically significant difference between groups 2 and 3 for fat g/kg. The average fat g/kg intake was 0.92 g/kg for group 2 and 1.4 g/kg for group 3. Those with the highest average energy balance also had the highest g/kg intake of fat. Despite the difference in energy balance between the groups, there was no significant difference in protein intake.

Energy Substrates and Body Composition.

Table 8: Substrate and body composition correlations (N=31).						
Spearman's rho	Protein (g/kg)	Protein (%)	Carb (g/kg)	Carb (%)	Fat (g/kg)	Fat (%)
Body Fat (%)	R	-.577	-.310	-.285	.300	-.530
	P	.001	.089	.120	.101	.002
FFM per kg	R	.579	.312	.285	-.299	.529
	P	.001	.088	.120	.102	.002

R=Correlation; P = Probability

Table 8 illustrates the relationship between body fat percent and the energy substrates. There were no significant correlations between body fat percent and % of any given substrate in the diet. Protein and fat intake (g/kg) were negatively associated with body fat percent.

Energy Substrates and Hunger

Hours spent in Optimal Satiety was associated with the percent of the diet that came from protein (R=.384, P=.033). Specifically, percent of the diet that came from protein was positively associated with satiety at 1 am (R= .866, P=.012) and 4pm (R=.424, P=.017). The higher the percentage of the diet that came from protein the more hours participants spent feeling satiated but not hungry or full. Hunger Average, Hours Spent in Negative Satiety, Hours Spent in Positive Satiety, Hours Spent at Satiety Score 1 (extremely hungry), and Hours Spent at Satiety Score 5 (extremely full) were not significantly associated with energy substrates.

For males, the number of g/kg of protein and percent of total calories from protein were significantly associated with their average hunger score (R=.806, P=.005 and

$R=.879$, $P=.001$), respectively. The more protein in their diet, the more satiated they felt. Carbohydrate and fat were not associated with any hunger variables for male participants. Interestingly, in females, percent of total calories from carbohydrate was positively associated with hunger average ($R= .479$, $P=.028$). The greater the percent of their diet that came from carbohydrate, the more satiated they felt. Females who had high levels of carbohydrate were more likely to feel extremely full than those who had less carbohydrate. High percent of carbohydrate was positively associated with hours spent in positive satiety (feeling full) and hours spent extremely full (with a satiety score of 5) ($R=.474$, $P=.030$ and $R=.480$, $P=.027$), respectively. However, percent carbohydrate was negatively associated with hours spent in optimal satiety (without a feeling of fullness or hunger) ($R=-.0470$, $P=.031$).

CHAPTER V

DICUSSION AND CONCLUSION

On average, participants had a 24-Hour Net Energy Balance of -333 kcals (± 725). According to the participants reported intake, on average, they consumed fewer calories than they expended. Participants spent more hours of the day catabolic than they did anabolic (16.71 ± 6.7 vs. 7.28 ± 6.7). A catabolic state occurs when the body is in negative energy balance and must therefore breakdown body tissue to utilize for energy. An anabolic state occurs when the body has a positive energy balance and therefore can utilize the energy to build body tissues. This is important because there is a statistically significant relationship for the entire population between energy balance average and body fat percent ($R = -0.376$; $P = 0.037$). Therefore, those people with higher energy balance averages spent less time catabolic. This means they spent less time breaking down body tissues, and more time building muscle thus resulting in a lower body fat percent and a higher percent of lean mass.

Contrary to common belief that energy deficits (i.e., reduced energy intakes) will result in a lower body fat, this study demonstrates that hours spent in energy deficient is positively associated with body fat percent ($R = 0.467$; $P = 0.008$). The more hours that people spent in a negative energy balance the higher their body fat percent. Interestingly, hours spent in energy surplus ($> +400$ kcals) was not significantly associated with body fat percent. This means periods of excessive energy balance did not result in excessive body fat.

Protein eating opportunities was negatively associated with percent body fat and negatively associated with FFM. This means the more times people ate 20 or more grams of protein during the day the higher their muscle mass and the lower their percent body fat.

Energy balance and satiety were negatively associated at 10am, 1pm, and 2pm. The more hungry people were the higher their energy balance. This was opposite of what was predicted. Perhaps this could be attributed to the fact that when people experience high levels of hunger they corrected the problem by consuming a large amount of food within the same hour period. However, energy balance and satiety were positively associated at 6pm, 7pm, and 8pm. That is to say, the more hungry people were the lower their real time energy balance.

The only time during the day that there was a significant correlation between body fat percent and hunger was at 5pm ($R = -0.391$, $P = 0.029$). This indicates that people who let themselves get extremely hungry before dinner had higher body fat percentages than those who maintained higher satiety levels in the late afternoon.

Males appeared to have better regulation of energy balance utilizing hunger cues. Hunger average was associated with hours spent in optimal energy balance ($R = .758$, $P = .011$). The more hours they spent in optimal energy balance between +400 and -400 kcals, the more satiated they felt. For females, this was not the case. For females the more hours they spent extremely full (satiety score 5), the more hours they spent in energy surplus ($R = .456$, $P = .038$). Thus suggesting females do not distinguish between energy deficit and optimal energy balance using hunger cues.

For males, 24HourNetEB was not related to macronutrient substrates. Females, however, experienced a negative association between 24HourNetEB and protein g/kg. The more protein females consumed the lower their 24HourNetEB, which means the lower their calorie intake per energy expenditure. Oppositely, the more carbohydrates (% of total calories) and the more fat (g/kg) females consumed the higher the 24HourNetEB.

For all participants, carbohydrate intake was not related to body composition. However, protein and fat intake (g/kg) were negatively associated with body fat percent and positively associated with FFM. This means higher intakes of protein and fat was related to lower body fat percentages. It is important to keep in mind that the average net energy balance was -333 kcals. This means that those with higher intakes of fat and protein may not be excessively consuming these macronutrients.

Different substrates were associated with hunger scores in males and females. The number of hours that males spent in optimal satiety, not hungry or full, was directly related to the percent of the diet that came from protein. The more protein in the diet the more hours people spent in optimal satiety. Carbohydrate and fat were not associated with any hunger variables for male participants. In females, the proportion of total calories from carbohydrate was positively associated with hunger average ($R = .479$, $P = .028$). The greater the percent of their diet that came from carbohydrate, the more satiated they felt. Additionally, females who had high levels of carbohydrate were more likely to feel extremely full than those who had less carbohydrate. In summary, hunger is related to carbohydrate intake in females, while it is related to protein intake in males.

Therefore, this study suggests that obtaining optimal hourly energy balance through increased eating frequency can result in lower body fat percent. Additionally,

hunger at 5pm impacts body composition. Hunger is driven primarily by protein intake in males and carbohydrate intake in females.

Other studies have assessed eating frequency, not hourly energy balance, so a comparison cannot be made. The only exception is a study by Deutz et al. (2000). Our study is consistent with their finding that there is a negative correlation between energy balance average and body fat percent.

STUDY LIMITATIONS

All diet records were self-reported by the participants and may have led to over-reporting and/or under-reporting. The participants' understanding of portion sizes and food label reading skills could have impacted their reported food intake.

FUTURE RESEARCH

Future research could look at hours of the day in relation to waking or sleeping instead of fixed time points. Additionally, if studying college age students, it would be helpful to obtain a 2 day food diary. In this study, many students did not go to sleep or stop eating at the 12am end-of-day cut point. Therefore, last time of food consumption before bed could not be analyzed with hunger or body composition variables.

It is also important to note that the hunger scale used in this study was not previously validated. Participants' average hunger scores did not vary widely. That, coupled with the relatively small sample size, could attribute to the small number of correlations seen with hunger variables.

CONCLUSIONS

It is evident that energy restriction as a method of lower body fat percentage is not effective. Large energy deficits caused by inadequate intake and/or high-energy expenditure may result in the breakdown of a high ratio of muscle to stored adipose tissue. This research calls for a shift in the weight loss paradigm. The traditional recommendation of end of day energy balance may result in desired weight loss. However, the proportion is high muscle loss to fat loss. This will not result in the desired long-term outcomes as basal metabolic rate will also decrease. Weight loss recommendations should shift away from typical energy restricting 'diet' strategies that focus on end-of-day calorie deficits and/or calorie surpluses. Instead they should focus on real-time energy balance. Use of such software could be used to help people track energy balance data in real time and, therefore, allow them to self-correct accordingly.

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APPENDIX 1

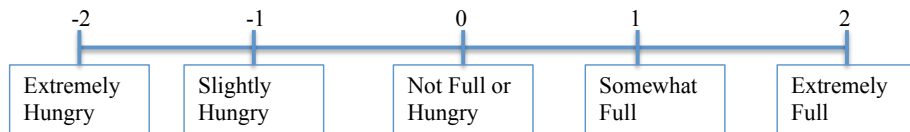
NutriTiming™ Data Entry Form

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	Resting, Reclining: Sleeping, reclining, relaxing				
1.5	Rest +: Normal, average sitting, standing daytime activity				
2.0	Very Light: More movement, mainly with upper body. Equivalent to tying shoes, typing, brushing teeth				
2.5	Very Light +: Working harder than 2.0				
3.0	Light: Movement with upper and lower body. Equivalent to household chores				
3.5	Light +: Working harder than 3.0; Heart rate faster, but can do this all day without difficulty				
4.0	Moderate: Walking briskly, etc. Heart rate faster, sweating lightly, etc but comfortable				
4.5	Moderate +: Working harder than 4.0. Heart rate noticeably faster, breathing faster				
5.0	Vigorous: Breathing faster and deeper, heart rate faster, must take occasional deep breath during sentence for conversation				
5.5	Vigorous +: Working harder than 5.0. Breathing faster and deeper, and must breath deeply more often to carry on conversation				
6.0	Heavy: You can still talk, but breathing is so hard and deep you would prefer not to. Sweating profusely. Heart rate very high				
6.5	Heavy +: 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	Exhaustive: 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 (Frozen-Kellogg)	3
				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	5.0	Jog 30 minutes	Gatorade	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)
5pm	6pm	4.0	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
End Example					

APPENDIX II

During each hour of the day that you are awake please rate your feeling of hunger/ fullness on the scale below.



Hour	Hunger Rating
12am	
1 am	
2 am	
3 am	
4 am	
5 am	
6 am	
7 am	
8 am	
9 am	
10 am	
11 am	
12 pm	
1 pm	
2 pm	
3 pm	
4 pm	
5 pm	
6 pm	
7 pm	
8 pm	
9 pm	
10 pm	
11 pm	

The following are questions to be answered at the end of the day, immediately before going to sleep. Please be sure to include am or pm in your answer

1. Please indicate the hour of the day during which you felt the most hungry.

2. Please indicate the hour of the day during which you felt the most full.

APPENDIX III

Protein Eating Opportunities Correlations

Spearman's rho		Protein Eating Opportunities	Fat (%)	FFM (kg)	FFM per kg	FFM to Height Ratio	24HrEBNet
Protein Eating Opportunities	R	1.000	-.363	.379	.363	.420	.377
	P	.	.045	.035	.045	.019	.037
Fat (%)	R	-.363	1.000	-.307	-1.000	-.293	-.266
	P	.045	.	.093	.000	.110	.149
FFM (kg)	R	.379	-.307	1.000	.306	.948	.202
	P	.035	.093	.	.094	.000	.276
FFM per kg	R	.363	-1.000	.306	1.000	.292	.265
	P	.045	.000	.094	.	.112	.150
FFM to Height Ratio	R	.420	-.293	.948	.292	1.000	.145
	P	.019	.110	.000	.112	.	.437
24HrEBNet	R	.377	-.266	.202	.265	.145	1.000
	P	.037	.149	.276	.150	.437	.

APPENDIX IV

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.931 ^a	.866	.839	4.5819398

a. Predictors: (Constant), HrOpt, Gender, Age (yr), Weight (kg), Height (cm)

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-14.310	20.060		-.713	.482
	Age (yr)	.305	.296	.081	1.031	.313
	Height (cm)	-.134	.102	-.141	-1.316	.200
	Weight (kg)	.419	.059	.591	7.160	.000
	Gender	18.256	2.556	.759	7.143	.000
	HrOpt	-.535	.166	-.250	-3.230	.003

a. Dependent Variable: Body Fat (%)

APPENDIX V

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.983 ^a	.965	.945	2.6696383

a. Predictors: (Constant), Fat (%), Weight (kg), OptSatiety, 24HrEBNet, HrOpt, Gender, Protein (%), Height (cm), Hrcatabolic, Satiety1Hrs, Carbohydrate (g/kg)

ANOVA^b

Model	Sum of Squares	df	Mean Square	F	Sig.
1 Regression	3782.544	11	343.868	48.249	.000 ^a
Residual	135.412	19	7.127		
Total	3917.957	30			

a. Predictors: (Constant), Fat (%), Weight (kg), OptSatiety, 24HrEBNet, HrOpt, Gender, Protein (%), Height (cm), Hrcatabolic, Satiety1Hrs, Carbohydrate (g/kg)
 b. Dependent Variable: Fat (%)

Coefficients^a

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
1 (Constant)	69.108	20.043		3.448	.003
Gender	13.543	1.676	.563	8.082	.000
Height (cm)	-.281	.068	-.297	-4.111	.001
Weight (kg)	.328	.041	.462	8.004	.000
24HrEBNet	.011	.002	.680	5.405	.000
HrOpt	-.777	.135	-.363	-5.750	.000
Hrcatabolic	.250	.127	.147	1.976	.063
OptSatiety	.905	.256	.243	3.542	.002
Satiety1Hrs	3.436	.675	.449	5.090	.000
Protein (%)	-.253	.111	-.132	-2.290	.034
Carbohydrate (g/kg)	-5.219	1.060	-.775	-4.924	.000
Fat (%)	-.674	.125	-.552	-5.393	.000

a. Dependent Variable: Fat (%)

APPENDIX VI

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.949 ^a	.901	.871	.04105

a. Predictors: (Constant), Protein (g/kg), Weight (kg), 24HrEBNet, Time of Least Hunger, Gender, Time of Highest Energy Balance, Hrcatabolic

ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.353	7	.050	29.930	.000 ^b
	Residual	.039	23	.002		
	Total	.392	30			

a. Dependent Variable: FFM per kg

b. Predictors: (Constant), Protein (g/kg), Weight (kg), 24HrEBNet, Time of Least Hunger, Gender, Time of Highest Energy Balance, Hrcatabolic

Coefficients^a

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	1.343	.077		17.483	.000
Time of Highest Energy Balance	2.700	.000	.153	1.708	.101
Time of Least Hunger	-2.447	.000	-.104	-1.385	.179
1 Hrcatabolic	-.005	.002	-.292	-2.746	.011
Gender	-.183	.019	-.762	-9.851	.000
Weight (kg)	-.003	.001	-.477	-5.934	.000
24HrEBNet	-5.396	.000	-.342	-3.068	.005
Protein (g/kg)	.023	.011	.167	2.130	.044

a. Dependent Variable: FFM per kg

APPENDIX VII

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.963 ^a	.927	.905	4.00400

a. Predictors: (Constant), Protein (g/kg), Weight (kg), 24HrEBNet, Time of Least Hunger, Gender, Time of Highest Energy Balance, Hrcatabolic

ANOVA^b

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4671.093	7	667.299	41.623	.000 ^a
	Residual	368.737	23	16.032		
	Total	5039.830	30			

a. Predictors: (Constant), Protein (g/kg), Weight (kg), 24HrEBNet, Time of Least Hunger, Gender, Time of Highest Energy Balance, Hrcatabolic

b. Dependent Variable: FFM (kg)

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	51.034	7.492		6.812	.000
	Time of Highest Energy Balance	.002	.002	.093	1.214	.237
	Time of Least Hunger	-.001	.002	-.028	-.439	.664
	Hrcatabolic	-.415	.177	-.215	-2.345	.028
	Gender	-16.047	1.814	-.588	-8.846	.000
	Weight (kg)	.445	.056	.553	7.992	.000
	24HrEBNet	-.004	.002	-.207	-2.156	.042
	Protein (g/kg)	1.555	1.047	.100	1.485	.151

a. Dependent Variable: FFM (kg)

APPENDIX VIII

Energy Balance and Hunger 9am-11am Correlations

Spearman's rho		9amEB	10amEB	11amEB	EB Morning Average
9amEB	R	1.000	.624	.165	.737
	P	.	.000	.374	.000
	N	31	31	31	31
10amEB	R	.624	1.000	.538	.936
	P	.000	.	.002	.000
	N	31	31	31	31
11amEB	R	.165	.538	1.000	.657
	P	.374	.002	.	.000
	N	31	31	31	31
EB Morning Average	R	.737	.936	.657	1.000
	P	.000	.000	.000	.
	N	31	31	31	31
9amH	R	.157	.169	.004	.126
	P	.465	.430	.987	.556
	N	24	24	24	24
10amH	R	-.326	-.402	-.329	-.473
	P	.091	.034	.087	.011
	N	28	28	28	28
11amH	R	-.077	.142	.394	.192
	P	.698	.471	.038	.328
	N	28	28	28	28
H Morning Average	R	-.276	-.193	.000	-.222
	P	.193	.365	1.000	.298
	N	24	24	24	24

APPENDIX IX

Energy Balance and Hunger 12pm-2pm Correlations

Spearman's rho		12pmEB	1pmEB	2pmEB	EB Early Afternoon Average
12pmEB	R	1.000	.536	.533	.756
	P	.	.002	.002	.000
	N	31	31	31	31
1pmEB	R	.536	1.000	.925	.923
	P	.002	.	.000	.000
	N	31	31	31	31
2pmEB	R	.533	.925	1.000	.934
	P	.002	.000	.	.000
	N	31	31	31	31
EB Early Afternoon Average	R	.756	.923	.934	1.000
	P	.000	.000	.000	.
	N	31	31	31	31
12pmH	R	.225	.222	.208	.264
	P	.241	.247	.279	.166
	N	29	29	29	29
1pmH	R	.187	-.378	-.387	-.214
	P	.314	.036	.031	.248
	N	31	31	31	31
2pmH	R	.051	.063	-.027	.041
	P	.787	.737	.887	.826
	N	31	31	31	31
Hunger Early Afternoon Average	R	.290	-.068	-.147	.059
	P	.127	.727	.447	.762
	N	29	29	29	29

APPENDIX X

Energy Balance and Hunger 6-8pm Correlations

			6pmEB	7pmEB	8pmEB	EB Early Evening Average
Spearman's rho	6pmEB	R	1.000	.850	.753	.881
		P	.	.000	.000	.000
		N	31	31	31	31
	7pmEB	R	.850	1.000	.847	.948
		P	.000	.	.000	.000
		N	31	31	31	31
	8pmEB	R	.753	.847	1.000	.942
		P	.000	.000	.	.000
		N	31	31	31	31
	EB Early Evening Average	R	.881	.948	.942	1.000
		P	.000	.000	.000	.
		N	31	31	31	31
	6pmH	R	.360	.240	.204	.223
		P	.047	.193	.270	.229
		N	31	31	31	31
	7pmH	R	.631	.487	.404	.466
		P	.000	.005	.024	.008
		N	31	31	31	31
	8pmH	R	.314	.399	.192	.317
		P	.086	.026	.300	.082
		N	31	31	31	31
Hunger Early Evening Average	R	.647	.606	.445	.546	
	P	.000	.000	.012	.001	
	N	31	31	31	31	

APPENDIX XI
Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Protein (g/kg)	1.00	9	1.191	.496	.165	.810	1.572
	2.00	13	1.617	.688	.191	1.201	2.033
	3.00	9	2.018	1.131	.377	1.148	2.887
	Total	31	1.610	.836	.150	1.303	1.916
Protein (%)	1.00	9	20.150	4.575	1.525	16.634	23.666
	2.00	13	20.318	5.653	1.568	16.902	23.734
	3.00	9	18.763	7.848	2.616	12.731	24.796
	Total	31	19.818	5.938	1.067	17.640	21.996
Carbohydrate (g/kg)	1.00	9	2.547	1.159	.386	1.656	3.438
	2.00	13	4.169	1.219	.338	3.432	4.905
	3.00	9	5.431	1.583	.528	4.214	6.648
	Total	31	4.064	1.696	.305	3.442	4.686
Carbohydrate (%)	1.00	9	43.863	13.595	4.532	33.413	54.313
	2.00	13	53.101	8.502	2.358	47.963	58.239
	3.00	9	50.956	7.877	2.626	44.901	57.011
	Total	31	49.796	10.510	1.887	45.941	53.651
Fat (g/kg)	1.00	9	.959	.571	.190	.520	1.398
	2.00	13	.922	.301	.083	.740	1.103
	3.00	9	1.410	.286	.095	1.190	1.630
	Total	31	1.074	.439	.079	.913	1.235
Fat (%)	1.00	9	35.986	13.630	4.543	25.508	46.463
	2.00	13	26.581	6.141	1.703	22.870	30.292
	3.00	9	30.279	5.264	1.755	26.232	34.325
	Total	31	30.385	9.365	1.682	26.950	33.820

Descriptives

		Minimum	Maximum
Protein (g/kg)	1.00	.56	2.11
	2.00	.71	2.78
	3.00	1.28	4.82
	Total	.56	4.82
Protein (%)	1.00	12.72	28.45
	2.00	9.10	28.16
	3.00	10.66	35.99
	Total	9.10	35.99
Carbohydrate (g/kg)	1.00	1.17	4.32
	2.00	2.42	6.36
	3.00	4.13	8.67
	Total	1.17	8.67
Carbohydrate (%)	1.00	21.91	57.30
	2.00	44.81	71.24
	3.00	40.02	66.16
	Total	21.91	71.24
Fat (g/kg)	1.00	.45	2.19
	2.00	.47	1.62
	3.00	1.02	1.96
	Total	.45	2.19
Fat (%)	1.00	22.19	65.37
	2.00	15.48	37.55
	3.00	23.18	40.55
	Total	15.48	65.37

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Protein (g/kg)	Between Groups	3.076	2	1.538	2.408	.108
	Within Groups	17.888	28	.639		
	Total	20.965	30			
Protein (%)	Between Groups	14.249	2	7.124	.191	.827
	Within Groups	1043.514	28	37.268		
	Total	1057.763	30			
Carbohydrate (g/kg)	Between Groups	37.683	2	18.842	10.848	.000
	Within Groups	48.632	28	1.737		
	Total	86.316	30			
Carbohydrate (%)	Between Groups	470.849	2	235.425	2.319	.117
	Within Groups	2842.396	28	101.514		
	Total	3313.246	30			
Fat (g/kg)	Between Groups	1.437	2	.719	4.631	.018
	Within Groups	4.346	28	.155		
	Total	5.784	30			
Fat (%)	Between Groups	470.536	2	235.268	3.049	.063
	Within Groups	2160.445	28	77.159		
	Total	2630.981	30			

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Protein (g/kg) is the same across categories of CatEBAvg.	Independent-Samples Kruskal-Wallis Test	.083	Retain the null hypothesis.
2	The distribution of Protein (%) is the same across categories of CatEBAvg.	Independent-Samples Kruskal-Wallis Test	.487	Retain the null hypothesis.
3	The distribution of Carbohydrate (g/kg) is the same across categories of CatEBAvg.	Independent-Samples Kruskal-Wallis Test	.001	Reject the null hypothesis.
4	The distribution of Carbohydrate (%) is the same across categories of CatEBAvg.	Independent-Samples Kruskal-Wallis Test	.551	Retain the null hypothesis.
5	The distribution of Fat (g/kg) is the same across categories of CatEBAvg.	Independent-Samples Kruskal-Wallis Test	.006	Reject the null hypothesis.
6	The distribution of Fat (%) is the same across categories of CatEBAvg.	Independent-Samples Kruskal-Wallis Test	.104	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Post Hoc Tests

Multiple Comparisons

Bonferroni

Dependent Variable	(I) Cat EBAvg	(J) Cat EBAvg	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Protein (g/kg)	1.00	2.00	-.426	.347	.688	-1.308	.457
		3.00	-.827	.377	.110	-1.786	.133
	2.00	1.00	.426	.347	.688	-.457	1.308
		3.00	-.401	.347	.772	-1.284	.482
	3.00	1.00	.827	.377	.110	-.133	1.786
		2.00	.401	.347	.772	-.482	1.284
Protein (%)	1.00	2.00	-.168	2.647	1.000	-6.909	6.573
		3.00	1.387	2.878	1.000	-5.942	8.715

	2.00	1.00	.168	2.647	1.000	-6.573	6.909
		3.00	1.554	2.647	1.000	-5.187	8.295
	3.00	1.00	-1.387	2.878	1.000	-8.715	5.942
		2.00	-1.554	2.647	1.000	-8.295	5.187
Carbohydrate (g/kg)	1.00	2.00	-1.622	.571	.025	-3.077	-.167
		3.00	-2.884	.621	.000	-4.467	-1.302
	2.00	1.00	1.622	.571	.025	.167	3.077
		3.00	-1.263	.571	.106	-2.718	.193
	3.00	1.00	2.884	.621	.000	1.302	4.467
		2.00	1.263	.571	.106	-.193	2.718
Carbohydrate (%)	1.00	2.00	-9.237	4.369	.131	-20.363	1.888
		3.00	-7.092	4.750	.440	-19.187	5.003
	2.00	1.00	9.237	4.369	.131	-1.888	20.363
		3.00	2.145	4.369	1.000	-8.980	13.271
	3.00	1.00	7.092	4.750	.440	-5.003	19.187
		2.00	-2.145	4.369	1.000	-13.271	8.980
Fat (g/kg)	1.00	2.00	.037	.171	1.000	-.398	.472
		3.00	-.451	.186	.065	-.924	.022
	2.00	1.00	-.037	.171	1.000	-.472	.398
		3.00	-.488	.171	.024	-.924	-.053
	3.00	1.00	.451	.186	.065	-.022	.924
		2.00	.488	.171	.024	.053	.924
Fat (%)	1.00	2.00	9.405	3.809	.060	-.295	19.104
		3.00	5.707	4.141	.537	-4.838	16.251
	2.00	1.00	-9.405	3.809	.060	-19.104	.295
		3.00	-3.698	3.809	1.000	-13.398	6.001
	3.00	1.00	-5.707	4.141	.537	-16.251	4.838
		2.00	3.698	3.809	1.000	-6.001	13.398

*. The mean difference is significant at the 0.05 level.