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## Within-Day Energy Balance and Protein Intake Affect Body Composition in Physically Active Young Adult Females

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## APPROVAL

This thesis, Energy Balance and Protein Distribution throughout the Day Affect Body Composition in Physically Active Young Adults, by Heather Hanson, 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 of Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee members, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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## ABSTRACT

**Title:** Within-Day Energy Balance and Protein Intake Affect Body Composition in Physically Active Young Adult Females

**Background:** Past studies suggest that individuals who eat smaller, more frequent meals are at a metabolic advantage when compared to those who eat larger, less frequent isocaloric meals. Studies also suggest that consumption of small amounts (~ 20 to 30 g) of protein evenly distributed during the day, may be a superior strategy for satisfying the protein requirement and improving muscle protein synthesis. It was, therefore, the purpose of this study was to assess the relationship between body composition and the distributed consumption of energy and protein in physically active young adult females.

**Methods:** Using an IRB approved protocol, physically active female volunteers were measured for height using a standard wall mount stadiometer; body composition and weight were measured using a multi-current 8-mode segmental bioelectrical impedance device (Tanita, Arlington Heights, Illinois USA, Model BC-418). The volunteers kept a food and activity journal for one day, which was assessed to determine hourly energy balance and hourly protein intake. Exertion was assessed using a relative intensity activity MET value scale that produces multiples of resting energy expenditure, which was predicted using the Harris-Benedict equation.

**Results:** The 28 females who volunteered for this study ranged in age from 19-24 years. Significant inverse associations were found between protein (grams/kg) consumption and fat mass ( $r=-0.42$ ;  $p=0.026$ ); and FFM and the ratio of protein to energy balance at 4pm ( $r=-0.376$ ;  $p=0.049$ ). There was a significant positive association between FFM and the ratio of protein to energy balance at 12pm ( $r=0.390$ ;  $p=0.040$ ) and 9pm ( $r=0.379$ ;  $p=0.047$ ). There was also a significant positive association between the ratio of FFM to height and the ratio of protein to energy balance at 12 pm ( $r=0.423$ ;  $p=0.025$ ). There was a significant association between highest daily peak energy balance and FFM to height ratio ( $r=0.402$ ;  $p=0.034$ ). Regression analysis determined that independent EB and protein variables could be used to predict the dependent variable FFM to Height ratio ( $r=0.727$ ;  $p=0.019$ ).

**Conclusions:** These findings demonstrate a significant positive association between highest daily EB and FFM to height ratio. The results also suggest that higher protein consumption per kg is inversely associated with fat mass. Similarly, when protein is consumed when in  $\pm 400$  kcal energy balance, is associated with higher FFM.

Additionally, energy balance and protein variables can be used to predict FFM to height ratio using a regression equation that accounts for 52.9% of variance. These data indicate that subjects spent far more hours in an energy balance deficit than surplus, making it difficult to assess the impact of protein intake distribution on body composition. It does appear that, from a relatively small subsample ( $n=28$ ) who did achieve a positive within-day energy balance and adequate protein intake, consumption of protein while in good energy balance may help physically active adult females achieve a body composition that is low in fat mass and high in lean muscle mass.

**Within-Day Energy Balance and Protein Intake Affect Body  
Composition in Physically Active Young Adult Females**

by

Heather L. Hanson

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

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## LIST OF ABBREVIATIONS

BF	Body Fat
BMI	Body mass index
CHO	Carbohydrate
DEXA	Dual-energy X-ray absorptiometry
EA=EI-EEE	Energy availability=energy intake-energy expended in exercise
EB	Energy balance
EB=EI-TEE	Energy balance=energy intake-total energy expenditure
FFM	Fat free mass
FM	Fat mass
kcal	Kilocalorie
kg	kilogram
mL	Milliliter
O <sub>2</sub>	Oxygen
p	Probability value
r	Correlation coefficient
RDA	Recommended Dietary Allowance
RMR	Resting metabolic rate
SD	Standard deviation
U.S.	United States
USDA	United States Department of Agriculture

## **CHAPTER I**

### **Introduction**

In 2010, 69.2% of Americans age 20 years and older were classified as overweight or obese, a percentage that has been on the rise for the past several decades.<sup>1</sup> Excess body fat has physical and psychological health implications, which include coronary heart disease, type-2 diabetes, cancer, hypertension, dyslipidemia, stroke, liver and gallbladder disease, sleep apnea, osteoarthritis, and gynecological problems.<sup>2</sup> The amount and source of energy (calories), and frequency of eating play a role in body weight and composition changes. Consuming smaller meals more frequently throughout the day is thought to decrease appetite, increase metabolism, maintain energy balance, and improve body composition.<sup>3</sup> The actual effects of increasing meal frequency on body composition are unclear, however, and several studies have suggested an inverse association between meal frequency and overweight/obese individuals.<sup>3,4</sup>

The current traditional eating pattern in the U.S. consists of consuming three meals per day; yet, there are data suggesting that individuals who eat smaller, more frequent meals are at a metabolic advantage when compared to those who eat larger, less frequent meals of the same caloric density.<sup>5</sup> Increasing meal frequency to maintain daily energy balance and improve body composition is important for physically active young

adults because body composition can affect an individual's ability to engage in physical activity. A high proportion of fat mass in physically active adults is associated with lower power to weight ratio, reduced acceleration, and increased energy expenditure.<sup>6</sup>

Alternatively, having a body fat mass proportion that is too low can reduce performance, potentially as a result of decreased energy availability.<sup>6</sup> There are studies suggesting that physically active individuals are predisposed to consuming a large volume of energy late in the day, a behavior that may compromise body composition and performance.<sup>4</sup>

However, few studies on physically active individuals have assessed the effects of consuming a high proportion of the daily energy requirements at one meal on within-day energy balance and body composition, and no study has assessed the effect of consuming a large volume of protein in one meal.

Factors influencing a change in body composition have captured the interest of researchers for many years. There is a body of evidence suggesting that a lower number of eating episodes is positively associated with an increase in obesity risks, loss of lean tissue mass, and an increase in the percentage of body fat.<sup>3,4</sup> Conversely, increasing meal frequency may aid in an increase in lean muscle mass, decrease the risk of becoming obese, and help regulate total energy intake throughout the day.<sup>3,4,7</sup> There is also evidence to suggest that an increased meal frequency, in conjunction with a diet that is higher in protein than the RDA of 0.80 g/kg, may result in decreased nitrogen excretion, an indication of lean tissue sparing and enhanced muscle protein synthesis.<sup>8</sup>

The distributions of macronutrients within each meal and the relationship between nutrient utilization and body composition have also been investigated. Research has

shown a positive correlation between body fat percentage and the total consumption of fat, saturated fat, and monounsaturated fat, and a negative correlation between body fat percentage and plant protein, total carbohydrate, and fiber intake.<sup>9</sup> Metabolism of these macronutrients is effected by the number of feedings per day, as research indicates that fat oxidation is increased, and fat and carbohydrate oxidation are stabilized with more frequent daily feedings.<sup>10, 11</sup>

The investigation of total daily protein has gained popularity due to its potential role in the improvement of body composition. Several studies have assessed protein intake and distribution in a variety of populations including overweight/obese, the elderly and healthy individuals. Research has determined that the consumption of a high protein diet when accomplishing weight loss can not only preserve, but also increase lean body mass over time.<sup>12, 13</sup> Also, higher intakes of protein more frequently throughout the day can decrease total body and abdominal fat, and increase postprandial thermogenesis and leptin, a hormone responsible for producing a reduction in hunger sensations.<sup>13</sup> The distribution of protein throughout the day has also been increasingly considered for its effects on body composition. More evenly 24-hour distribution of protein has been shown to increase muscle synthesis rate and decrease muscle catabolism, resulting in decreased muscle loss.<sup>14, 15</sup>

Meal timing and within-day energy balance may also play an integral role in fluctuations in body composition. Within-day energy balance deficits have been positively associated with body fat percentage, while energy balance surpluses are associated with a lower amount of body fat percentage.<sup>16</sup> Consuming a high fat diet during a low energy expenditure circadian phase has been associated with increased

weight gain and fat mass percentages. Similarly, greater fat intake at night may be associated with higher body fat percent, BMI, and waist circumference, whereas food intake in the morning is associated with lower levels of these same anthropometric values.<sup>17</sup>

Studies that have assessed the relationship between nutrition and weight have typically utilized overweight and/or obese populations, or have minimized the potential impact of physical activity on body composition. Therefore, it is important to investigate the relationship between fluctuation in within-day energy balance and body composition in a physically active population. The purpose of this study will be to assess if consuming disproportionately large amounts of energy and protein at any one meal negatively affects body composition in young adult, physically active females. It is hypothesized that individuals who consume disproportionately large amounts of energy and protein at one meal, as opposed to a more even distribution throughout the day, will have a higher body fat percentage, higher fat mass, and lower lean body mass. Finding a relationship between eating frequency and the resultant within-day energy balance will provide valuable information that can help physically active adults improve body composition, and their ability to engage in moderate to vigorous physical activity without fear of compromising lean body mass.

### ***Hypotheses***

H<sup>1</sup>. Consuming a high proportion of daily calorie requirements in one meal is associated with a high percentage of body fat and lower lean tissue mass in physically active females age 19-30 years.

$H^1$  null. Consuming a high proportion of daily calorie requirements in one meal is not associated with a high percentage of body fat and lower lean tissue mass in physically active adults age 19-30 years.

$H^2$ . Consuming greater than 1/3 of daily protein requirements in one meal is associated with a high percentage of body fat in physically active females age 19-30 years.

$H^2$  null. Consuming greater than 1/3 of daily protein requirements in on meal is not associated with a high percentage of body fat in physically active adults age 19-30 years.

## CHAPTER II

### Review of Literature

#### *Meal Frequency and Body Composition*

Within-day meal distribution has gained popularity throughout recent years. Many medical and nutrition professionals have come to the conclusion that increased meal frequency can produce beneficial effects in body composition. It has been reported that consuming smaller meals more frequently throughout the day, as opposed to larger meals less often, can decrease appetite, increase metabolism, maintain energy balance, and result in improved body composition.<sup>3</sup> The effects of increasing meal frequency on body composition remain unclear. However, there have been several recent studies suggesting an inverse association between meal frequency and overweight/obese individuals.<sup>3,4</sup> Therefore, the investigation of meal frequency and its effect on body composition is often carried out in overweight or obese populations.

Data from the Seasonal Variation of Blood Cholesterol Study were used to assess the relationship between eating patterns and obesity.<sup>18</sup> Individuals who were included in this study were men and women between the ages of 20-70 years who met the following criteria: 1. Not taking cholesterol-lowering medications; 2. Not currently on lipid lowering or weight control diets; 3. Free from possible causes of secondary

hypercholesterolemia; 4. Not working a night shift job; 5. Free from chronic illnesses such as cancer, renal disease, and heart failure. The study participants were seen for an initial baseline interview and then every three months over the next year. During each three-month review, body weight and blood samples were collected. The participants also completed three 24-hour dietary recalls including assessments of food intake on two weekdays and one weekend day selected randomly, within 42 days surrounding each clinic visit (from -28 to +14 days). The study found that the number of eating episodes was inversely associated with the risk of obesity. Participants who reported eating four or more times per day experienced a 45 percent lower risk of obesity when compared to participants who ate 3 or fewer times per day. The study also determined that participants who regularly skipped breakfast had 4.5 times the risk of obesity when compared to those who regularly consumed breakfast, which was a statistically significant association.<sup>3</sup>

The association between meal patterns and overweight/obesity and metabolic syndrome has also been assessed. A population-based sample of 16-year-old males and females from The Northern Finland Birth Cohort 1986 were used to determine if an association between breakfast consumption and meal frequency and obesity and metabolic syndrome exists.<sup>19</sup> At the 16 year follow up, the parents and children filled in self-administered food frequency questionnaires to examine three different meal patterns: five meals including breakfast,  $\leq$  four meals per day including breakfast, and  $\leq$  four meals per day without breakfast. The participants were clinically assessed one time by a trained nurse, who obtained anthropometric measurements, blood pressure, and drawn

blood samples to fasting plasma glucose and serum lipid concentrations. Meal consumption was assessed from the question “Do you usually have the following meals: breakfast, lunch, snack, dinner, and evening snack on weekdays?” which was answered as only “yes” or “no.” The questionnaires were then categorized into five meals including breakfast,  $\leq$  four meals per day including breakfast, and  $\leq$  four meals per day without breakfast for analysis. Adolescents who ate five meals per day were at lower risk for overweight/obesity, abdominal obesity, and hypertriglyceridemia after adjustment for early life factors. The five meals per day eating pattern was also associated with decreased risks of overweight/obesity and abdominal obesity in boys after the adjustment of later childhood factors.<sup>19</sup>

The investigation of meal frequency in a similar population determined that reducing the number of meals to three per day in older school aged boys, and even more so in older school aged girls, led to an increased tendency to form and deposit fat reserves when compared with equal aged individuals who were given five to seven smaller meals per day.<sup>20</sup> The study purpose was to determine whether the frequency of food intake has a significant effect on body weight and changes in skinfold thickness in children and adolescents. The study was conducted over a one-year period, and utilized 226 boys and girls aged six to sixteen who were living in three Prague boarding schools. Three schools were selected to be involved in this study, and each was assigned a particular dietary pattern. School A provided three meals per day, school B provided seven meals per day, and school C provided five meals per day. A dietitian visited the schools each week and provided menu guidelines that were the same for all three schools. Growth of these

children was evaluated according to Kapalin's standards based on average values for Czech children. The deviation from ideal weight and height corresponding to sex and age were assessed in every child at the beginning and end of the experiment, and these changes were expressed by sigma values. Skinfold thickness was assessed using calipers. Boys ages 11-16 years and girls ages 10-16 years had a significantly greater percentage of weight-height proportionality change in favor body weight when given three meals per day as opposed to five or seven meals per day. Also, the increment of the skinfold thickness was significantly greater in the children consuming three meals per day when compared with those consuming five or seven meals per day.<sup>20</sup>

In another study investigating meal frequency, 4370 German male and female children ages 5 and 6 were assessed for stature and weight.<sup>21</sup> The participants were children who were required to attend a school entry health examination in local public health offices to assess deficits that might influence school performance, such as impaired vision, but can be easily corrected. Parents of 8741 children that were being assessed in six German communities from September 2001 to August 2002 were invited to participate in a self-completed questionnaire as part of their child's mandatory examination. Approximately 80% of these parents completed the surveys, however, only children of German nationality ages 5 and 6 years were included in the study. Further inclusion criteria were full information on anthropometric measurements, meal frequency, and potential confounding factors. The purpose of the study was to assess the relationship between meal frequency and childhood obesity. Trained nurses measured stature and weight with subjects in light clothing. Overweight and obesity were defined

according to sex and age specific BMI cut off points set in place by the International Obesity Task Force, which are equivalent to the widely used cut-off points of 25 and 30 kg/m<sup>2</sup> for adults. The frequency of meals was assessed via the question: “How many meals per day does your child consume?” And Meals were defined given examples that represented meals that are conventionally served on a plate. The potential confounding factors for the association between main meal frequency and childhood obesity included: parental education, parental obesity, watching television or playing video games, physical activity level, breastfeeding, eating snacks while watching television, having main meals alone, child’s consumption of instant food, and smoking during pregnancy. 43.4% of children that participated in this study consumed four meals per day, whereas 39% consumed five meals per day. Only 2.9% of the children had more than five meals each day, and 14.7% of the children had a maximum of three meals per day. Overweight and obesity prevalence decreased by the number of meals per day. When assessed against potential confounders, frequent daily meals was associated with high educational levels, decreased prevalence of having main meals alone, no more than 1 hour of daily television watching, no regular snacking in front of the television, having siblings, non-smoking during pregnancy, and breastfeeding for more than 1 month. Obesity at school entry was most strongly associated with parental obesity, followed by physical activity, watching television, smoking during pregnancy, and snacking in front of the television. Overall, an increased meal frequency was inversely related to the prevalence of childhood overweight and obesity, suggesting that frequent meals may be protective of obesity.<sup>21</sup>

The concept of energy balance has been a basis of research in the field of nutrition for many years. Energy balance can be defined as dietary energy intake minus total energy expenditure ( $EB=EI-TEE$ ). Energy balance is the amount of dietary energy added to or lost from the body's energy stores after the body's physiological systems have done work for the day.<sup>22</sup> Increasing meal frequency may enable improved maintenance of energy balance throughout the day.<sup>14</sup> Forty-two United States National team artistic and rhythmic gymnasts and 20 national and/or internationally ranked middle and long distance runners participated in a study assessing energy balance and body composition. The relationship between energy balance and body composition in elite female gymnasts and runners showed a significant relationship between the number of daily energy deficits that were greater than 300 kcal of energy balance and DEXA derived body fat percent for gymnasts and runners. In other words, the athletes that were in a high calorie deficit several times throughout the day had a higher body fat percentage than the athletes who had fewer or no caloric deficits  $\geq 300$  calories of energy balance. There was also a negative relationship between the largest daily energy surplus and body fat percentage for gymnasts. These data suggest that within-day energy deficits are associated with higher body fat percentage in both anaerobic and aerobic athletes, possibly from an adaptive reduction in resting energy expenditure.<sup>16</sup>

Energy availability can be defined in exercise physiology as dietary energy intake minus the energy expended in exercise ( $EA=EI-EEE$ ). Energy availability is an input to the body's physiological systems and may depict nutritional status of athletes more accurately. Factors for competitive success is sport specific, and athletes engage in

different diet and exercise behaviors that impact energy availability to achieve these factors. For example, in endurance sports, prolonged exercise training greatly reduces energy availability, unless energy intake is increased to replace the energy that has been expended. In contrast, in sports where less energy is expended, dietary restrictions may be more prevalent for reducing energy availability to modify body size and composition. Experimental evidence indicates that athletes should follow diet and exercise regimens that provide energy availabilities of 30-45 kcal/kg of fat free mass per day while training to reduce body size or fatness.<sup>22</sup>

Meal Frequency and distribution throughout the day has been shown to affect total daily energy intake, thus affecting body composition. A study investigating the relationship between altered feeding frequencies, perceived hunger, and subsequent food intake in obese men age 19-56 determined that men who were given one large meal (single preload), as opposed to an isoenergetic meal that was divided into 5 meals (multi preload), consumed more ad libitum after the meal. The study participants were given an isoenergetic meal that was comprised of 70% carbohydrate, 15% protein, and 15% fat. The meal was administered as either a single meal or divided evenly over five meals given each hour. Five hours after the first administration was given, the participants were allowed to eat a test meal ad libitum to determine if a difference in the amount of energy that was consumed between the two eating patterns existed. Participants who were given the single preload meal consumed 27% more energy in the ad libitum test meal compared to the study participants given the multi preload meal despite no significant change in subjective hunger ratings.<sup>23</sup> These findings indicate that consuming one large meal in

comparison to more frequent smaller meals of the same caloric density may facilitate increased subsequent energy intake even if there is no difference in perceived hunger.<sup>23</sup>

In a similar randomized crossover study, intakes of a buffet style meal provided ad libitum after altered feeding patterns was assessed. Participants included fourteen normal weight women, aged 24.4 with a standard deviation of 7.1. The participants were invited to have lunch at the Institut Paul Bocuse Research Center to familiarize them with the environment and the foods that would be used during the study. After the first visit, participants were invited to participate in four other experimental sessions that were separated by at least seven days. The participants reported to the center at 7:00 am in a fasted state, and were then given a 674.8 kcal breakfast, either in one 20 minute long eating episode at 8:00 am or in four 10 minute long identical spaced eating episodes provided every hour. Each subject received the breakfast in one eating episode on two occasions and four eating episodes on two occasions. Participants reported being less hungry and more satiated after consuming the four eating episode breakfast, and the participants consumed 61 less calories during the ad libitum buffet meal. The study supports the idea that an isocaloric increase of eating frequency may contribute to improving appetite control by reducing hunger, decreasing ghrelin concentrations, and reducing fat intake in the subsequent meal.<sup>9</sup>

### ***Macronutrient Distribution and Body Composition***

The distribution of macronutrients within meals or daily has been associated with nutrient utilization and body composition, but the exact relationship is unclear. A randomized crossover study showed a positive correlation between body fat percentage and the total consumption of fat, saturated fat, and monounsaturated fat, and a negative correlation between body fat percentage and plant protein, total carbohydrate, and fiber intake. The study consisted of two experimental conditions in which subjects underwent 36-hour sessions in energy balance in a respiration chamber for measurements of energy expenditure and substrate oxidation. The subjects were given either two meals per day (lunch omitted) or three meals per day. There was no difference in total energy expenditure between the two meal or three meal groups. However, eating three meals as opposed to two meals per day increased 24-hour fat oxidation, and the participants reported increased satiety feelings when given the three meals per day.<sup>10</sup>

Similarly, the relationship between habitual meal timing and subsequent energy intake and appetite was assessed to determine intermeal interval timing based on specific previous macronutrient intakes. The study used a high-fat preload that had been previously shown to be followed by an intermeal-interval of about 2 hours, whereas the high-carbohydrate preload given in this study had been shown to be followed by an intermeal interval of about 1 hour. Participants were twenty healthy, non-smoking, normal males between the ages of 18-31 years. The study protocol consisted of two visits to a clinic separated by at least one week. During each visit the volunteer was isolated

from time cues to eliminate habitual meal patterns so that meal responses were based mainly on physiological cues. At baseline, and in random intervals throughout the day, the subjects completed ratings of hunger, satiety, and desire to eat. Throughout the day the subjects could eat and drink ad libitum from a cooler in the room. The cooler contained one of the two preload drinks, which was consumed entirely. On subsequent meals, large portions of high fat and high carbohydrate food choices were available. The study determined that healthy men with a high habitual meal frequency showed lower 24 hour energy intake and smaller differences in energy intake after macronutrient specific preloads, compared to participants with a low habitual meal frequency, and that the percentage of energy from carbohydrate or fat explained the variation in habitual meal frequency. A relatively high proportion of CHO in energy intake leads to relatively high average blood glucose levels during baselines, and shorter intermeal-intervals. The proportion of CHO in the diet is inversely related to the energy density of food intake and therefore inversely related to energy intake.<sup>24</sup>

The daily number of meals may affect substrate partitioning and weight control due to the effect meal frequency has on postprandial glucose and insulin responses. Munsters et al investigated the effects of meal frequency on substrate partitioning and metabolic profiles in 12 lean healthy males who were less than 40 years of age. The study included two intervention periods that lasted 36 hours in the respiration chamber. All of the subjects randomly received the same diet with a low meal frequency (3 meals) or a high meal frequency (14 meals). Both meal groups contained 2400 had a macronutrient composition of 15% protein, 30% fat, and 55% carbohydrate. The low meal frequency

intervention included breakfast, lunch, and dinner distributed at 08.00h, 12.00h, and 17.00h, respectively. The high meal frequency intervention included an eating episode every hour between 08.00h and 21.00h. Physical activity was carefully controlled while participants were in the respiratory chamber and included three times of stepping for 15 minutes each time. Blood was sampled just before the consumption of the first meal, 30 minutes postprandially, and then every hour until 21.30 hours to determine plasma insulin, glucose, free fatty acids, and triglycerides. Glucose and insulin profiles showed greater fluctuations in the low meal frequency intervention. There were no observable differences in fat and carbohydrate oxidation between the two interventions, however, protein oxidation and RMR were significantly increase in the low meal frequency intervention. These findings indicate that a low meal frequency could result in the breakdown of muscle mass which can compromise lean body composition.<sup>25</sup>

The metabolism of macronutrients is potentially influenced by the pattern of food intake.<sup>11</sup> The effect of meal frequency on human energy expenditure and its components was assessed in a study of eight young adult males using two separate meal patterns. For two weeks, daily intake was consumed at two meals and the following two weeks, intake was consumed over six evenly spaced meals each day. The participants occupied a whole body calorimeter and follow a prescribed activity pattern for two 31 hour periods while on each diet regimen. There was no discernible effect of meal frequency on energy expenditure in either eating pattern, and the total expenditure in the calorimeter on both regimens was less than the energy intake.<sup>26</sup>

The potential role of macronutrient distribution on body composition has also been assessed in weight loss and control studies. Participants of one study were individuals who had failed to lose weight or maintain weight within the desirable range under outpatient supervision. These individuals were offered a place in a metabolic unit with strict dietary supervision. The study was divided into three experimental categories: High-protein (15% protein-energy) vs. low protein (10% protein energy), frequent meals (5/day) v. infrequent meals (1/day), and high-protein high-frequency vs. low-protein low frequency (combination of experiment one and two). Nitrogen loss was significantly less in the high protein week than the low protein week, and the same results were found in the frequency group compared to the infrequent group, and the high protein high frequency group compared to the low protein low frequency group. The results of this study indicate that higher protein concentrations are more protein sparing, even at low energy intakes. High concentrations of protein in the diet has a significant Nitrogen sparing effect, therefore, since weight loss related to metabolic rate, and metabolic rate to lean body mass, it is appropriate to try to conserve lean body mass during weight loss.<sup>11</sup>

One study determined a connection between energy availability and carbohydrate and fat oxidation. The study was conducted to investigate the independent effects of energy availability and exercise stress on the diurnal leptin rhythm in healthy young women. Leptin is secreted by adipocytes and correlates highly with body mass index, body adiposity, and fat mass. Therefore, it was first hypothesized to signal information about fat stores, however, later reports indicated that profound fluctuations in leptin occur before changes in body adiposity in response to fasting, dietary restriction, refeeding after

dietary restriction, and overfeeding. These findings led to the hypothesis that leptin signals information about dietary energy intake, particularly carbohydrate intake. Young, healthy, habitually healthy, nonsmoking, normal weight women were recruited from Ohio University and the surrounding community to participate in this study. Subjects with a history of menstrual or thyroid disorders, diabetes, or other known health problems, and individuals taking any medications were excluded from the study. Additional inclusion criteria were dietary intake between 35 and 55 kcal/kg of lean body mass per day, a body composition between 18-30% body fat, no recent history of dieting or weight loss, and maximal aerobic capacity of  $<42 \text{ ml O}_2/\text{kg}$  of body weight per minute, and habitual aerobic activity of  $<60 \text{ min/week}$ . For this study, energy availability was defined as dietary energy intake minus exercise energy expenditure. Exercise stress was defined as everything, physiological and psychological, associated with exercise except energy cost. Subjects were assigned to sedentary and exercising groups, and each subject was examined twice, once under balanced energy availability and once under low energy availability. At baseline, balanced energy availability was achieved by administering a dietary intake of 45 kcal/kg of lean body mass per day, and low energy availability was achieved by restricting dietary intake to 10 kcal/kg of lean body mass per day. After two baseline days without treatments, treatments were administered for 4 days beginning on *days 5, 6, or 7* of the menstrual cycle. In the exercising group, the low energy availability was achieved by administering a dietary energy intake of  $I = 40 \text{ kcal/kg}$  of lean body mass per day and an exercise workload of  $E = 30 \text{ kcal/kg}$  of lean body mass per day. The balanced energy availability condition was achieved by raising

dietary energy intake to  $I = 75$  kcal/kg of lean body mass per day in compensation for the same exercise workload. To assess 24-h energy expenditure, subjects wore an activity monitor during all waking hours, except while showering, throughout the experiment. When the sedentary women were administered the balanced energy availability treatment, a significant diurnal rhythm was detected in each subject. After the balanced energy availability treatment, leptin concentrations were substantially higher at the end of the 24-h frequent blood sampling period than at the beginning. This occurred in both the sedentary and exercising women although the increase was approximately twice as great in the sedentary women. After the low energy availability treatment, leptin concentrations were also higher in the sedentary women at the end of the frequent blood-sampling period; however, this rise had no effect on the change in 24-h mean leptin levels in either group. When comparing exercising women (X) to sedentary women (S) at the same energy availabilities, exercise stress had no suppressive effect on either the 24-h mean or the amplitude of the diurnal leptin rhythm. Furthermore, low energy availability strongly suppressed both the 24-h mean and amplitude of the diurnal rhythm of leptin. Low energy availability blunted the amplitude of the leptin rhythm by  $>10\%$  in all seven sedentary women, however, the rhythm was maintained in all nine of the exercising women during the low energy availability treatment and the amplitude was blunted by  $>10\%$  in only two. In summary, low energy availability can suppress 24-h mean and amplitude of the diurnal rhythm of leptin. The effects of low energy availability by exercise energy expenditure were smaller than the effects of low energy availability caused by dietary restriction. Both balanced energy availability treatments in this

experiment provided ~1,000 kcal/day of carbohydrate availability, but skeletal muscle altered its fuel selection in response to the low energy availability treatment, oxidizing less carbohydrate and more fat during exercise. As a result, carbohydrate availability was 57% higher in women whose energy availability was reduced by exercise than in our women whose energy availability was reduced by dietary restriction. Thus, the smaller effects of low energy availability on the diurnal rhythm of leptin in exercising women as opposed to women partaking in dietary restriction may be explained by a greater availability of glucose to adipose tissue.<sup>27</sup>

### ***Meal Timing and Body Composition***

Limited information has been revealed about the best times of day to consume meals. It has been indicated that certain physiological functions such as the rate of gastric emptying, intestinal blood flow, hormonal responses to food intake, insulin sensitivity, and glucose tolerance are less efficient at night and more efficient in the morning hours of the day.<sup>28, 29, 30, 31</sup> For example, one study determined that eating earlier in the day could reduce the total amount of energy consumed for the entire day because morning hours are associated with higher metabolic and physiological efficiency.<sup>32</sup> Another study examined the association between energy intake in the morning, midday, and evening with body mass index. Three 24 hour dietary recalls were used to assess energy intake and were stratified by time of day: morning 0-11 hours, midday 11-17 hours, and evening 17-0 hours. Participants who consumed  $\geq 33\%$  of their daily energy intake at 12 hours

were less likely to be overweight or obese. Participants who consumed  $\geq 33\%$  of daily energy intake in the evening were two times more likely to be overweight or obese. These findings indicate that higher daily intake at midday is associated with a lower risk of overweight or obesity, whereas higher daily intake in the evening is associated with a higher risk.<sup>33</sup>

Food intake timing may contribute to physiological changes in digestion, absorption, and utilization of nutrients which could help to regulate long term body weight.<sup>17</sup> A rodent study indicated that food intake during a 12 hour light phase is associated with a significant weight gain when compared with mice fed during a 12 hour dark phase.<sup>34</sup> This hypothesis suggests that obese individuals consume most of their energy intake in the latter half of the day when compared to their leaner counterparts.<sup>34,35,36</sup> There has also been research showing that night workers consuming a majority of their food at night have a greater risk of becoming obese.<sup>37,38,39</sup>

Dattilo et al. (2011) used three-day food records and anthropometric data to evaluate the correlation between distribution of energy and macronutrient intake and body composition in healthy men and women. The study determined that there was a positive correlation seen in men between night fat intake and body mass index, body fat percentage, and waist circumference. Negative correlations were seen between morning energy and macronutrient intake and body mass index, body fat percentage, and waist circumference. These results suggest that fat intake at night is associated with higher

body mass index, body fat percentage, and waist circumference, while morning food intake is possibly associated with lower anthropometric values.<sup>17</sup>

Meal timing and frequency are also important factors to consider when examining physical performance. Increasing meal frequency can allow physically active individuals to increase their energy intake to optimize fuel availability during training sessions. Increased carbohydrate intake can provide fuel for exercising individuals and promote muscle glycogen re-synthesis after exercise. Appropriate timing and frequency of carbohydrate consumption can optimize fuel availability and enhance exercise capacity. Timing of this consumption is crucial for its effectiveness. Pre- exercise nutrition can be used to optimize liver glycogen stores, avoid GI discomfort, and avoid prevent the possibility of rebound hypoglycemia. Carbohydrate consumption in the first two hours after exercise has been shown to produce the highest rate of muscle glycogen synthesis, whereas carbohydrate consumption delayed for 2 hours after exercise had stopped produced a lower muscle glycogen synthesis rate. The repletion of muscle glycogen synthesis has been found to be unaffected by the frequency of food intake as long as the total amount of carbohydrate consumed after exercise is sufficient. These findings indicate that the timing of intake are crucial determinants for optimizing exercise capacity which could result in an improved body composition.<sup>40</sup>

### ***Eating Frequency and Hormones***

Eating frequency may also play a role in the regulation of certain hormones that have an effect on body composition. The hormone ghrelin is responsible for controlling

appetite and can be affected by the types of foods eaten and frequency of eating episodes.<sup>41</sup> For example, there are data that suggest large intakes of refined or simple carbohydrates may result in hyperinsulemia which can fail to shut down the ghrelin hormone.<sup>42, 43, 44</sup> The continued presence of ghrelin can result in greater food consumption because appetite has not been properly controlled.<sup>41</sup> An infrequent eating pattern that allows for a drop in blood sugar levels can also be a culprit for a hyperinsulinemic response at the next eating opportunity.<sup>45, 46</sup> Eating an excessively large meal will result in excess insulin production which can lead to improper maintenance of the hormone ghrelin.<sup>47, 48</sup> Reduced meal frequency can influence leptin and ghrelin in unexpected ways. Larger portion sizes, consumption of foods with hidden fats, and decreased meal frequency influence ghrelin and leptin and also contribute to obesity.<sup>49</sup> Decreased meal frequency in particular is associated with increased daily energy intake, possibly resulting from an up-regulation of appetite and increased fat consumption.<sup>50, 51</sup>

Additionally, infrequent eating episodes and the consumption of excessively large meals results in higher body fat storage even if the total caloric intake is equivalent to a person who has spread the energy intake evenly throughout the day. This is thought to be a consequence of greater insulin production subsequent to large bolus meals and blood sugar drops.<sup>52, 16</sup> Insulin, blood sugar, and leptin are better controlled with smaller, more frequent meals that adequately sustain energy balance.<sup>53</sup> If the need for more energy is not satisfied and blood sugar drops, the body will go into a state of gluconeogenesis, breaking down lean tissue mass to sustain normal body functions.<sup>41</sup>

### ***Total Protein and Protein Distribution and Body Composition***

Increased dietary protein has been shown to promote favorable changes in body composition in several studies. Various studies have assessed the effects of total protein intake and protein distribution on body composition. For example, in a 12-week study assessing Normal vs High Protein weight loss diet, men randomized to the high protein diet group lost less lean body mass than the normal protein group. The high protein diet consisted of 25% calories from protein and the normal protein diet consisted of 15% of calories from protein and contained 750 kcal less than daily energy needs.<sup>12</sup> The men in both diet groups lost comparable body weight and fat, however, the consumption of the higher protein diet preserved lean body mass over time.<sup>12</sup> Increasing protein intake has also been recognized as a more effective method for reducing body fat percentage when compared to a high carbohydrate diet in overweight and obese individuals.<sup>54</sup>

Similarly, increased protein intake has been shown to decrease total body fat, abdominal fat, increase lean body mass, and increase postprandial thermogenesis. This study involved randomizing 30 overweight individuals into three groups: two high protein groups (35%) consumed as three meals or six meals and one group consumed three meals per day of traditional protein intake (~15%) to compare the effects of protein distribution on abdominal fat, postprandial thermogenesis, and cardiometabolic markers during 28 days of energy balance and deficit. Subjects began consuming their respective diets after a 5-day baseline control period. The diets were consumed throughout a 56 day

intervention consisting of 2, 28 day phases. The first phase was an energy balance phase, which was followed by an energy deficit phase (75% of needs). Body weight remained stable throughout the control and balance periods in all groups. Body fat and abdominal fat decreased in the high protein groups and lean body mass and leptin increased in the high protein 6 meals per day group. Body weight decreased in all groups during the deficient period. Also during this period, body fat, abdominal fat and leptin decreased in both high protein groups. Lean body mass and postprandial thermogenesis were highest in the high protein six times per day group, and the consumption of increased protein more frequently throughout the day was shown to favorably affect adipokines more than current recommendations for macronutrients consumed over 3 meals per day in overweight individuals.<sup>13</sup>

Engaging in physical activity, whether it be endurance or resistance training, results in a subsequent increase in muscle anabolism. Therefore, it is important to properly nourish the body after exercise to promote muscle protein synthesis. The amount of protein and the timing of protein intake are important considerations for maximizing the rate and duration of muscle protein synthesis. Research studies have suggested that 6 to 10 grams of essential amino acids immediately following exercise is the most beneficial for stimulating maximal muscle protein synthesis.<sup>55, 56, 57</sup>

The current recommended dietary allowance (RDA) of 0.8 grams of protein/kg body mass, was determined to cover basal losses of nitrogen for 97.5% of the population. Several studies have indicated that the RDA established for protein is not enough to meet

the needs of regular exercisers and athletes. Individuals who regularly engage in strength training activities may require more dietary protein in order to synthesize new muscle or repair muscle that has been damaged as a result of this type of training.<sup>58, 59, 60</sup> One study that assessed protein requirements for trained strength athletes determined that protein requirements in these individuals are greater than for sedentary individuals. 13 young Canadian males volunteered to participate in this study. The volunteers were divided into two groups to be assessed for the study. The first group, designated as the “strength athletes,” consisted of habitual exercisers (>4 days/week) that performed >70% of total exercise as circuit weight training to increase strength for >= two months before the study. The second group consisted of sedentary age-matched control subjects. All subjects participated in three, 13 days experiments, with a mean 8 day diet washout period. During each experimental period the subjects were randomly assigned dietary protein at one of three levels: Low protein (0.86 g/kg), moderate protein (1.4 g/kg), and a high protein (2.4 g/kg). The diets were designed to meet the habitual caloric intake of each volunteer which was determined from weighted food records collected immediately before the study. All subjects completed the three 13 day experimental periods. Each period consisted of 6 days in which the subjects were provided with food exchanges to match the protein and energy intake of that particular phase, a 4 day meat free period, and a 3 day nitrogen balance period. The volunteers were instructed to strictly follow each diet and were asked to record consumption of all foods and liquids. The sedentary group maintained their physical inactivity throughout the study and the strength athletes performed their habitual circuit training routine. Nitrogen balance was assessed via urine

assay and whole body protein synthesis, and leucine oxidation was determined from leucine turnover. The study determined that the low protein diet did not provide adequate protein for the strength athletes and resulted in a decrease in whole body protein synthesis. The moderate protein diet resulted in an increase in whole body protein synthesis in the strength athletes. The high protein diet did not result in increased whole body protein synthesis, however leucine oxidation significantly increased indicating nutrient overload. The low protein diet provided adequate protein for the sedentary group and the moderate and high protein diets did not increase whole body protein synthesis. These findings indicate that protein requirements for strength training individuals are greater than the requirements of sedentary individuals, and that these requirements are higher than the current US and Canadian recommended daily protein intake requirements.

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Endurance exercise may also promote increased protein needs due to the associated increase in amino acid oxidation, specifically leucine, from this type of training.<sup>61, 62, 63, 64, 65</sup> There is an increase in the proportion of carbohydrate oxidation and relative decrease in the proportion of leucine oxidized during acute endurance training. This may result in an absolute increase in amino acid oxidation due to the increase in total energy requirements secondary to intensive endurance exercise. A study by Meredith et al. (2004) was conducted to determine whether endurance trained men consuming enough energy to maintain constant body weight have dietary protein requirements that exceed the RDA of 0.8 g/kg/day and to establish if age had any effect on these protein needs. The study also determined if there was a relationship between

whole body protein turnover, myofibrillar protein turnover, and protein requirements in these men. Twelve physically active men (6 men aged 22-30 years and 6 men aged 48-59 years) that has been training between 2 and 40 years volunteered for this study. All men were assessed via a physical examination and were considered to be in good health. The men lived in a metabolic research unit throughout three 10 day periods that consisted of a synthetic creatinine free diet with varying levels of protein intake (0.6 g/kg, 0.9 g/kg, or 1.2 g/kg). Egg and milk solids made into a drink with water and sugar supplied the dietary protein. The “non-protein energy” was 64% carbohydrates and 36% fat which was supplied by low protein cookies, pudding, and beverages made from wheat starch, corn starch, oil, sugar, and other flavorings. The men were asked to maintain habitual activity and keep a record of their daily exercise. During each diet period, total urine and stool were collected for analysis, and at the end of each period, an amino acid tracer study was conducted to assess whole body protein turnover. Nitrogen balance was calculated for the final five days of each diet period from the difference between daily nitrogen intake and nitrogen losses. Nitrogen measurements in diet, urine, and stool showed that the men were in negative nitrogen balance at a protein intake of 0.6 g/kg/day. The estimated protein requirement for the men in this study was  $0.94 \pm 0.05$  g/kg/day with no effect of age. The study also determined that protein intake affected whole body protein flux and synthesis but not 3-methylhistadine excretion. These findings indicate that habitual endurance exercise is associated with dietary protein needs greater than the current Recommended Dietary Allowance of 0.8 g/kg/day.<sup>66</sup>

Energy restriction is a common practice among female athletes and habitual exercisers that can lead to a relative increase in protein oxidation to satisfy the energy requirement. Protein balance can be improved in young women performing daily endurance exercise by increasing total energy intake. However, studies have indicated that a high protein diet is associated with a decrease in energy intake throughout the day. Weigle et al (2005) showed that a high protein diet consisting of 30% of energy from protein produced a sustained decrease in ad libitum energy intake and related weight loss.<sup>67</sup> Researchers hypothesize that this effect may be due to the highly satiating effect of protein. The protein leverage hypothesis suggests that protein intake is regulated more strongly than energy intake or that protein intake automatically varies with energy intake. Therefore, female athletes and exercisers consuming high protein diets may have an unintentional subsequent decrease in energy intake.<sup>68</sup>

A single-blind crossover study by Martens et al (2013) investigated ad libitum energy intake, body weight changes, and appetite profile in response to protein-to-carbohydrate plus fat ratio over 12 consecutive days and in relation to age, sex, BMI, and type of protein. 79 volunteers participated in this research study. The volunteers consisted of 40 men and 39 women with BMI ranging from 18.2-33.9 and were between the ages of 18-70 years. The volunteers were nonsmoking, consuming >10 alcoholic drinks/week, weight stable, and not using medications or oral supplements. Body weight was measured using a digital balance, and height was measured by using a wall-mounted stadiometer. The volunteers completed questionnaires related to health, smoking behavior, medication, alcohol use, physical activity, anxiety, and liking the of the study meals. The validated

Dutch translation of the Baecke Activity Questionnaire was used to measure habitual physical activity, and eating behavior was analyzed using a validated Dutch translation of the Three-Factor Eating Questionnaire. The study included 3 randomly sequenced experimental conditions that differed in relative protein content of the provided meals. The volunteers consumed all meals ad libitum at the university for 12 consecutive days in each condition. The subsequent test session started 8 weeks after the preceding one with a 6 week washout period. The volunteers were assigned to either a whey-protein group or a soy protein group. The 3 applied conditions consisted of protein content of 5% of energy, 15% of energy, or 30% of energy from protein. All conditions contained 35% of energy from fat, and the carbohydrate content varied depending on the protein set for that group. All food items and the energy density, weight, and volume of the meals were the same between conditions. The meals were served as ready-to-eat to prevent selective consumption of food items within meals, and the volunteers were provided with individual boxes with snack items for ad libitum consumption at home. Urinary nitrogen excretion was measured at baseline, day 5, and day 11 to determine protein intake. Age, sex, BMI, and type of protein did not have an effect on differences in energy and macronutrient intake between conditions. Total energy intake was significantly lower in the high protein condition than in the low protein and normal protein conditions. In conclusion, the study indicated that individuals under ate relative to energy balance from diets containing higher amounts of protein.<sup>68</sup>

The distribution of protein throughout the day has also been increasingly considered for its effects on body composition. The distribution of protein intake

throughout the day has been associated with the rate of muscle synthesis in healthy young individuals. A 7 day crossover feeding study with a 3- day washout period was conducted to examine this association. Changes in muscle protein synthesis in response to isoenergetic and isonitrogenous diets with protein at breakfast, lunch, and dinner distributed evenly (31.5 +/- 1.3, 29.9 +/-1.6, and 32.7 +/-1.6) or skewed (10.7 +/- 0.8, 16.0 +/-0.5, and 63.4 +/- 3.7) were measured. On days 1 and 7, venous blood samples and vastus lateralis muscle biopsy samples were obtained on a 24-h periods. The 24-h mixed muscle protein fractional synthesis rate was 25% higher in the even protein distribution groups. The consumption of a moderate amount of protein at each meal stimulated 24-h muscle protein synthesis more effectively than skewed protein intake towards the evening meal. Therefore, a more even distribution of protein throughout the day can stimulate muscle synthesis and result in improvements in body composition.<sup>15</sup>

The way protein is distributed throughout the day could also to be associated with the action of protein anabolism. This linkage was investigated in an elderly population involving 194 community-dwelling seniors using Food Frequency Questionnaires. Frailty was defined as the presence of at least three of the following criteria: weight loss, exhaustion, low physical activity, low handgrip strength, and slow walking speed. Pre frailty was defined as the presence of one or two of those criteria. Frail participants consumed significantly less protein in the morning (11.9 vs 14.9 vs 17.4%) but more at noon (61.4 vs 60.8 vs 55.3%) than pre frail and non-frail. The conclusions of the study indicate that the amount of protein was not associated with frailty, however the distribution of protein intake was significantly different between frail, pre frail, and non-

frail participants. Elderly individuals who distributed protein intake more evenly throughout the day were less likely to be considered as part of the frail category.<sup>14</sup>

## **CHAPTER III**

### **Methods**

Using an IRB-approved protocol, volunteers for this study were recruited on the Georgia State University campus as well as throughout the Atlanta area. Flyers were distributed via email and around Georgia State's campus to recruit Georgia State University students. Social media and word of mouth were used to inform individuals not currently enrolled at Georgia State University about the opportunity to become involved in this study.

#### Subjects

We recruited 28 females who volunteered to participate in this study. To satisfy our exclusion criteria, the volunteers were free of any metabolic conditions including dyslipidemia, diabetes, cardiovascular disease and hypertension. As part of the inclusion criteria, each female that participated in this study was physically active for at least 150 minutes of moderate physical activity, or 75 minutes of vigorous physical activity each week. Subjects included both Georgia State University students (n=18) and non-Georgia State University students (n=10) who reside in the greater Atlanta area. Subjects ranged

in age from 19-24 years and the racial background of subjects included Caucasians (n=27) and one African American (n=1)

### Data Collection

Subjects were measured for height in inches using a standard wall mount stadiometer. Height was then converted to centimeters for analyses. Body composition and weight were measured using a multi-current 8-mode segmental bioelectrical impedance device (Tanita, Arlington Heights, Illinois USA; Model BC-418). This assessment provided information on subject weight, body fat percent, BMI, total body water, and lean muscle mass, in addition to segmental (trunk, arms, legs) body composition values. The volunteers were instructed to keep a thorough food and activity journal for one day to assess dietary intake and exercise patterns. The volunteers were asked to provide as much detail as possible about the foods and beverages consumed, including brand names, recipes for foods cooked at home, and portion sizes. The information provided was then assessed for hourly and 24-hour nutrient composition and energy balance using NutriTiming®. (NutriTiming® uses the USDA food database version 25.) Exertion was predicted using a relative intensity activity MET value scale that produces multiples of resting energy expenditure, which was obtained from the Harris-Benedict equation. The activity intensity scale is included in Appendix I.

### Statistical Analysis

All statistical analyses were performed using SPSS (version 20.0, SPSS Inc. Chicago, IL) statistical analysis software. Descriptive statistics were reported as mean and standard deviation and included: age in years, height in centimeters, weight in kilograms, body fat percent, fat mass in kilograms, fat free mass in kilograms, fat free mass to height ratio, and total body water in kilograms. Two-tailed Spearman correlations were used to assess the following relationships: Energy Balance and body fat percent, fat mass, fat free mass, and fat free mass to height ratio; total protein intake and body fat percent, fat mass, and fat free mass; protein intake per kilogram and body fat percent, fat mass, fat free mass, and fat free mass to height ratio; ratio of protein to energy balance in the late AM and body fat, fat mass, fat free mass, and a ratio of fat free mass to height; ratio of protein to energy balance in the early PM and body fat, fat mass, fat free mass, and a ratio of fat free mass to height; ratio of protein to energy balance in the late PM and body fat, fat mass, fat free mass, and a ratio of fat free mass to height; ratio of protein to energy balance in the early evening and body fat, fat mass, fat free mass, and a ratio of fat free mass to height; and ratio of protein to energy balance in the late evening and body fat, fat mass, fat free mass, and a ratio of fat free mass to height. Mann Whitney U-Tests (Appendix II-V) were used to compare a variety of independent groups which included: body fat percentage and protein to energy balance ratio in five different time categories throughout the day; Fat free mass in kilograms and protein to energy balance ratio in five different time categories throughout the day; a ratio of fat free mass in kilograms to height and protein to energy balance ratio in five different time categories throughout the

day; and fat mass and protein to energy balance ratio in five different time categories throughout the day. Kruskal-Wallis tests (Appendix VI-IX) were used to compare more than two independent groups to determine differences between body compositions that were categorized by z-scores. Z-scores were assigned for the following variables: body fat percent, fat mass, fat free mass, and fat free mass to height ratio. Z-scores represent the number of standard deviations a datum is above the mean, thus a positive z-score represents a datum above the mean, while a negative z-score represents a datum below the mean. The z-scores for each variable were further divided into three groups to compare scores from different normal distributions. Significance was set at  $p < 0.05$  for all statistical tests.

Energy balance variables included:

1. The ratio of energy in to energy out to produce 24 hour energy balance net.
2. Hours in energy balance of  $\pm 400$  kcal energy balance.
3. Hours in energy balance deficit, which considered to be  $> 400$  kcal below energy balance.
4. Hours anabolic, which is the considered to be the total number of hours each day that the participant spent obtaining energy for growth and development.
5. Hours catabolic, which is considered to be the total number of hours each day the participant spent breaking down complex materials, resulting in the release of energy.
6. Highest energy balance, which represents the highest kcal amount achieved for energy balance.

7. Lowest energy balance, which represents the lowest kcal amount sustained in energy balance.

The energy balance variables were reported as mean and standard deviation. Body composition variables being assessed include percentage of body fat, fat mass in kilograms, fat free mass in kilograms, and a ratio of fat free mass in kilograms to height in centimeters.

## CHAPTER IV

### Results

#### Subjects

Subject characteristics for all study volunteers are shown in Table 1. All study volunteers were female. The average age of the subjects was 21.57 years (min=18, max=24). The mean height of the participants was 169.0 centimeters and the mean weight in kilograms was 63.42. Mean body fat percent of the total body was 23.1%. Mean fat mass for all participants was 14.9 kilograms, while the average fat free mass was 48.56 kilograms. Mean total body water was 35.6 kilograms.

<b>Table 1. Subject Age, Height, Weight, and Body Composition Characteristics (N=28)</b>				
	<b>Min</b>	<b>Max</b>	<b>Mean</b>	<b>Std. Deviation</b>
<b>Age (yr)</b>	18	24	21.57	1.83
<b>Height (cm)</b>	154.94	179.07	169.00	7.12
<b>Weight (kg)</b>	48.64	81.09	63.42	7.14
<b>Body Fat (%)</b>	10.70	41.10	23.08	6.92
<b>Fat Mass (kg)</b>	6.45	33.64	14.86	5.62
<b>FFM (kg)</b>	39.55	59.55	48.56	5.35
<b>TBW (kg)</b>	29.00	43.64	35.55	3.92

Note: FFM= Fat free mass. TBW= Total body water

<b>Table 2. Subject Energy Balance Findings (N=28)</b>		
	<b>Mean</b>	<b>Std. Deviation</b>
<b>Calories In</b>	1769	497.26
<b>Calories Out</b>	2455	315.46
<b>24HrEBNet</b>	-684	434.55
<b>EB Hr <math>\pm</math> 400 kcal</b>	16.89	5.48
<b>EB Hr Deficit</b>	6.46	5.65
<b>EB Hr Anabolic</b>	4.71	5.05
<b>EB Hr Catabolic</b>	19.29	5.05
<b>EB Highest</b>	221	188.12
<b>EB Lowest</b>	-723	336.99
Note: 24HrEBNet= ratio of calories in to calories out to produce 24 hr EB net. EB Hr $\pm$ 400 kcal = Number of hours spent $\pm$ 400 kcal of EB. EB Hr Deficit= Number of hours spent >400 kcal below EB. EB Hr anabolic= number of hours spent in anabolic state. EB Hr catabolic= number of hours spent in catabolic state. EB Highest= Highest average daily peak energy balance. EB Lowest= Lowest average daily energy balance.		

Energy balance (EB) variables represented in Table 2 are defined as: 1. *Calories in* represents the mean number of calories consumed during the day. 2. *Calories out* represents the mean amount of calories expended throughout the day. 3. *24 Hr EB Net* is the ratio of calories in to calories out to produce 24-hour energy balance net. 4. *EB Hr  $\pm$ 400 kcal* represents the mean number of hours spent within  $\pm$ 400 kcal of energy balance. 5. *EB Hr Deficit* represents the mean number of hours spent >400 kcal below energy balance. 6. *Hours anabolic* represents the mean number of hours each day that the volunteers spent obtaining energy for growth and development. 7. *Hours catabolic* represents the mean number of hours each day the volunteers spent breaking down complex materials, resulting in the release of energy. 8. *Highest energy balance* represents the average of the highest kcal amount achieved for energy balance. 9. *Lowest energy balance* represents the average of the lowest kcal amount sustained in energy balance.

The energy balance findings are as follows: mean amount of kilocalories consumed was 1769. The mean kilocalories expended was 2455, thus the average 24 hour energy balance net was -684 kilocalories. An optimal range of energy balance is defined by NutriTiming® as  $\pm 400$  kilocalories of energy balance, based on each individual's needs. The mean number of hours spent in an energy balance range of  $\pm 400$  kcal was 16.9 hours. Energy balance deficit is defined as being greater than 400 kilocalories below energy balance. The mean number of hours spent in an energy deficit was 6.5 hours. The mean number of hours that these participants spent in an anabolic state was 4.7 and the mean hours spent in a catabolic state was 19.3. Therefore, the participants appear to have spent more time breaking down tissue for energy than they spent obtaining energy for growth and development. The mean highest energy balance was 221 kilocalories, and the mean lowest energy balance -723 kilocalories.

	Mean (SD)	Mean (SD)	
Time	EB (kcal)	Protein (g)	EB:Protein Ratio
12am	98 (98.59)	0 (0)	n/a
1am	37 (99.05)	0 (0)	n/a
2am	-24 (99.29)	0 (0)	n/a
3am	-86 (99.86)	0 (0)	n/a
4am	-147 (100.45)	0 (0)	n/a
5am	-195 (125.33)	0 (0)	n/a
6am	-191 (175.86)	3.35 (7.90)	-57.01
7am	-293 (153.38)	1.38 (4.48)	-212.32
8am	-194 (289.14)	8.91 (11.36)	-21.77
9am	-149 (227.45)	5.68 (8.46)	-26.23
10am	-222 (252.20)	1.77 (7.60)	-125.42
11am	-266 (275.40)	2.88 (5.60)	-78.47
12pm	-142 (285.59)	14.42 (16.36)	-9.85
1pm	-184 (339.48)	3.18 (7.51)	-57.86
2pm	-248 (343.81)	4.77 (13.55)	-51.99
3pm	-356 (372.52)	3.88 (8.24)	-91.75
4pm	-415 (370.42)	3.84 (7.49)	-108.07
5pm	-456 (391.16)	4.50 (9.86)	-101.33
6pm	-440 (451.07)	8.70 (14.05)	-50.57
7pm	-362 (372.30)	15.51 (24.47)	-23.34
8pm	-358 (421.48)	7.50 (14.96)	-47.73
9pm	-409 (458.57)	1.77 (3.27)	-231.07
10pm	-436 (486.26)	1.73 (4.75)	-252.02
11pm	-524 (487.78)	0.47 (2.22)	-1114.89

Mean energy balance was negative during all hours of the day except the hours of 12 a.m. and 1 a.m. Negative energy balance began at 2 a.m. and got progressively worse until 8 a.m. which is consistent with the first eating episode of the day. Although the energy balance deficit decreased at this time, the mean energy balance was still negative. This same trend was seen at hours 12 p.m. and 7 p.m. which are times associated with lunch and dinner consumption.

Mean daily protein intake was highest at 8 a.m. (8.91 g), 12 p.m. (14.42 g), and 7

p.m. (15.51 g), which are the times that most of the volunteers consumed breakfast, lunch, and dinner, respectively. These averages indicate a relatively even distribution of protein intake between the lunch and dinner meal, with a slightly lower intake during the first meal of the day. Because the average protein intakes at these times is relatively low, it is important to consider protein intake during the hours surrounding these three time periods to get an accurate idea of average protein intake at meal time. The ratio of energy balance to protein is also shown in Table 3. The more negative ratios are a result of higher energy balance deficits in conjunction with lower protein intake during that hour.

<b>Table 4. Total Daily Protein Intake and Protein Intake/kg (N=28)</b>		
	<b>Mean</b>	<b>Std. Deviation</b>
<b>Total Protein</b>	95.05 g	34.26
<b>Protein / kg</b>	1.51 g	0.59

The mean total daily protein intake was 95.05 grams, and the average protein intake per kg was 1.51 grams per day. This indicates protein intake well above the current RDA of 0.86 g/kg/day for protein. Although the distribution of protein throughout the day was not significantly associated with body composition, total protein intake showed a significant relationship which is indicated in Table 5.

**Table 5. Relationships (Spearman rho) between protein intake and body composition values (N=28)**

		Body Fat (%)	Fat Mass (kg)	FFM (kg)	FFM to Height Ratio
<b>Body Fat (%)</b>	R	1.000	.954	-.465	-.515
	P	n/a	<.001	.013	.005
<b>Fat Mass (kg)</b>	R	.954	1.000	-.218	-.272
	P	<.001	n/a	.265	.162
<b>FFM (kg)</b>	R	-.465	-.218	1.000	.942
	P	.013	.272	n/a	<.001
<b>Total Protein</b>	R	-.285	-.253	.167	1.000
	P	.142	.195	.394	n/a
<b>Protein / kg</b>	R	-.372	<b>-.419</b>	-.037	-.052
	P	.051	<b>.026</b>	.853	.793

Note: FFM= fat free mass

Spearman correlations between protein intake and body composition in all subjects revealed no significant associations between total protein intake and body fat percent, total protein intake and fat mass, total protein intake and fat free mass (kg), or total protein intake and FFM to Height ratio. There was also no significant association between protein consumption per kg and body fat percent, protein consumption per kg and FFM, or protein consumption per kg and FFM to height ratio. There was an inverse association between protein consumption per kg and kilograms of fat mass ( $r=-0.419$ ;  $p=.026$ ), indicating that as protein consumption per kg decreased, kg of fat mass increased.

<b>Table 6. Relationships (Spearman rho) between energy balance variables and body composition values (N=28)</b>					
		<b>Body Fat (%)</b>	<b>Fat Mass (kg)</b>	<b>FFM (kg)</b>	<b>FFM to Height Ratio</b>
<b>Body Fat (%)</b>	R	1.000	.954	-.465	-.515
	P	n/a	<.001	.013	.005
<b>Fat Mass (kg)</b>	R	.954	1.000	-.218	-.272
	P	<.001	n/a	.265	.162
<b>FFM (kg)</b>	R	-.465	-.218	1.000	.942
	P	.013	.265	n/a	<.001
<b>FFM to Height Ratio</b>	R	-.515	-.272	.942	1.000
	P	.005	.162	<.001	n/a
<b>24HrEBNet</b>	R	-.164	-.199	-.051	-.030
	P	.403	.311	.797	.879
<b>EB Hr ±400 kcal</b>	R	-.160	-.167	-.055	.011
	P	.417	.396	.780	.955
<b>EB Hr Deficit</b>	R	.229	.227	-.038	-.092
	P	.241	.245	.847	.640
<b>EB Hr Anabolic</b>	R	-.232	-.175	.250	.312
	P	.234	.373	.199	.106
<b>EB Hr Catabolic</b>	R	.232	.175	-.250	-.312
	P	.234	.373	.199	.106
<b>EB Highest</b>	R	-.342	-.280	.319	<b>.402</b>
	P	.075	.148	.098	<b>.034</b>
<b>EB Lowest</b>	R	-.130	-.174	-.114	-.088
	P	.510	.375	.562	.658

Note: FFM=Fat free mass. 24HrEBNet= ratio of calories in to calories out to produce 24 hr EB net. EB Hr ±400 kcal = Number of hours spent ±400 kcal EB. EB Hr Deficit= Number of hours spent >400 kcal below EB. EB Hr anabolic= number of hours spent in anabolic state. EB Hr catabolic= number of hours spent in catabolic state. EB Highest= Highest average daily peak energy balance. EB Lowest= Lowest average daily energy balance. R= correlation coefficient; P=probability

There was a significant association between highest daily peak energy balance and FFM to height ratio. Highest daily peak energy balance is considered the highest kcal amount achieved for energy balance. Therefore, this finding indicates that highest kcal amount accomplished during energy balance is associated with increased lean tissue

mass.

	<b>Mean</b>	<b>Std. Deviation</b>
<b>Ratio Protein:EB Late AM</b>	-.13	.48
<b>Ratio Protein:EB Early PM</b>	.01	.14
<b>Ratio Protein:EB Late PM</b>	-.02	.05
<b>Ratio Protein:EB Early Evening</b>	.01	.20
<b>Ratio Protein:EB Late Evening</b>	-.00	.01
Note: EB= Energy Balance		

The average ratios of protein to energy balance from late morning to late evening are represented in Table 6. Late AM is considered to be the hours of 9:00am- 11:00am and the mean protein to energy balance ratio for this time period was -.13. Early PM represents 12:00pm-2:00pm with a mean protein to energy balance ratio of .01. Late PM represents the hours of 3:00pm-5:00pm and had a mean protein to energy balance ratio of -.02. Early evening hours were considered to be 6:00pm-8:00pm with a mean protein to energy balance ratio of .01. Late evening presented the hours of 9:00pm-11:00pm with a mean protein to energy balance ratio of -.00.

The average ratios of protein to energy balance from late morning to late evening are represented in Table 6. Late AM is considered to be the hours of 9:00am- 11:00am and the mean protein to energy balance ratio for this time period was -.13. Early PM represents 12:00pm-2:00pm with a mean protein to energy balance ratio of .01. Late PM

represents the hours of 3:00pm-5:00pm and had a mean protein to energy balance ratio of -.02. Early evening hours were considered to be 6:00pm-8:00pm with a mean protein to energy balance ratio of .01. Late evening presented the hours of 9:00pm-11:00pm with a mean protein to energy balance ratio of -.00.

<b>Table 8. Correlation Matrix: Ratio of Protein to Energy Balance and Body Composition (N=28)</b>					
		<b>Body Fat (%)</b>	<b>Fat Mass (kg)</b>	<b>FFM (kg)</b>	<b>FFM to Height Ratio</b>
<b>Ratio Prot:EB 12pm</b>	R	-.016	.066	<b>.390</b>	<b>.423</b>
	P	.934	.740	<b>.040</b>	<b>.025</b>
<b>Ratio Prot:EB 4pm</b>	R	.065	-.019	<b>-.376</b>	-.351
	P	.742	.923	<b>.049</b>	.067
<b>Ratio Prot:EB 9pm</b>	R	-.147	-.021	<b>.379</b>	.374
	P	.454	.914	<b>.047</b>	.050
Note: EB=Energy Balance					

At noon ( $r=0.390$ ;  $p=0.040$ ) and at 9pm ( $r=.379$ ;  $p=0.047$ ) the ratio of protein consumption to energy balance was positively associated with fat free mass which indicates that protein consumption, while in an energy balance range of  $\pm 400$  kcal, is associated with higher FFM. There was also a positive association between the ratio of FFM to height and the ratio of protein to energy balance at 12 pm ( $r=.423$ ;  $p=0.025$ ). Conversely, at 4pm ( $r=-0.376$ ;  $p=0.49$ ) the ratio of protein consumption to energy balance was negatively associated with FFM. This result suggests that as the ratio of protein to energy balance during this hour increases, FFM decreases.

Regression analysis determined that independent EB and protein variables could be used to predict the dependent variable FFM to Height ratio. These independent

variables include: EB Hr  $\pm$  400 kcal, EB Hr Catabolic, Protein/kg, Total Protein, Ratio Protein:EB Late AM, Ratio Protein:EB Early PM, Ratio Protein:EB Late PM. These variables can predict FFM to height ratio using the following equation:

$$\mathbf{FFM/Ht} = \mathbf{EB_{Hr Opt} (.001) + EB_{Hr Cat} (-.001) + Protein/kg (-.082) + Total Protein (.001) + Ratio Protein:EB_{Late AM} (.018) + Ratio Protein:EB_{Early PM} (.032) + Ratio Protein:EB_{Late PM} (-.050) + .304}$$

$$\mathbf{SEE=0.02036}$$

$$\mathbf{R=.727}$$

$$\mathbf{R^2=.529}$$

$$\mathbf{P=.019}$$

## CHAPTER V

### **Discussions and Conclusions**

#### Meal frequency and Body Composition

This study assessed hourly EB for the study volunteers. EB findings indicated that the volunteers were in negative energy balance from 2 a.m. to 12 a.m. Research suggests that more frequent eating episodes may promote better within day energy balance, and previous studies indicate that increased meal frequency is associated with a reduced risk for obesity, decreased abdominal obesity, decreased body fat percent, reduction in total daily energy intake, and improved appetite control.<sup>3,9,14,19,21,23</sup> We found a significant positive association between highest daily energy balance and FFM to height ratio. It is also important to note that many of the study participants were in an extreme energy balance deficit for several hours of the day. This deficit could have influenced the results of the statistical analyses. All of the study volunteers recorded physical activity as part of the food and activity log that was assessed for this study. The energy balance deficits that are evident in this population may not be as extreme on days when these individuals are not physically active, therefore restoring normal energy balance in this specific population.

### Protein intake and Body Composition

Endurance and resistance training stimulates muscle anabolism and the subsequent need to replenish the body with adequate protein to promote muscle protein synthesis.<sup>55, 56, 57</sup>

Studies show that individuals who regularly exercise have higher protein needs than their sedentary counterparts.<sup>60, 66</sup> Increasing protein intake above the current RDA of 0.80

g/kg/day can better meet the needs of this population and also help improve body composition.<sup>60, 66</sup> Total average daily protein consumption in this population was 94.05

grams, and average total protein/kg was 1.51 g/kg. This indicates protein intake well above the current RDA for protein. Total protein intake throughout the day was not

significantly associated with body composition when the subject population was assessed as a whole. However, there was an inverse association between protein intake and FM

when protein intake was assessed per kg of body weight for each subject ( $r = -.419$ ;  $p = 0.025$ ). These results indicate that higher protein consumption per kg is associated with

lower fat mass. This finding is also consistent with the results from other studies which have indicated that increased protein intake is effective for reducing body fat percentage, decreasing abdominal fat, increasing lean body mass, and stimulating muscle synthesis.

<sup>12, 13, 15</sup> Additionally, regression analysis determined that energy balance and protein variables could be used to predict FFM to Height ratio with a low standard error of estimation ( $SEE = \pm 0.02036$ ). The independent variables for this equation included:

Energy balance hours  $\pm$  400 kcal, Energy balance hours Catabolic, Protein/kg, Total Protein, Ratio Protein:Energy Balance Late AM, Ratio Protein:Energy Balance Early

PM, Ratio Protein:Energy Balance Late PM. This regression equation model explains 52.9% of the variability of the response data around its mean. In other words, nearly half of the variance in FFM to height ratio can be explained from the independent variables used in this equation.

### Protein Distribution and Body Composition

Various studies have suggested a relationship between daily protein distribution and body composition. Specifically, Bollwein et al. (2013) determined that the consumption of a moderate amount of protein at each meal stimulates muscle synthesis, which improves body composition.<sup>15</sup> Therefore, we hypothesized that consuming greater than 1/3 of daily protein requirements in one meal is associated with a high percentage of body fat in physically active adults aged 19-30 years. We were unable to reject the null hypothesis, but there were some significant relationships between protein and energy balance found in this study. At noon ( $r=0.390$ ;  $p=0.040$ ) and at 9pm ( $r=.379$ ;  $p=0.047$ ) the ratio of protein consumption to energy balance was positively associated with fat free mass which indicates that protein consumption, while in and energy balance range of  $\pm 400$  kcal, is associated with higher FFM. There was also a positive association between the ratio of FFM to height and the ratio of protein to energy balance at 12 pm ( $r=.423$ ;  $p=0.025$ ). Conversely, at 4pm ( $r=-0.376$ ;  $p=0.049$ ) the ratio of protein consumption to energy balance was negatively associated with FFM. This result suggests that as the ratio of protein to energy balance during this hour increases, FFM decreases. This negative

association could be the result of a high mean energy balance deficit (-415 kcal) along with low mean protein intake (3.84 g) during this hour.

### Limitations

Perhaps the greatest limitation of this study was the size and homogeneity of the population that was assessed. Because there were only 28 volunteers with similar diet and exercise regimens, it was difficult to establish significant relationships or differences from the data. If more individuals had volunteered for the study, we may have been able to better assess our hypotheses, particularly if a greater proportion of the subjects were in better EB during more hours of the day. Another limitation of this study was that the volunteer's diet and physical activity levels were only assessed for one day and were self-reported. One 24-hour food and activity journal may not accurately depict the dietary habits of the volunteer, and further information about the subject's diet and body composition history was not considered. Further research is necessary to determine relationships between protein intake, meal frequency, and body composition because this analysis was primarily based on Caucasian females and may not be generalizable to a broader population.

### Conclusions

The results of this study show a significant positive association between highest daily EB and FFM to height ratio. The results also indicate that higher protein consumption per kg is inversely associated with fat mass in physically active young adult females. Similarly,

adequate protein consumption, in conjunction with an energy balance range of  $\pm 400$  kcal of EB, is associated with higher amounts of FFM at specific times of the day.

Additionally, energy balance and protein variables can be used to predict FFM to height ratio using a regression equation that accounts for 52.9% of the variance in the FFM to height ratio. These findings suggest that, in the assessed population, total protein intake has a greater effect on body composition in physically active individuals than the distribution of protein throughout the day. Additionally, achieving positive within-day energy balance, along with adequate protein intake, may help physically active females achieve a body composition that is low in fat mass and high in lean muscle mass.

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## APPENDIX I

### Relative Intensity Activity MET Value Scale

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

## APPENDIX II

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of HiLoFatPercent.	Independent-Samples Mann-Whitney U Test	.277	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of HiLoFatPercent.	Independent-Samples Mann-Whitney U Test	.120	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of HiLoFatPercent.	Independent-Samples Mann-Whitney U Test	.355	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of HiLoFatPercent.	Independent-Samples Mann-Whitney U Test	.846	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of HiLoFatPercent.	Independent-Samples Mann-Whitney U Test	.514	Retain the null hypothesis.

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

### APPENDIX III

#### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of HiLoFFMkg.	Independent-Samples Mann-Whitney U Test	.158	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of HiLoFFMkg.	Independent-Samples Mann-Whitney U Test	.890	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of HiLoFFMkg.	Independent-Samples Mann-Whitney U Test	.079	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of HiLoFFMkg.	Independent-Samples Mann-Whitney U Test	.550	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of HiLoFFMkg.	Independent-Samples Mann-Whitney U Test	.343	Retain the null hypothesis.

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

## APPENDIX IV

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of HiLoFFMkgtoht.	Independent-Samples Mann-Whitney U Test	.438	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of HiLoFFMkgtoht.	Independent-Samples Mann-Whitney U Test	.491	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of HiLoFFMkgtoht.	Independent-Samples Mann-Whitney U Test	.248	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of HiLoFFMkgtoht.	Independent-Samples Mann-Whitney U Test	.183	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of HiLoFFMkgtoht.	Independent-Samples Mann-Whitney U Test	.962	Retain the null hypothesis.

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

## APPENDIX V

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of HiLoFM.	Independent-Samples Mann-Whitney U Test	.686	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of HiLoFM.	Independent-Samples Mann-Whitney U Test	.889	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of HiLoFM.	Independent-Samples Mann-Whitney U Test	.162	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of HiLoFM.	Independent-Samples Mann-Whitney U Test	.403	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of HiLoFM.	Independent-Samples Mann-Whitney U Test	.085	Retain the null hypothesis.

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

## APPENDIX VI

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of ThreeCatBFPercent.	Independent-Samples Kruskal-Wallis Test	.356	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of ThreeCatBFPercent.	Independent-Samples Kruskal-Wallis Test	.601	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of ThreeCatBFPercent.	Independent-Samples Kruskal-Wallis Test	.115	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of ThreeCatBFPercent.	Independent-Samples Kruskal-Wallis Test	.265	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of ThreeCatBFPercent.	Independent-Samples Kruskal-Wallis Test	.077	Retain the null hypothesis.

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

## APPENDIX VII

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of ThreeCatFFMkg.	Independent-Samples Kruskal-Wallis Test	.153	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of ThreeCatFFMkg.	Independent-Samples Kruskal-Wallis Test	.999	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of ThreeCatFFMkg.	Independent-Samples Kruskal-Wallis Test	.381	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of ThreeCatFFMkg.	Independent-Samples Kruskal-Wallis Test	.592	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of ThreeCatFFMkg.	Independent-Samples Kruskal-Wallis Test	.702	Retain the null hypothesis.

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

## APPENDIX VIII

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of ThreeCatFFMkgtoht.	Independent-Samples Kruskal-Wallis Test	.223	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of ThreeCatFFMkgtoht.	Independent-Samples Kruskal-Wallis Test	.547	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of ThreeCatFFMkgtoht.	Independent-Samples Kruskal-Wallis Test	.691	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of ThreeCatFFMkgtoht.	Independent-Samples Kruskal-Wallis Test	.577	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of ThreeCatFFMkgtoht.	Independent-Samples Kruskal-Wallis Test	.399	Retain the null hypothesis.

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

## APPENDIX IX

### Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Ratio Protein: EB Late AM is the same across categories of ThreeCatFMkg.	Independent-Samples Kruskal-Wallis Test	.671	Retain the null hypothesis.
2	The distribution of Ratio Protein: EB Early PM is the same across categories of ThreeCatFMkg.	Independent-Samples Kruskal-Wallis Test	.773	Retain the null hypothesis.
3	The distribution of Ratio Protein: EB Late PM is the same across categories of ThreeCatFMkg.	Independent-Samples Kruskal-Wallis Test	.111	Retain the null hypothesis.
4	The distribution of Ratio Protein: EB Early Evening is the same across categories of ThreeCatFMkg.	Independent-Samples Kruskal-Wallis Test	.119	Retain the null hypothesis.
5	The distribution of Ratio Protein: EB Late Evening is the same across categories of ThreeCatFMkg.	Independent-Samples Kruskal-Wallis Test	.241	Retain the null hypothesis.

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