Summer 6-19-2014

The Relationship between Moderate, Within Day Protein Intake and Energy Balance on Body Composition of Collegiate Sand Volleyball Players

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ACCEPTANCE

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- January to May 2013: Graduate Teaching Assistant, provided technological support in an online general nutrition course

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ABSTRACT

Title: The Relationship between Moderate, Within Day Protein Intake and Energy Balance on Body Composition of Collegiate Sand Volleyball Players

Background: Achieving an ideal body composition with relatively low fat mass and relatively high fat-free mass (FFM) is desirable for virtually all competitive athletes. Some studies suggest that protein intake, depending on quality, amount, and timing, may improve relative musculature by stimulating muscle protein synthesis, but some issues related to timing and amount of protein intake remain unclear. Current evidence suggests that frequent consumption of moderate amounts of protein is useful for muscle building.

Purpose: The purpose of this study was to simultaneously assess energy balance and protein intake to determine if these factors are associated with body composition in a population of collegiate sand volleyball players.

Methods: In a cross sectional, observational study, players completed a food intake and activity form for a 24-hour period to serve as the basis of energy balance and protein intake assessment. The assessment day was representative of a typical day during the regular training season. These data were entered into a software program providing total and hourly energy balance and nutrient content of the consumed foods. Athletes were measured for body composition via a multi-current bioelectrical impedance scale to predict weight, BMI, fat mass and fat free mass. Height was measured using a standard wall-mounted stadiometer. Data analyses included descriptive and frequency statistics, Spearman correlations and regression analyses.

Results: Twelve women from the GSU sand volleyball team participated in the study using an IRB-approved protocol. The mean BMI was 22 kg/m^2 (±3 kg/m^2) and the mean body fat percentage was 18% (±7%). The mean protein intake for all participants was 132 grams (±52 g). Protein intake distribution was skewed, on average, toward the latter half of the day with approximately 19% of protein consumed in the morning and 34% consumed in the evening. The mean net energy balance at the end of the 24-hour assessment period was -404 (±385) kcal. Athletes, on average, spent 17 hours in a catabolic energy balance state (< 0 kcal). No significant correlation was found between energy balance per gram of protein consumption and body composition. However, regression analyses indicated that energy balance and protein variables explain a significant proportion ($p=.037$) of the variance in body fat percentage.

Conclusions: Sand volleyball players in this study spent a high proportion of time in a negative energy balance, which may have compromised the potential benefit that frequent protein consumption may have had on FFM. Since both energy balance and protein explain a significant proportion of the variance in body composition, these athletes might

benefit from improving within-day energy balance as a strategy for optimizing body composition.

The Relationship between Moderate, Within Day Protein Intake and Energy Balance on Body Composition of Collegiate Sand Volleyball Players

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

MASTER OF SCIENCE
IN
HEALTH SCIENCE
WITH A CONCENTRATION IN NUTRITION

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2014

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ACKNOWLEDGEMENTS

I'd like to express my heartfelt appreciation for my thesis chair, Dr. Dan Benardot. His vision made this thesis possible and I am truly grateful to be guided by someone who has such a profound passion for research. I would like to acknowledge Dr. Anita Nucci for her enthusiasm and her dedication to helping her students maximize their potential and grow as researchers. Thank you, Dr. Walt Thompson, for hopping on board in the last few months and providing invaluable feedback and encouragement. It was also a pleasure working with Dr. Marugi Dnirangu, to whom I am thankful for the insight and wealth of knowledge. I'd also like to acknowledge Ms. Catherine McCarroll. From the beginning, Ms. McCarroll has put in an endless amount of effort to ensure my curiosities and interests in the field of nutrition are fulfilled. I will be forever grateful for her compassion. Lastly, I'd like to thank my fellow classmates in Cohort XII, a group of individuals that contributed greatly to my most memorable times over the last two years and provided the best support system anyone could ask for. I couldn't be luckier to be part of a group like you all!

Chris, Tracey and Johnny, John, Ben, and Jake, to you six I dedicate this thesis. Without your love and continuing encouragement, I would not be where I am today.

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ABBREVIATIONS

AA	Amino Acid
BMI	Body Mass Index
BCAA	Branched Chain Amino Acid
BW	Body weight
Cm	Centimeter
EA	Energy Availability
EB	Energy Balance
EI	Energy Intake
EAA	Essential Amino Acid
FFM	Fat Free Mass
FM	Fat Mass
FSR	Fractional Synthetic Rate
ISSN	International Society of Sports Nutrition
Kcal	Kilocalorie
Kg	Kilogram
LBM	Lean Body Mass
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
SVP	Sand Volleyball Player

TEE	Total Energy Expenditure
VP	Volleyball Player

Chapter I

INTRODUCTION

Being primarily an anaerobic sport, sand volleyball induces frequent damage to skeletal muscles during play (Buśko et al., 2013). When athletes perform vigorous activity, adequate protein intake is crucial to maintain and/or build muscle mass (West et al., 2011). While it is common for athletes to consume as much as 3 grams of protein per kilogram of body weight (BW) per day, the Recommended Dietary Allowance (RDA) suggests an intake of 0.8-1.0 g/kg per day for the average, healthy individual (Phillips et al., 2007). Studies have shown that the lower end of the RDA's range will not adequately support protein synthesis in a working athlete (Layman, 2009). The Position Statement from the Dietitians of Canada, the American Dietetic Association, and the American College of Sports Medicine recommends a daily protein intake of between 1.2-1.7 g/kg of BW to support muscle use in athletes ("Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and Athletic Performance" 2009).

An athlete's body composition can directly affect their level of performance. A ratio of high lean body mass to fat mass is desirable (Hinton et al., 2004). To achieve this ratio, identifying appropriate protein intake is necessary in addition to routine physical activity. Protein is an important nutrient involved in the synthesis of muscle mass, therefore, optimizing its intake is of interest for the diet of a typical athlete (Phillips et al., 2011). The quality, amount, and timing of protein intake all play a significant role in body composition (Wilborn et al., 2013). The benefits of protein ingestion include an increase in satiety, an increase in thermogenesis and an increase in lean body or muscle mass (Paddon-Jones et al., 2008; Symons et al., 2009). A number of factors influence the synthesis of muscle mass, including: amino acid content of protein, the distribution of fat and carbohydrates, energy availability, timing of protein consumption, and the allotment and portion of consumption. Numerous studies are presently examining the optimal amount and distribution of protein intake for the synthesis of muscle tissue (Symons et al., 2009; Witard et al., 2013; Mamerow et al., 2014).

In addition to the optimal amount of protein intake in athletes, how an athlete should distribute the consumed protein across the day remains unclear in current literature. Studies have found that in athletics, higher frequencies of protein intake are more beneficial for an ideal body composition than consuming larger amounts less often (Phillips et al., 2007). In a regularly trained athlete, protein is consistently being utilized to heal damaged muscle and increase muscle cell size. While frequent exercise increases lean body mass, it is the proper intake of protein that is directly related to the increase in muscle protein synthesis (MPS) (Layman et al., 2005). Some athletes report having protein intakes as high as 175 grams per day, believing this amount is necessary to increase muscle mass (Paddon-Jones et al., 2008; Fox et al., 2011).

PURPOSE AND HYPOTHESIS

Recent studies suggest that frequent intakes of protein consumption in moderate amounts over the course of the day is associated with a higher lean body mass than similar amounts of protein consumed less frequently and in larger amounts. Therefore, the goal of this study is to assess if different protein intake patterns and the ratio of energy balance to protein intake in female collegiate sand volleyball athletes are associated with different body compositions. In addition, factoring in energy balance (EB) will be helpful in determining if the participant's protein intake is effective in increasing muscle mass when considering energy availability at the time of consumption.

Hypothesis: Athletes consuming meals with 25g of protein frequently throughout the day have a higher fat-free mass and a lower body fat percentage than subjects consuming larger amounts of protein less frequently throughout the day.

Null: Athletes who consume meals with 25g of protein frequently throughout the day will not have a higher fat-free mass and a lower body fat percentage than subjects consuming larger amounts of protein less frequently throughout the day.

Chapter II

LITERATURE REVIEW

Through intensive training, strict diet regimens, and commitment, competitive athletes will take various routes to achieve success. In sand volleyball, a number of factors play into that success rate such as finding the ideal body type for each position, meeting and exceeding the physiological demands of play, and following strict dietary and exercise routines to get there. One of the most commonly argued topics of an athlete's diet is adequate protein intake. The following is a review of the current literature covering the ideal body types of sand volleyball players (SVPs), the typical dietary habits of female athletes, and the evidence behind the effect of varying amounts and distribution of protein intake on body composition. Energy balance is also taken into account as it directly affects the role of consumed protein on muscle synthesis and breakdown.

Ideal Traits of Sand Volleyball Players

A higher lean body mass to fat mass ratio is an ideal quality of an athlete. Lidor et al. (2010) found that more successful volleyball players (VPs) averaged three kilograms(kg) less fat mass than those VPs who are less successful (Lidor et al., 2010). A higher fat free mass (FFM) to fat mass ratio can contribute to both anaerobic power and aerobic capacity. Volleyball is a largely anaerobic sport and typically that anaerobic power is positively associated with high lean body mass (Buśko et al., 2013; Anderson 2010). `

Sand volleyball is one of many sports that are more successfully played with specific body types (Martín-Matillas et al., 2013). In addition to muscle mass, the top team players are typically characterized by tallness and longer arm span (Martín-Matillas et al., 2013). Beyond the benefit of height, certain physical attributes will vary from position to position. An individual player will have unique characteristics to excel in that position and maximize the efficiency of the team. A volleyball team is made up of five positions: setter, opposite, center, hitter and libero (Martín-Matillas et al., 2013). In attempt to classify ideal body types for each volleyball position, Matillas et al. (2013) assessed 148 female players from the highest Spanish league. Anthropometrics, body composition and somatotypes were measures based on performance and playing positions. Overall lean body mass is ideal for enhanced performance, but skills and tactics also play a role. According to the study findings, players on the national level were typically taller, heavier and had a relatively higher percentage of lean body mass. In comparing position to position, setters are usually lighter, shorter and have less lean body mass. Opposites and centers have higher muscle mass than setters and liberos. Liberos have higher mesomorphy and lower ectomorphy (Martín-Matillas et al., 2013). Centers are typically more endurance trained while setters are fast and agile (Buśko et al., 2013).

A study by Buśko et al. (2013) examined 14 Division II female volleyball players and assessed various body types and physical attributes and their relevance to success. The participants in the study were college aged, averaging 21 years old. According to the findings, the highest performing volleyball players tend to be taller, more muscular and have a higher jumping ability, and velocity. Other ideal characteristics included hand eye coordination, strength and elevation to block, speed, spiking ability, endurance, and technical ability to understand strategy of the game (Buśko et al., 2013). It is with extensive training and practice, that players are able to achieve these ideal traits.

Physiological Demands of Sand Volleyball

One goal of athletic training is to generate physiological and physical changes in an effort to reduce fatigue and increase physical ability specific to the sport. In a typical game of volleyball, energy is expended through short and frequent bouts of high-intensity exercise with low intensity periods and recovery time in between. Performance in these highly intense moments includes jumping and reaching (Sheppard et al., 2008). The extensive training for college and elite level volleyball players contributes to a naturally greater amount of energy expenditure in addition to increased muscle mass (Eliakim et al., 2013). In a single volleyball match, numerous myofibrallar injuries to the muscles will take place and in turn, synthesize muscle proteins (Pilaczyńska-Szcześniak et al., 2011).

Playing sand volleyball takes more strength and endurance than playing volleyball on a hard court (Muramatsu et al., 2006). Unlike indoor volleyball, sand and beach volleyball are typically performed outside in the heat of the sun. Matches require constant motion with jumping and running on the sand, which alone require more energy expenditure than on hard surface (Pilaczyńska-Szcześniak et al., 2011).

To compare the output in sand to hard surfaces, a study measured energy expenditure on sand versus hard surfaces in eight male university volleyball players (Muramatsu et al., 2006). The participants performed three sets of ten repetitive jumps on sand and on a hard platform, jumping every two seconds during a set with 20 seconds in between. Energy expenditure of jumping is 1.2 times greater on sand than hard surface, 37 kcal on sand and 31.2 kcal on firm. The ankle extension, especially, on sand creates energy loss with a lower contribution of plantar flexion. As a result of the expended energy, oxygen requirement is higher on sand than on firm surfaces. Overall results of this study showed that jump height on sand surfaces were not as high as on firm surfaces proving the difficulty to gain height on the sand. With the uneven level of sand and the lack of resistance, the difficulty of running and jumping requires more exertion and power, leading to greater expenditure (Muramatsu et al., 2006).

To research the anabolic response in physically intense sports such as sand volleyball, Eliakim et al. (2013) measured hormone levels during the first seven weeks of training in national level female volleyball players (N=13). The study group had an average age of 16 years old. Measurements were taken during the first seven weeks of the volleyball training season. Training included both endurance and resistance exercises along with speed drills and technical drills. Fitness was measured by vertical jumps, anaerobic capacity and aerobic power. Growth hormone, insulin-like growth factor (IGF), IGF-binding protein, cortisol and pro and anti-inflammatory markers were measured via blood tests. The blood samples were collected before and after a sixty-minute volleyball practice session. Results from the tests showed a dominance of anabolic response in the sample population of national level volleyball players. The changes in hormone levels in response to training show exercise-related anabolic adaptations. Interlukin-6 was one hormone that when increased, may indicate muscle damage repair. Due to these increases, training is shown to reduce the catabolic and inflammatory response to exercise. These results imply that training improves fitness and increases muscle mass in addition to improving hormonal balances (Eliakim et al., 2013).

Nutritional Habits of Female Athletes

Building lean body mass (LBM) is not only brought on by exercise, but is also influenced by a proper diet (Pilaczyńska-Szcześniak et al., 2011). An extensive amount of research has been done to find the 'gold standard' of nutrient requirements for enhancing performance in athletes. One review evaluates the best methods to achieve maximum performance in athletes. The review's findings are showing that energy needs vary, depending on the sport and the athlete. Those who exercise three to five times per week at 30 minutes a session are typically satisfied with 1,800 to 2,400 kcals per day on average. Elite athletes, on the other hand, may need anywhere from 6,000 to 12,000 kcals per day. In the context of protein, findings show that intensely trained athletes are allowed up to two times the RDI of protein, which is equivalent to five servings of lean meat per day (Teta et al., 2013). The extensive training in elite athletes create an increased need for both excess calories and protein, providing that nutritional habits will vary based on the lifestyle and athletic performance of the individual.

Energy consumption of sand volleyball players is researched to be lower than other competitive sports. In the case of this study by Papadopoulou et al. (2002), high school aged athletes were evaluated to find the average macronutrient consumption.

High school aged teenagers and adolescents are likely to have similar caloric intake levels to college-aged individuals (Beals, 2002). Evidence from Papadopoulou et al. (2002) provides that sand volleyball players, ages 14 to 19 years old had an intake of less than the RDA for active women (44 kcal/kg) and less than other female athletes in track and field, swimming, rowing, gymnastics, figure skating and running. The adolescent's carbohydrate intake was also low, at 45% of calories, below the recommended 3 g/kg. Fat intake was higher than recommended in all populations. More results from the study show a minimal intake of 1 g of protein per kg of body weight, averaging 62 grams of protein per day. This amount reflects 6% of the protein-related energy intake to expenditure. Protein should provide 10% of needs in endurance sports. Growth spurts are also considered in this adolescent and young adult population. To take growth into account, the 10% recommendation would increase to 12-15% for this particular study population (Papadopoulou et al., 2002).

Depending on the sport, athletes are likely to consume similar energy intakes to their teammates. Taking the ideal body type for the sport into consideration, intakes will vary (Beals, 2002). Gymnasts, for example, are typically of lean body stature while swimmers have a broader and more muscular build. Beals (2002) compared energy intakes in various sporting teams, following female sand volleyball players, ages 14 to 17 years old as they self-reported their diets. The study measured estimated energy needs per athlete and analyzed their self-reported diet recalls. The study's population, represented by adolescent females in competitive sports, generally does not meet the DRI for energy requirements. The athletes, on average, were in a negative energy balance at the end of the day. In addition, their diets' micronutrient content is of concern as they are not meeting the DRIs. The SVPs ate less protein than swimmers and highly active women, but ate more than lean athletes such as gymnasts. Body fat percentages were within normal range for the SVPs. In addition to measuring energy levels, weight behaviors and feelings were inquired from all athletic groups. Forty percent of the SVPs had high body dissatisfaction scores. In pursuing weight control behaviors, some of these adolescent athletes reported vomiting and fasting (Beals, 2002).

Eight NCAA Division II college female volleyball players provided a 3-day diet record in an intervention study measuring the impact of dietary feedback on nutrient intake and body composition (Anderson, 2010). Body composition measurements were taken by air displacement plethymography at baseline, peak-season, and one-week post-season in addition to three day diet recalls at those time periods. The study compared two seasons of volleyball: the first without any nutrition education and the other with nutrition education. A specific component of the education suggested a protein intake of 1.2-1.4 g/kg. A significant result showed that at baseline, the protein intake increased in the season including education. There was no significant difference in carbohydrate and fat intake. The study concludes that feedback resulted in overall high consumption of protein and various micronutrients such as vitamin C and calcium at baseline of the season. Peak and post measurements showed no changes other than an increase in fiber. Body composition measurements did not show any significant differences at any point in the study. Caloric intake increased overall in the volleyball players from 35 to 40 kcal per kg. Seventy five percent of the athletes were below protein recommendations at baseline. Education and feedback improved this level briefly through the season, but it lowered back again by peak. In many competitive sports, traveling is a constant factor affecting the intake of athletes. This study found that traveling and eating out during the peak of the season may have affected intake and may be related to lack of sustaining dietary changes from the feedback (Anderson, 2010).

The typical lifestyle and routine of a college athlete is likely to differ than that of any other competitive athlete. College athletes have to eat around class schedules, practice, and like all students, have limited access to food and food preparation equipment (Hinton et al., 2004). To assess the diet behavior of a typical college athlete, Hinton et al. (2004) evaluated food frequency questionnaires from 345 NCAA Division One university athletes. Findings showed a marginal 15% had adequate intake of carbohydrate and protein based on recommendations for athletes. Like other studies, the athletes were asked about eating habits related to body satisfaction. Unfortunately, female athletes are consuming far below the recommended calories in an effort to achieve the optimal body composition. Over half of the females (62%) were limiting their intake to lose weight, an energy deficit that will likely lead to a loss in muscle mass (Hinton et al., 2004). Almost 70% of female VPs wanted to lose weight, while 20% wished to maintain their weight and a mere 10% were aiming to gain weight. Studies imply a lack of nutritional knowledge in college athletes, which is resulting in restriction of energy intake and insufficient carbohydrate intake (Hinton et al., 2004).

Multiple studies are finding that females in particular, but not limited to those in athletic sports, are undernourished (Beals, 2002; Hinton et al., 2004) Depending on the age range, research is implying that several factors influence energy balance and nutrient requirements (Aerenhouts et al., 2013). One factor under much scrutiny is protein. A study performed on 60 adolescent male and female sprint athletes used a 7-day dietary and activity recall to estimate energy balance and protein intake. Other measurements included anthropometrics and body composition. Growth curves were noted at peak height and a urine sample was collected to determine nitrogen balance. Results showed that the mean protein intake ranged from 1.4 to 1.6 grams per kilogram. Height, LBM, and weight increased throughout the study period in both males and females. In determining the effects of the nitrogen balance on growth spurts, findings showed that growth only contributes to a small percentage of the protein needs in these individuals. A 'ceiling effect' shows that excessive protein intake is not used by the body. Peak periods of muscular growth in this population does not support the need for increased protein intake (Aerenhouts et al., 2013).

Malaguti et al. (2008) tested two diets in 11 male and female volleyball players. The first group (n=5) had the Mediterranean diet which is primarily a plant based diet, high in fruits and vegetables, nuts, fish, and oils. The second group (n=6) had a high protein, low calorie diet supplemented with omega-3 fatty acids. Anthropometrics were taken at baseline and end of the 2-month long study. The second, higher protein group had a lower BMI and a lower body fat percentage after the short term study period. The lower BMI is most likely a result of the lower calorie intake in the high protein group versus the higher caloric intake in the plant based group. The lower body fat percentage can also be related to the higher intake of protein in the second group. Protein is lower in calories per gram than fat. The results imply that body composition and anthropometrics may vary by consuming different macronutrient distributions (Malaguti et al., 2008).

Aside from protein alone, carbohydrates may play a crucial role in muscle building. Insulin's response to carbohydrate intake can either promote or inhibit muscle protein synthesis. Deutz et al. (2013) considered protein intake as a part of a full meal, studying the anabolic mechanisms behind protein and carbohydrate absorption. An increase in insulin levels resulting from the co-ingestion of carbohydrates and protein can suppress the amount of protein breakdown and therefore limit the amino acid pool (Deutz et al., 2013). A study assessing weight loss in adult women suggest that a lower carbohydrate and higher protein diet will increase lean body mass and reduce fat mass (Layman et al., 2005). This research takes into consideration the body's response to combined macronutrient ingestion whereas most studies focus on protein intake alone.

In conclusion, the nutritional habits of female athletes, college level athletes, and VPs alike tend to be structured in an attempt to optimize weight, body composition and therefore, performance. It is also evident from some studies that young female athletes have negative body images, which are likely to adversely alter their eating habits.

Energy Balance

To satisfy the desired, steady energy balance, the International Society of Sports Nutrition (ISSN) recommends including four to six meals per day in consistently timed intervals (Teta et al., 2013). A stable energy balance is important to keep in mind, especially when maximizing the role of MPS stimulating foods such as protein. Energy balance is the difference between energy intake and energy expenditure. Evidence is showing that inadequate energy availability is commonly seen in athletes, especially females. With decreased energy intake and increased exercise, the deficient energy balance may affect important functions including the anabolic effects of muscle protein synthesis (Mountjoy et al., 2014).

A benefit of a steady energy balance is improving the ability to build muscle. When an individual is consistently in a catabolic state, protein intake will only contribute calories for expenditure versus stimulating muscle protein synthesis. This continuous catabolic state may also lead to increasing body fat (Deutz et al., 2000). When energy availability is low, the body tries to compensate by reducing energy expenditure. Energy availability (EA), otherwise known as energy balance, is an important factor in athletic performance and performing other routine activities after exercise (Mountjoy et al., 2014). EA is calculated by measuring energy intake and total energy expenditure. Having a low EA can result in a number of negative effects on health (e.g. menstrual dysfunction, low iron intake, reproductive disorders, etc.) (Woodruff et al., 2013). In a consensus statement by the International Olympic Committee, it was found that such events will lead to physiological changes, such as hormonal imbalances and disruption of metabolic functioning (Mountjoy et al., 2014).

Some studies are reviewing the average intake and expenditure for female VPs (Beals, 2002). Findings show that EI is consistently low among the population, which is resulting in a negative energy balance for these competitive athletes. In a review of multiple studies, the focus was on self-reported data of intake and expenditure (Woodruff et al., 2013). Woodruff et al. (2013) followed 10 participants from the women's Canadian University volleyball team. The mean age was 20 years old, the mean weight was 75 kg, the mean FM was 19.4 %, and the mean FFM of the participants was 55.6%. The participants' body composition was measured using a Bod Pod®. A multi-system sensor armband tracked total energy expenditure and a dietary recall assessed energy intake, both occurring over a 7-day period. In evaluating the measurement tools, findings showed that the armband underestimated the caloric expenditure in high intensity exercise and measured more accurately on moderate exercise. Due to high energy expenditure, participants were encouraged to consume energy during training to stay in a positive EB. To calculate EA, the study used the equation as follows: EA = (EI kcal – ExEE kcal)/kg FFM. Results found that the VPs expended around 3,479 kcal per day on average while consuming 3,435 kcal. TEE was just 1,000 calories above the estimated 2,400 kcal daily expenditure for the athletes (based on a recommended intake of 30/kcal per kg per day). In this specific study, the participants on average, had a healthy EB to contribute to weight maintenance and muscle build. These findings conflicted with other studies where elite female VPs are averaging a negative EBs (Woodruff et al., 2013).

Erdman et al. (2013) performed a study assessing 324 high performance Canadian athletes to evaluate their energy requirements. The population, 64% of which whom were females, provided 3-day dietary records of food, fluid and supplement intake and time of consumption. Kilocalories and macronutrient intake were compared by gender, age, meal versus snack, and training versus rest days. Almost all subjects ate three meals per day while on average these athletes ate roughly five times per day. Fewer snacks were consumed on rest days than on training days. The athletes were thought to have higher energy intakes and due to a higher metabolic rate at rest, greater expenditure. This is known as the thermic effect. The athletes exceeded protein requirements, but were under for intake of carbohydrate requirements. However, it is important to note that carbohydrate requirements will vary by athlete due to volume and intensity of training.

On average, the athlete's greatest calories were consumed at dinner and/or meals at night and the least amount of calories were consumed at breakfast, with the female gender especially. Twenty four percent of total energy intake came from snacking. On training versus rest days, meal frequency did not increase, but rather an increase in portion size by 1.5 times. Morning snack was omitted and EI was reduced on rest days. Results indicated that Americans greater than 19 years old, in the general population, intake around 472 calories per day in snacks. Among other factors, the more numerous and the more evenly dispersed the eating opportunities, the greater the reduction in gastrointestinal upset and irritation. Frequent feeds help keep total energy intake up for athletes and will then maintain normal blood glucose levels. The study expresses the importance of carbohydrate ingestion when there is extensive physical exercise. Unfortunately, the individuals in this study are not fulfilling their physiological requirements and energy needs relevant to their exercise volume, frequency, and intensity (Erdman et al., 2013).

Protein Intake and Distribution

There is a lack of evidence between the relationship of protein consumption and body composition in the female population. While protein absorption is high (up to 95% of that consumed), the mechanism behind a maximized anabolic response to muscle protein synthesis is still not fully understood. The average individual will eat the majority of their daily protein at dinner, with steak, chicken or fish being the primary sources. For maximal protein utilization, past studies suggest that this high protein dinner should be redistributed among at least three meals a day (Mamerow et al., 2014;

Symons et al., 2009). Numerous benefits can be associated with a balanced protein intake among meals such as early satiety, decreased energy intake, and repletion of the body's proteins.

Another benefit of protein consumption, commonly focused on by competitive athletes, is the stimulation of muscle protein synthesis (MPS) (Paddon-Jones et al., 2008). The muscle protein fractional synthetic rate (FSR) represents the rate at which ingested amino acids (AA) are synthesized into protein. MPS is a result of protein ingestion when muscle proteins are synthesized versus being broken down (Witard et al., 2013). This muscle building mechanism occurs in an anabolic or fed state, when the body utilizes amino acids from an intracellular pool. The ingestion of amino acids while in the anabolic energy balance results in a positive nitrogen balance, which is one step toward gaining lean muscle mass. If an individual is in a catabolic state, on the other hand, AAs will be sent to catabolic pathways (Witard et al., 2013). AAs can then be used as a source of energy for the body, they can be oxidized and denitrogenated for urea production, or AAs can contribute to plasma proteins or other derivatives such as neurotransmitters (Phillips et al., 2007).

The balance between MPS and muscle protein breakdown (MPB) determines muscle build over prolonged periods of time. When MPS exceeds MPB, it is defined as net gain (Deutz et al., 2013). Protein turnover takes place when breakdown or catabolism of protein occurs faster than the anabolism or synthesis of the protein. With resistance exercise, muscle enters a hypertrophic state where the muscle proteins are being synthesized. Depending on the weight of resistance load, muscle protein synthesis and breakdown will either increase or decrease. Once completing any kind of enduring exercise, nutrient intake is important to maintain a balance between MPS and MPB. The stimulation of resistance exercise on the muscle will bring the muscle into a breaking down period post workout and in the absence of calories and protein, net muscle protein balance will remain negative and hypertrophy will not occur (Koopman et al., 2007).

While resistance training is specifically recommended to build muscle, the effects of aerobic exercise on muscle building are not yet established. Research agrees that heavy resistance exercise leads to hypertrophy of the muscle while aerobic exercise may not have the same effect. In a study examining MPS in 12 untrained men, the idea was to mimic mild exercise similar to walking on the beach (Sheffield-Moore et al., 2004). The population included older (n=6) and younger (n=6) men. Measurements included muscle phenylalanine kinetics pre and post-exercise using blood samples and muscle biopsies. Results of the study showed that moderate intensity exercise, such as walking, induces short-term increases in post-exercise MPS in both post absorptive younger and older men. The mixed muscle fractional synthetic rate increased the most for older and young men in 10 minutes post-exercise (Sheffield-Moore et al., 2004).

Resistance exercise stimulates myofibrillar MPS with a greater duration and amplitude than eating alone. In sarcoplasmic MPS, on the other hand, both consuming calories and exercise provide the same effect and rate of stimulation. Moore et al. (2009) looks at muscle protein synthesis with eating alone and with eating followed by resistance exercise in seven healthy young men. To test fasting MPS, the men were infused with phenylalanine which aided in measuring protein synthesis. The participants then performed high intensity resistant exercise followed by consumption of 25 grams of whey protein. Results showed that with ingestion of protein, the amino acid pool is stimulated rapidly one half hour after eating. Findings also showed that once five hours passed, MPS is back to baseline (Moore et al., 2009).

To increase MPS, decrease MPB and improve exercise recovery, recommendations from the ISSN are to supplement with branched chain amino acids (BCAAs). BCAAs leucine, isoleucine and valine, make up 1/3 of the muscle proteins and therefore have the greatest effect on MPS stimulation. Another recommendation by the ISSN is to consume 10 to 20 grams of amino acids, within three hours post-exercise and immediately before exercise to maximize stimulation of MPS (Teta et al., 2013) While any amino acid will stimulate MPS, essential amino acids will stimulate MPS at the same rate and in smaller amounts (6g) for 1-2 hours post-exercise. However, such small amounts will not contribute to maintenance of an anabolic state for prolonged periods of time (Koopman et al., 2007).

When considering sources of protein, it is important to remember plants are not all complete proteins, being deficient in EAAs. Therefore, plants are less likely to contribute as greatly to MPS. In a study reviewing the effects of EAAs on MPS, 15 grams of EAAs were administered to six young and seven elderly participants (Paddon-Jones et al., 2004). Methods included constant infusion of phenylalanine, an EAA that is neither synthesized nor metabolized in skeletal muscle, during which blood samples and muscle biopsies were taken. Both mixed FSR and muscle protein kinetics were measured before and after ingestion. The fractional synthetic rate measures actual incorporation of phenylalanine into protein. Net phenylalanine uptake and net balance of phenylalanine only translate to synthesis if there is subsequent uptake from the intracellular pool.

Results of the study suggest that EAA ingestion increases FSR in both young and elderly (Paddon-Jones et al., 2004).

In addition to the content of the protein consumed, the distribution of protein intake is being looked at in relationship to muscle building. According to a number of studies, the muscle protein FSR is maximized at approximately 20 to 30 grams of protein (Symons et al., 2009; Witard et al., 2013; Deutz et al., 2013; Mamerow et al., 2014). The amino acid has other functions in the body if not utilized for MPS. For instance, the attached nitrogen is removed and the remaining carbon chains are oxidized for energy or stored as fat. This process is known as the 'muscle full effect.' Researchers are still not entirely certain on how this mechanism is controlled (Atherton et al., 2010). Phillips et al. (2011) in addition to other studies are finding that an intake of 30 grams of protein at each meal leads to maximum stimulation of protein synthesis.

In a recent study by Witard et al. (2013), testing was done on the post absorptive effects of 10g, 20g, and 40g doses of whey protein on myofibrillar MPS. Myofibrillar proteins are involved in muscle hypertrophy and their synthesis is directly stimulated by resistance exercise. The study found that young men weighing around 80 kg had the highest rate of MPS after intake of 20 g of whey protein. Any amount higher resulted in ureagenesis and amino acid oxidation, suggesting there is a utilization of protein for energy rather than to support lean tissue mass. The findings imply that myofibrillar MPS can reach an upper limit resulting in discontinuing the synthesis of proteins (Witard et al., 2013). In another study, a population sample of six healthy individuals were infused with mixed amino acids and monitored for latency of MPS. Again, results showed that overfeeding protein does not speed up the rate of MPS because the excess amino acids are oxidized versus synthesized (Bohé et al., 2001).

In a study by Symons et al. (2009), two portions of lean beef, 113 g (30g intact protein) and 340 g (90 g intact protein) were given to both younger and older participants to determine the effect of MPS with varying protein doses. The goal was to compare MPS rates in aging and younger individuals based on either 30 grams or 90 grams of high quality protein intake. Blood samples were taken to assess glucose and insulin concentrations. Stable infusion of an isotope of L-phenylalanine tracked essential amino acid levels. Using these amounts, an equation to compute FSR measured the rate of mixed MPS in each group. The results indicated in both the 30 g and 90 g groups, the FSR increased at around 50%. The study concludes that more moderate portions of protein are just as effective in stimulating muscle growth as the larger amounts in resting individuals (Symons et al., 2009).

A study by Mamerow et al. (2014) measured fractional synthetic rate in evenly distributed protein diets and skewed protein diets. The evenly distributed diet contained approximately 30 grams of protein at breakfast, lunch and dinner. The skewed diet administered 10 gram, 16 grams and 60 grams of protein at breakfast, lunch, and dinner respectively. The sample size included eight healthy adult men (n=5) and women (n=3). The participants followed each diet, even and skewed, for seven days with a 30 day washout period. On days one and seven, blood samples were drawn and compared. An L-phenylalanine isotope allowed researchers to measure the infusion of the EAA into muscle, providing numeric data to calculate FSR. Findings showed there was 25% greater FSR in the even versus skewed protein diets. These results imply that MPS is stimulated more effectively with moderate amounts of protein consumption over the course of 24 hours (Mamerow et al., 2014).

Background research from a study by Paddon-Jones et al. (2004) suggests that after amino acids are ingested, there is limited time for MPS to be stimulated, ranging from one to two hours. In addition, when continuously ingesting protein, MPS will return to baseline levels regardless of the amount of protein consumed (Atherton et al., 2010). When amino acids leave circulation, they may enter the muscle intracellular pool and from there, they can be incorporated into protein. Another route of essential amino acids, such as phenylalanine, is to leave the plasma pool to quickly expand the intracellular pool, all before being released back to circulation. With the availability of exogenous amino acids, insulin has been shown to play a role in MPS. With additional carbohydrate intake after exercise, insulin levels will rise in the blood. These insulin levels promote protein synthesis and inhibit breakdown, but only minimally if there is an absence of amino acid ingestion. This evidence suggests that a combination of protein and carbohydrate is most beneficial after exercise (Koopman et al., 2007). Age will have an effect on sensitivity to insulin, however. Younger groups tend to have a stronger response of insulin to AA concentrations than the elderly (Paddon-Jones et al., 2004).

Carefully timing protein intake throughout the day with a focus on before and after exercise has numerous benefits. Protein uptake may depend on the type and intensity of exercise and also on the type of protein. Several studies review the differences between commonly supplemented proteins such as whey and casein. Whey protein breaks down faster and provides a lot of initial protein to the body. Casein breaks down slower and takes longer to stimulate MPS. Whey and casein protein are both beneficial in building muscle as they both have a high bioavailability. Sudicky (2012) suggests that whey is more beneficial in increasing LBM. Ideal meals should consist of slowly digested protein such as casein, but the faster digesting whey protein is a better option for performance recovery (Teta et al., 2013). Whey is digested rapidly, breaking down into amino acid form which is then readily available for MPS (Wilborn et al., 2013).

Pre- and post-exercise protein supplementation is capable of significantly changing body composition and performance in a controlled setting. A study population of 16 NCAA Division III female basketball players consumed 24 grams of either whey or casein protein. The treatment was taken immediately both before and after each workout over the course of eight weeks. The study results show no difference between whey and casein. Whey and casein showed the same response in muscle development when supplemented pre- and post-exercise. The immediate uptake of AAs in whey and the slow uptake over long periods of time in casein are proving to have the same result in this study. Whey breaks down quickly so the uptake is quick while casein is slow so the AAs are provided in little amounts over a long period of time (Wilborn et al., 2013).

In defining the varying influences on muscle strength and BC, Joy et al. (2013) evaluated the effects of different types of protein. A study shows the effect of whey protein on body composition in male athletes. The study population was made up of 24 college-aged, resistance trained males divided into two treatment groups. The treatment groups included 48 grams of either animal protein (whey protein isolate) or plant protein (rice protein isolate). Both treatments were administered during the eight week training program. The whey or rice supplements were taken immediately after exercise. A dualenergy X-ray absorptiometry (DXA) determined BC, and a leg and bench press determined strength. There was no significant difference in findings (recovery, body composition, strength) between either group for whey or rice protein treatments (Joy et al., 2013). This study suggests that evidence will still vary as to the ideal source of protein to maximize muscle building.

Some research contradicts the theory of associating frequent and moderate amounts of protein intake with higher muscle mass. West el al. (2011) performed a study administering whey and casein to represent either eating one large meal with high protein in a day or several moderate amounts of protein in multiple meals throughout the day. The study treated eight healthy men. Being slowly absorbed, casein represents more of a frequent and moderate protein intake. Whey is ingested rapidly so it is referred to as bolus intake, providing high amounts at one time. The bolus treatment included one 25 gram dose of whey protein or repeated smaller doses of 2.5 grams every 20 minutes. This pulse dosage mimicked a more slowly digested protein like casein. Both MPS and phosphorylation were measured at rest and following resistance exercise. The study found that a high peak of aminoacidemia after exercise will enhance MPS. Upon administering whey to the study population, hyperaminoacidemia resulted and MPS was more likely to be induced. With slowly absorbed amino acids, the casein prolonged aminoacidemia. MPS was stimulated to a lesser degree in casein (represented by PULSE) than whey (BOLUS) during recovery and at rest after exercise. This research suggests that MPS is optimal when stimulated with rapidly absorbed amino acids at large amounts after exercise (West et al., 2011).

Aside from the importance of muscle building, the type of protein can be associated with micronutrient sufficiency and deficiency. Endurance athletes of the female gender are more susceptible to iron deficiency anemia. A study divided 28 male and female Division-I cross country runners into a control group (typical diet) and an intervention group (typical diet with nine ounces of weekly lean beef supplementation). Measurements included dietary intake, body composition, VO2 max and iron status. The exact effect of meat on body composition was unclear, but the relationship with meat intake and iron levels show a significantly positive correlation (Burke, 2012).

Consuming large amounts of protein at one time or excessively throughout the day can ultimately be detrimental to the body's organs. Depending on the intensity and gender, exercise in addition to the consumption of protein may induce proteinuria. As proteinuria can lead to kidney damage, it is crucial that athletes take all risks and side effects into consideration when increasing or changing their protein consumption (Ayca et al., 2006).

This literature review demonstrates the continuing influences of diet, specifically protein, and body composition on collegiate level female athletes. To achieve that desirable body composition of higher lean mass to fat mass, proper protein intake is continuously being studied to identify the most efficient ways to incorporate it into an athlete's diet. While the findings for the content and administration of protein continue to vary, more and more evidence is suggesting moderate and frequent amounts of intake are the most effective in building muscle mass.

Chapter III

METHODS

Population

Twelve members of Georgia State University's sand volleyball team participated in an IRB-approved one-year observational study. The female participants averaged 20 years of age (±2 yr.) and had a mean weight of 68 kg (±7 kg). Eligibility requirements are that all participants must be athletically trained as well as active students at Georgia State University. Participants must also be healthy, having no preexisting medical conditions and falling within a healthy BMI range (18-24.9 kg/m^2). Subjects must also adhere to the guidelines of the study, invited via a consent form with specific detail of what is required to participate. Exclusion factors for this study included anyone not on the Georgia State University women's sand volleyball team. Signed informed consent was received from all participants prior to the beginning of the study.

Materials

A standardized form was provided to each participant for completion of a diet recall. Height was measured via a standard sliding wall-mounted stadiometer. Weight and body composition were measured via Tanita® bioelectrical impedance analysis scale (Barreira et al., 2013). These data were entered into the NutriTiming® software program, a nutrient analysis based on the U.S. Department of Agriculture's National Nutrient Database for Standard Reference Release 26 (NutriTiming® Nutrient and

Energy Analysis 2.1, NutriTiming LLC, 2014). Basal metabolic rates for each participant were derived by the Harris Benedict equation. The software program produces an hourly energy balance status based on the activity completed and energy intake for each hour.

Procedures

Data for this study were collected at the 6-month midpoint of a one-year observational study. The athletes were asked to complete an in depth form to provide dietary intake and activity over the course of a 24-hour period. Forms were filled out on a typical practice day and a day convenient to the participant during the competitive season of GSU sand volleyball. Food and beverage intake and activity levels were self-reported and confirmed through a follow-up interview. Participants were asked to provide as much detail as possible in their recalls. Food items were to be specified by type and amount at the time eaten. Any physical activity performed had an associated activity level. The numeric level ranged from one being the least active to six being the most active. Upon completion of their 24 hour form, participants were assessed for height, weight and body composition. See Appendix L for an example of the dietary recall form provided to the participants.

Data Analysis

This thesis involved a secondary analysis of collected data. Participant names were coded numerically to ensure confidentiality. Descriptive variables included age, height, weight, fat mass, fat free mass, and body fat percentage. Dietary and activity data were entered into a software program that provided a calculated energy balance over the course of 24 hours. Within the 24-hour period, energy balance was provided from hour to hour. The analysis determined the degree to which variables such as energy and/or protein intake distribution, total energy consumed, total energy expended and percent of energy requirement were achieved over the 24-hour data collection. We expected the results to show the association of the pattern of protein intake (amount and timing of consumption) and energy balance with the fat free mass in these subjects.

To perform this analysis, we began with numerical values provided by the nutrient database software program to create variables for comparison: total protein intake, lowest energy balance, highest energy balance, hourly energy balance, and hourly grams of protein intake. We assessed energy balance with a protein index score (ProtIS). The ProtIS was created to assess how closely the protein consumed matches the hypothetical ideal pattern. The ideal pattern was determined from the total protein requirement (1.5 g/kg) multiplied by the subject's total mass, and dividing that total by 25 grams to obtain the ideal number of protein eating opportunities of 25 grams during a typical day. The more closely a subject consumes protein compared to the ideal, the higher the ProtIS score.

The 24-hour period was divided into zones to compare the amount of protein consumed within varying time ranges across the day. The protein intake for each participant was allocated four different ways into three, four, six and eight time zones. The three-zone time periods are as follows: six am to twelve pm, twelve pm to six pm and six pm to midnight. The four-zone time periods are as follows: twelve pm to six am, six am to twelve pm, twelve pm to six pm and six pm to midnight. The six-zone time periods are as follows: six am to nine am, nine am to twelve pm, twelve pm to three pm, three pm to six pm, six pm to nine pm, and nine pm to midnight. The eight-zone time periods are as follows: twelve am to three am, three am to six am, six am to nine am, nine am to twelve pm, twelve pm to three pm, three pm to six pm, six pm to nine pm, and nine pm to midnight. Ratios were also created to portray the relationship between protein intake and energy balance at a specific point in time. The ratios were calculated by dividing the energy balance in calories by the grams of protein for each hour. This value was then averaged within morning (six am to noon), afternoon (noon to three pm) and evening (six pm to midnight) timeframes.

Anthropometric data collected for each participant were compared to these variables. Frequencies and descriptive statistics were used to describe characteristics of the sand volleyball players participating in this study. Spearman Correlations assessed the relationships between these variables. Regression analyses will be used to assess relationships between body composition, protein intake and EB. IBM SPSS was used to run statistical tests (version 20.0, SPSS, Inc., Chicago, IL).

Chapter IV

RESULTS

Participants

The descriptive characteristics of the 12 members of the Georgia State University sand volleyball (SV) team are shown in Table 1. The population age ranged from 18 yrs. to 22 yrs., with a mean age of 20 (± 1.17) yrs. Participant heights ranged from 165.1 cm to 179.07 cm, with a mean height of 172.51 (± 4.56) cm. Their weights ranged from 57.91 kg to 74.18 kg, with a mean weight of 65.23 (±5.78) kg. BMI ranged from 19.5 kg/ m^2 to 24.9 kg/m^2, with a mean BMI of 21.92 (±1.8) kg/m^2.

Table 2 describes body composition variables for the SVPs. The 12 SVPs had body fat percentages ranging from 10.7% to 25.4%, with a mean body fat percentage of 18.3 (±3.9) %. Fat mass ranged from 17.82 kg to 6.45 kg, with a mean fat mass of 12.09 (±3.3) kg. Fat free mass ranged from 59.55 kg to 47.55 kg, with a mean fat free mass of 53.15 (± 3.66) kg. Fat free mass was measured per kg of body weight, ranging from 0.75 kg to 0.89 kg, with a mean of 0.82 (±0.04) kg. The amount of fat free mass in kg was measured per cm of height ranging from 0.34 kg/cm to 0.28 kg/cm, with a mean fat free mass to height ratio of 0.31 (±0.02) kg/cm.

Protein Intake and Eating Opportunities

Table 3 contains protein intake and eating opportunity variables. We assessed a 24 hour recall and measured protein intake and energy balance. The SVPs' recalls were also evaluated for the number of times they met 25 grams of protein in one eating opportunity. Results show that protein intake ranged from 183.71grams to 85.64 g, with a mean protein intake of 130.99 (±34.39) g. The suggested amount of protein intake for the participants is 1.5 grams per kg. A ratio was found between the actual protein consumption per kg and the recommended 1.5 grams per kg. The ratio ranged from 0.82 g to 2.06 g, with a mean ratio of 1.37(±0.44) g per kg. Eating opportunities represents the number of times the participant consumed any type of caloric food or beverage. The number of eating opportunities ranged from 3 to 8 times, with a mean number of eating opportunities of 5.58 (±1.44) times. The ratio of protein (g) intake and the number of eating opportunities ranged from 11 g/EO to 46 g/EO, with a mean of 26.06 (±10.51) g/EO. Each eating opportunity was assessed for protein intake and one variable was derived to show the number of times the athlete consumed food with 25 grams of protein. The number of eating opportunities with 25 grams of protein ranged from 1 to 4 times, with a mean of 2.08 (±0.79) times. To portray the ideal number of times the athletes were to consume 25 grams of protein in a day, a ratio was developed using body mass in kg and the recommended 1.5 grams of protein per kg to create a Protein Index Score (ProtIS). The ProtIS for the SVPs ranged from 4.45 to 3.48 recommended times, with a mean protein index score of 3.91(±0.35) times. The ratio of eating opportunities with 25 grams of protein to a ProtIS has a mean of 0.55(±0.25) times. This number shows that on average, the athletes are eating 25 grams of protein around only half the ideal number of times recommended in the ProtIS.

Protein Intake in Zones

Table 4 and Appendix A provides protein intake for three time ranges. From six am to noon, the SVPs had a mean protein intake of 30(±15.62) grams. This morning time range is the only range in which all 12 SVPs had eating opportunities with protein, specifically a minimum of 10 grams. From noon to six pm, SVPs had a mean protein intake of 63(±44.48) grams. From six pm to midnight, SVPs ate a mean of 39(±30.76) grams. The mean percentage of total protein intake from six am to noon is 24(±15) %. Almost half of the mean protein intake was during mid-day as the percentage of total protein intake from noon to 6 pm is 45(±26) %. The mean percentage of total protein intake from 6 pm to midnight is 24(±23) %.

In Table 5 and Appendix B, protein intake for the SVPs was assessed for six time ranges across the day. In each of the time zones, at least one participant had zero eating opportunities with protein. From six am to nine am, volleyball players had a mean protein intake of 19(±11.98) grams. From nine am to noon, SVPs had a mean protein intake of 11(±16.81) grams. From noon to three pm, SVPs ate a mean of 29(±29.35) grams. From 3 pm to 6 pm, SVPs ate a mean of 33(±27.72) grams. From six pm to nine pm, SVPs ate a mean of 35(±29.35) grams. From nine pm to midnight, SVPs ate a mean of 4(±4.63) grams of protein. The total protein intake by each of the six time zones were then divided by the total protein intake for the day, creating a percentage of total protein intake. The mean percentage of total protein intake from six am to nine am is 14(±8) %.

The mean percentage of total protein intake from nine am to noon is 10(±4) %. The mean percentage of total protein intake from noon to three pm is 20(±17) %. The mean percentage of total protein intake from three pm to six pm is 25(±19) %. The mean percentage of total protein intake from six pm to nine pm is 28(±22) %. The mean percentage of total protein intake from nine pm to midnight is 4(±4) %.

Energy Balance

The reported energy balance over the course of a 24-hour period is shown in Table 6. Energy balance is represented in the optimal, anabolic, catabolic, surplus, and deficit. If a SVP is in an anabolic state, her energy balance would be between 0 and 100 kcal. If she is in a catabolic state, it would be below zero and to -400 kcal. A surplus is when energy availability is above 400 kcal. A deficit is below -400 kcal. An optimal energy balance is in the anabolic or catabolic state (+/- 400 kcal). The net energy balance is the total calories consumed in the 24-hour period less the total calories expended. The participants had a net energy balance mean of negative 404(±385.28) kcal, falling in a deficit. The hours spent in an optimal state over the course of a day were a mean 18(±3.5) hours. A surplus state had a mean of 2(±3.28) hours. Deficit had a mean of 5(±3.55) hours. SVPs were in an anabolic state for a mean of 7(±4.92) hours and a catabolic state for a mean of 17(±4.95) hours. The peak energy balance was at an average 320(±230.45) kcal, falling in an anabolic state. The lowest balance on average is -720(±280.01) kcal, being a deficit for the SVPs. Please see also Appendices F and G for a histogram showing the frequency of hours spent in both anabolic and catabolic states.

Relationship between Energy Balance and Protein Intake with Body Composition

At each time a SVP consumed protein, they were at a specific energy balance. In Table 7, the energy balances to protein consumption ratios are provided. The total number of eating opportunities with protein was counted for each participant. The participants had eating opportunities with protein ranging from three to seven times, with a mean of 5(±1.14) times. Each participant was also assessed for the number of times in the day they met or exceeded 25 grams of protein. At each time they met 25 grams, their energy balance value was noted and a ratio was created to portray what kind of balance they were in when consuming protein. The average energy balance at 25 gram protein eating opportunities ranged from 19 kcal to -14 kcal, with a mean of 1.8(±7.99) kcal. The net energy balance divided by total protein consumption created a ratio of energy balance to protein, providing a mean of -0.92(±11.59) EB per gram of protein. This number tells us that the players were, on average, in a negative energy balance when consuming protein. The average energy balance for the entire day was divided by the number of protein eating opportunities to provide a mean of -13.58(±84.62). The lower the number, the lower the energy balance and fewer protein eating opportunities the participant is partaking. From six am to noon, the mean energy balance to protein EOs is -7.03(±23.93). The mean EB to protein EO ratio from noon to six pm is -.083(±19.35). The mean EB to protein EO ratio from six pm to midnight is -2.21(±214.86). Appendices C and D provide a comparison between protein intake and energy balance.

Relationships between Protein, EB and Body Composition

The correlations between protein intake, energy balance and body composition using 2-tailed Spearman's Correlations are shown in Appendices H, I, J, and K. Appendix H shows relationships between body composition and protein intake. Relationships prove inverse for several variables. The more protein consumed the lower the BMI ($r=-0.594$; $p<0.05$). The less EOs with 25 grams of protein per protein index score, the higher the BMI ($r=-0.757$; $p<0.05$). FFM to height ratio is lower the more protein consumed ($r= -.839$; $p<0.05$). The higher the number of times participants were eating more than 25 grams of protein, the lower the FFM to height ratio ($r=-0.663$; $p<0.05$). These relationships suggest that protein consumption is negatively correlated with muscle mass.

Energy balance to protein ratios in relation to body composition are shown in Appendix I. There were no significant correlations between the variables ($p<0.05$). In Appendix J, protein intake is broken into time ranges for morning (six am to noon), afternoon (noon to six pm) and night (six pm to midnight). Relationships are shown in the afternoon hours with a correlation between higher protein intake in the afternoon is associated with lower FFM per kg in the participants ($r= -0.629$). All other relationships are insignificant between body composition and morning, afternoon, and night protein intake. In Appendix K, relationships are established between body composition and six time ranges: six am to nine am, nine am to noon, noon to three pm, three pm to six pm, six pm to nine pm and nine pm to midnight. Significant correlations are found in the early morning and mid-morning time zones. From six am to nine am, the higher the protein intake, the lower the FFM per kg BW and FFM per HT ratio. Interestingly enough, the next time zone shows quite the opposite. From nine am to noon, the higher the protein intake in this time frame is the higher the FFM per kg. All other correlations are insignificant for protein intake in later time zones of the day.

Regression Analysis between Frequency of Protein Intake and Body Composition

A multiple regression model predicted body fat percentages and total fat free mass in the 12 participating sand volleyball players. See below for the regression equation that predicts percentage of body fat. The number of eating opportunities, body weight (kg), height (cm), protein intake (kg), and percent of protein in diet all predict a significant amount of variance (94.7%) in body fat percentage.

EOs (-.728) + Wt_kg (.808) + Ht_cm (-.279) + ProIn (.118) + %Pro (-.181) + 6.391

P = .006

R = .947

R^2 = .896

SEE = 1.679

Variables in the next regression analysis show that the number of eating opportunities, hours in an anabolic state, the highest energy balance, weight (kg) and age (yr.) predict a significant amount of variance (89.9%) in percentage of body fat.

EOs(-1.649) + EBHrANA(.800) + Wt_kg(.463) + EBHighest(-.014) + Age_yr(-1.28) + 21.07

P = .037

R = .899

$R^2 = .808$

SEE = 2.288

The percent of protein, protein intake (kg), hours in anabolic energy balance, and hours in catabolic energy balance can all predict a significant amount of variance (87.6%) in total fat free mass. These variables were applied to a regression equation for percent of body fat and no significant variance was found.

%Pro(.041) + Pro_kg(-5.149) + EBHrANA(-.405) + EBHrCAT(-.400) + 72.528

P =.022;

R = .876;

$R^2 = .767$;

SEE = 2.216

The ratio of energy balance to protein intake in the morning hours (six am-noon), afternoon hours (noon-six pm) and night hours (six am-midnight) were tested to predict both body fat percentage and fat free mass to height ratio. The average energy balance to protein intake ratios did not predict a significant amount of variance in either body fat percentage or fat free mass per cm of height in the sample population.

RESULTS TABLES

Table 1:

Anthropometric Characteristics of SVPs at Georgia State University (N=12)

	Min	Max	Mean	Std. Deviation
Age (yr.)	18	22	20	1.17
Height (cm)	165	179	172.51	4.56
Weight (kg)	57.91	74.18	65.23	5.78
BMI	19.56	24.94	21.92	1.80

Table 2: Body Composition (N=12)

	Min	Max	Mean	Std. Deviation
Body Fat (%)	10.7	25.4	18.33	3.85
Fat Mass (kg)	6.45	17.82	12.09	3.3
Fat Free Mass (kg)	47.55	59.55	53.15	3.66
Fat Free Mass per kg	0.75	0.89	0.82	0.04
Fat Free Mass to Height Ratio	0.28	0.34	0.31	0.02

Table 3: Protein Intake and Eating Opportunities (N=12)

	Min	Max	Mean	Std. Deviation
Protein(g)	86	184	131	34.39
Protein(g)/Protein Recommended(g)	0.82	2.06	1.37	0.44
Eating Opportunities	3	8	5.58	1.44
Protein(g)/Eating Opportunities	12	46	26.06	10.51
Eating Opps w 25g Pro	1	4	2.08	0.79
Protein Index Score	3.48	4.45	3.91	0.35
Eating Opps w 25g Pro/ProtIS	0.23	1.15	0.55	0.25

Table 4: Protein Intake in Three Time Zones (N=12)

	Min	Max	Mean	Std. Deviation
Protein(g) Intake: 6am-12pm	10	68	30	15.62
Protein(g) Intake: 12pm-6pm	0	153	63	44.48
Protein(g) Intake: 6pm-12am	0	113	39	30.76
% of Tot Protein(g): 6am-12pm	0	67	24	0.15
% of Tot Protein(g): 12pm-6pm	0	83	45	0.26
% of Tot Protein(g): 6pm-12am	0	69	32	0.23

Table 5: Protein Intake in Six Time Zones (N=12)

	Max	Mean	Std. Deviation
Protein(g) Intake: 6am-9am	38	19	11.98
Protein(g) Intake: 9am-12pm	59	11	16.81
Protein(g) Intake: 12pm-3pm	89	29	29.35
Protein(g) Intake: 3pm-6pm	98	33	27.72
Protein(g) Intake: 6pm-9pm	99	35	29.35
Protein(g) Intake: 9pm-12am	13	4	4.63
% of Tot Protein(g): 6am-9pm	29	14	0.08
% of Tot Protein(g): 9am-noon	58	10	0.04
% of Tot Protein(g): noon-3pm	5	20	0.17
% of Tot Protein(g): 3pm-6pm	53	25	0.19
% of Tot Protein(g): 6pm-9pm	64	28	0.22
% of Tot Protein(g): 9pm-12am	1	4	0.04

Table 6: Energy Balance (N=12)

	Min	Max	Mean	Std. Deviation
Total Kcal In	1792	3042	2375	361.15
24-Hour EB	-1001	291	-404	385.28
Hours +/- 400 Kcal EB	12	22	18	3.5
Hours > 400 Kcal EB	0	10	2	3.28
Hours < 400 Kcal EB	0	10	5	3.55
Hours > 0 Kcal EB	0	18	7	4.92
Hours < 0 Kcal EB	6	24	17	4.95
Highest EB Peak	-11	734	320	230.45
Lowest EB Peak	-1077	-206	-720	280.01

Table 7: Energy Balance at Eating Opportunities of Protein (N=12)

	Min	Max	Mean	Std. Deviation
# of Times Eaten Protein	3	7	5	1.14
AvgEbAt25 (kcal)	-14	19	1.8	7.99
Ratio of Energy Balance to Protein	-28	18	-0.92	11.59
Avg EB/Protein All Day	-221	167	-13.58	84.62
Avg EB/Protein 6am-12pm	-59	32	-7.03	23.93
Avg EB/Protein 12pm-6pm	-54	21	-0.83	19.35
Avg EB/Protein 6pm-12am	-439	551	-2.21	214.86

Chapter V

DISCUSSION AND CONCLUSIONS

This study aimed to investigate the relationship between protein intake and energy balance and their combined and separate effects on body composition. In an average diet, protein is typically skewed towards the end of the day (Mamerow et al., 2014). It was the goal of this study to relate this back loading of protein to decreased muscle mass or show a relationship between increased muscle mass with balancing protein intake throughout the day. However, we failed to reject the null hypothesis that moderate and frequent protein intake throughout a single day will lead to increased fat free mass. A contributing factor could be that the twelve sand volleyball players participating were a relatively homogeneous population with small variances in height, weight and body composition.

Protein Intake

Descriptive statistics showed protein intake to be anywhere from two to three times the recommended daily intake of 0.8-1.0 grams of protein per day. To support a positive nitrogen balance and include factors of growth, multiple researchers suggest the amount of protein to be around 1.5 g/kg/day (Aerenhouts et al., 2013; Teta et al., 2013). The Position Statement from the Dietitians of Canada, the American Dietetic Association, and the American College of Sports Medicine recommend a protein intake from 1.2-1.7 g/kg/day to support the muscle development in athletes. In this study, 75% of athletes consumed well above the suggested amount of protein. The participants' extremely high protein intake may support evidence that muscle protein synthesis has a "muscle full effect" and will not continue to synthesize muscle after certain amounts (Atherton et al., 2010). However, from the data, it is difficult to determine exactly how the distributed protein is relating to the participants' fat free mass. A protein index score represents the suggested number of times a participant should eat 25 grams of protein during the day based on the 1.5 g/kg of protein and their body weight. The players' scores ranged from 3 to 5 times per day. The average amount of times athletes were meeting this recommendation was only 2 times per day which is almost half the recommended. Considering the hectic lifestyle of a college athlete with class, studying and training, it may be difficult for these athletes to find the opportunity to spread their protein evenly and moderately throughout the day.

Protein intake distribution across the day was studied by dividing the day into three and six time zones The three time zone distribution shows that SVPs were eating almost half their protein, at 44.5%, from noon to six pm. Given the large time frame of six hours, this zone could contain more than one meal, especially considering the SVPs training typically being held during mid-morning. Athletes could be loading up on protein multiple times thereafter so the results may be skewed to the second zone of the three time zone assessment. In the six time zone split, the participants' intake almost doubled in protein consumption with their evening meals (34.62 g) compared to their morning meals (19.1 g). The average distribution of protein is steady in the afternoon and evening hours, but relatively lighter in the morning. Again, this skewed distribution may be related to class and training in the morning which give less time for the athletes to cook a meal.

Energy Balance

In evaluating their energy balance, the SVPs were in an overall catabolic state. Evidence shows that these numbers may support the inverse correlations between protein intake and fat free mass (Deutz et al., 2000). The average energy balance to protein ratio for the entire day was -13.58 kcal/g. For each gram of protein eaten, these athletes were not using their protein anabolically. Athletes spent up to 17 hours of the day on average at below a 0 kcal energy balance level, leaving most protein consumption to be utilized for energy and not muscle building. Considering energy balance will then support the results of protein intake directly relating to more fat mass, which is discussed further in the following section.

Relationships between Protein and Body Composition

Surprisingly, we found primarily inverse correlations in the tests between protein intake and fat free mass, possibly because so much time was spent in an energy deficit state. One correlation found with this increasing intake significantly related the athletes to a lower BMI ($p<0.05$). This finding is supported by the evidence that because protein has less calories per gram than carb or fat, higher content in the diet may support a lower BMI (Malaguti et al., 2008).

Results showed that the more protein these volleyball players were consuming, the less muscle mass they had per kg and per cm of height. A similar relationship was found between body composition and the number of times the volleyball players met 25 grams of protein per day. Contrary to our hypothesis, statistical testing found that the higher the number of eating opportunities with 25 grams of protein or more correlated with less fat free mass per cm of height. The explanation for these outcomes can be related to the energy availability at the time these participants are consuming protein. Given that the majority of the food recalls were on training days, the athletes had a break mid-morning for practice which put them at an energy deficit. The SVPs then came home to have a large meal with protein that was more than likely being used as calories versus being broken down for MPS.

One significant correlation was found between fat free mass and protein intake in the second of six time zones. The more protein these participants were eating from nine am to noon, the higher the amount of fat free mass. This one relationship supports the findings that back loading protein does not support adequate muscle protein synthesis.

Although it was difficult to relate increased muscle mass with moderate and frequent protein intake in this population, these variables, along with energy balance and anthropometrics, were used to significantly predict body fat percentage and fat free mass in the population. These findings suggest that the eating and exercise habits of these athletes are able to predict body composition and may increase the likelihood for future studies.

Limitations

The most significant limitation of this study is the small sample size. The GSU female sand volleyball team has only 13 players, 12 of which participated in the study. The athletes also have similar ages, BMIs, and body compositions. These similarities narrow the variance between the participants, making it difficult to represent a larger and more generalized population (See Appendix E). Self-reported dietary recalls also create a limitation in the methods of the study. Participants are likely to underreport, especially considering the age and gender of the population. Additionally, misclassification of the recalls is a possibility. When entering the data into the nutrient and energy balance analysis software program, food items and recipes could have been misclassified resulting in inaccurate representation of calories and macronutrient distributions. Lastly, a single 24 hour period dietary recall will not appropriately characterize the eating habits of the population, creating another limitation.

Conclusions

The current study finds no significant relationship between a moderate and frequent within day protein intake distribution with increased FFM. The negative energy balance at the time of protein consumption was a significant factor likely to cause the inverse association of protein intake and FFM. Although the findings retained the null hypothesis, this study can provide a basis to perform further, more in depth research. Assessing body composition over the course of several months to a year, versus the cross sectional design in this study, would provide a more accurate representation of the diet's effects on muscle mass.   Interestingly, regression analyses found that in the sample population, dependent variables such as body fat % and fat free mass can be predicted by independent variables such as energy balance, anthropometrics, percent of protein and total protein intake. Findings such as these signify the importance of protein consumption and energy balance to achieve an ideal body composition.

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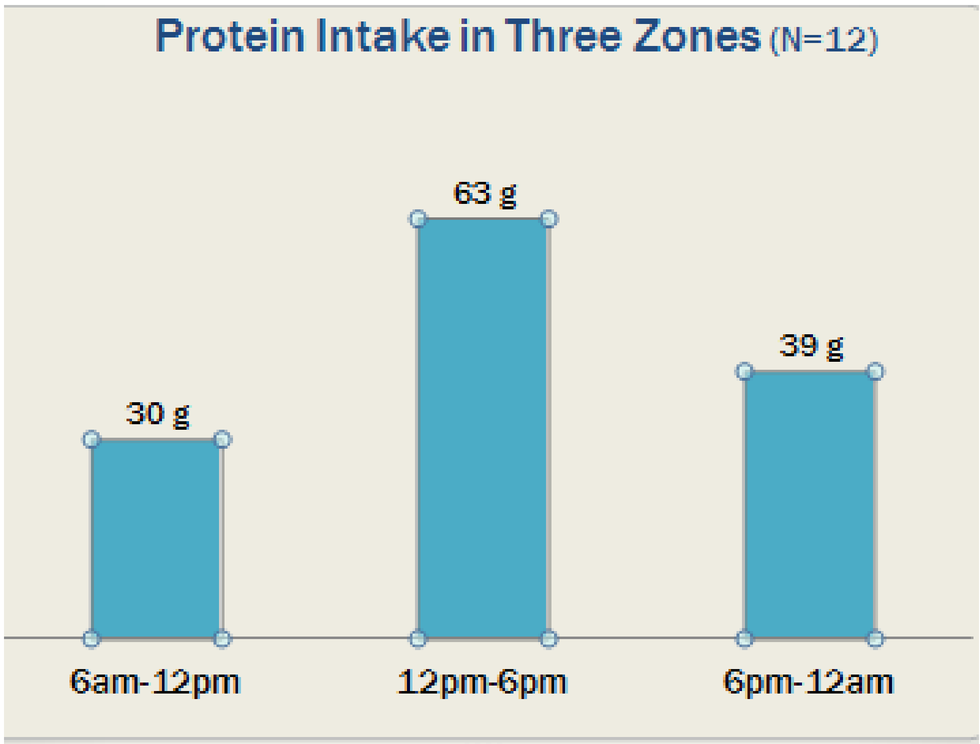
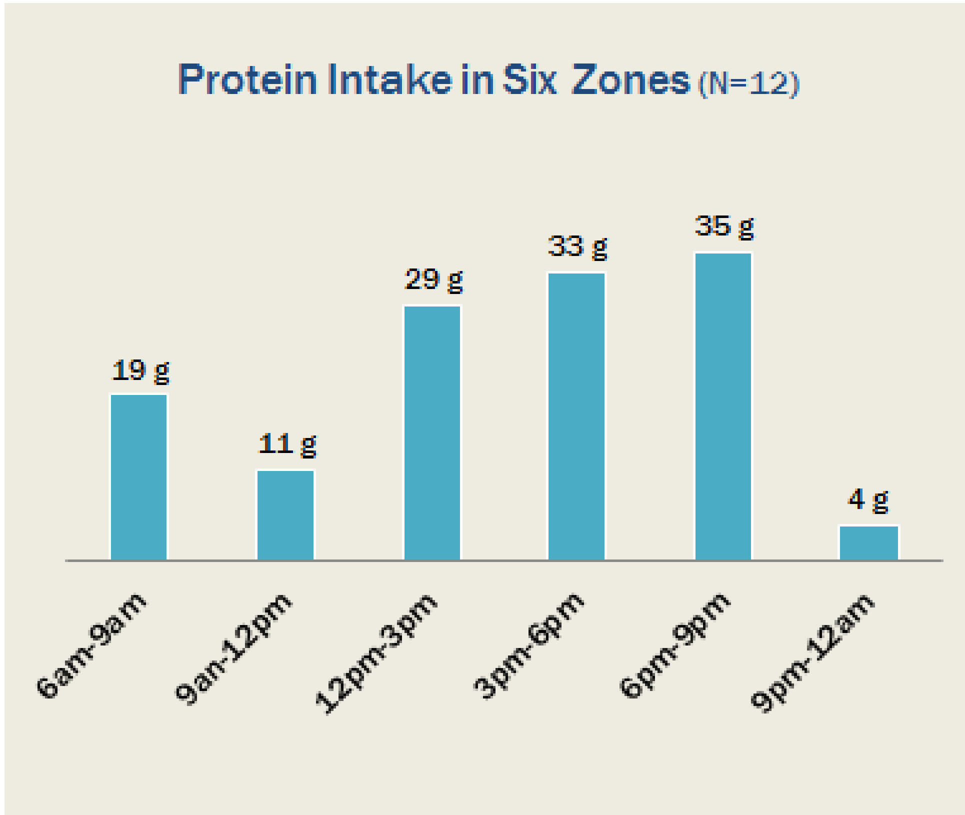
APPENDICES

APPENDIX A



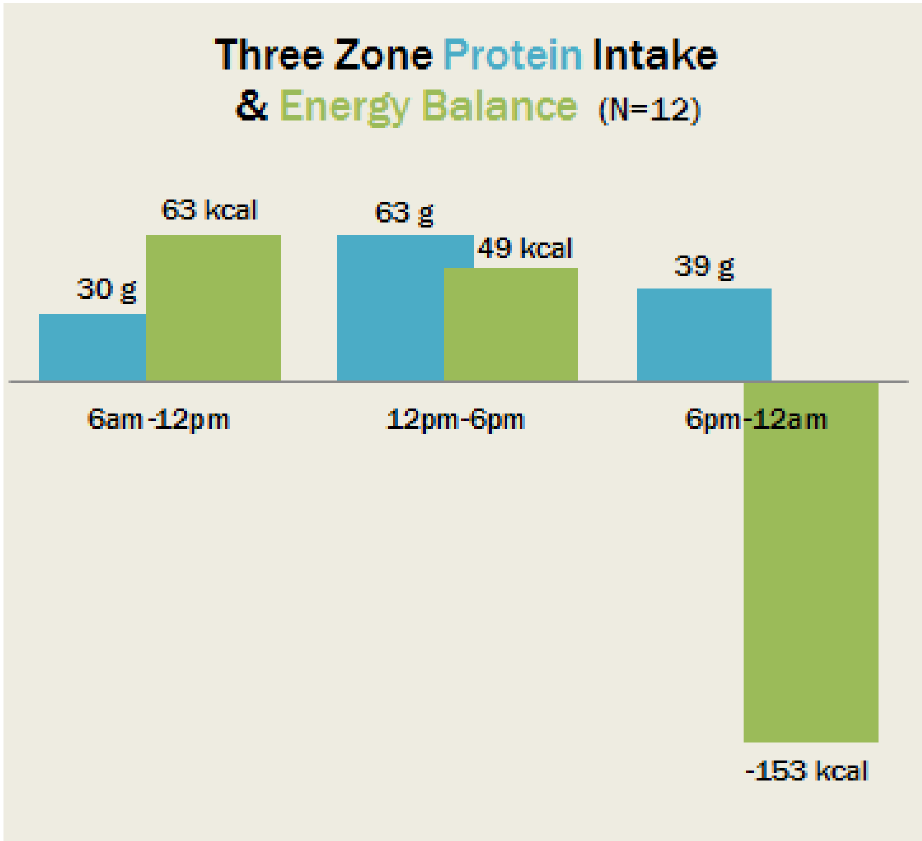
Appendix A represents the mean grams of protein intake for the 12 volleyball players in three time zones across a 24 hour day. From 12 am to 6 am in the hours previous to what is shown here, no intake was reported for the participants.

APPENDIX B



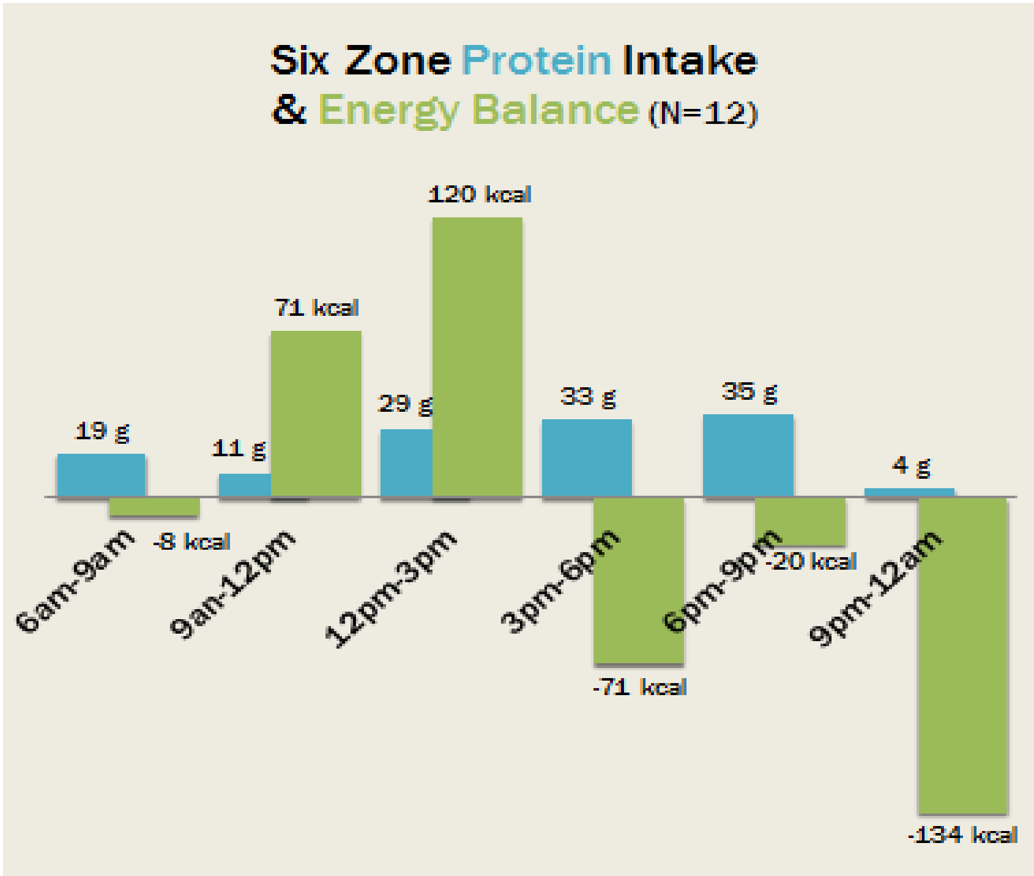
Appendix B represents the mean grams of protein intake for the 12 SVPs in six time zones across a 24 hour period. From 12 am to 6 am in the hours previous to what is shown, no intake was reported for the participants.

APPENDIX C



Appendix C represents a comparison of mean protein intake in the left hand columns and mean energy balance in the right hand columns for the 12 SVPs. The comparison is done in three time zones in one 24 hour period.

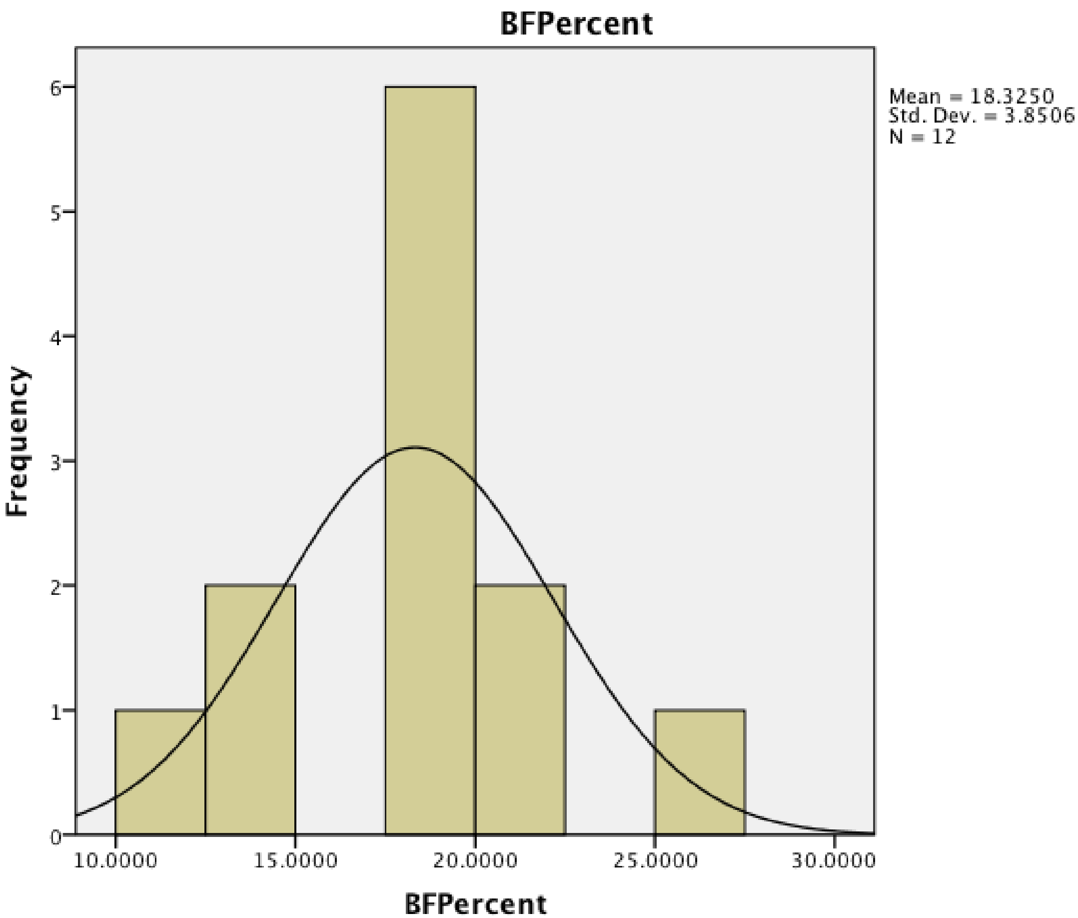
APPENDIX D



Appendix D represents a comparison of mean protein intake in the left hand columns and mean energy balance in the right hand columns for the 12 SVPs. The comparison is done in six time zones in one 24 hour period.

APPENDIX E

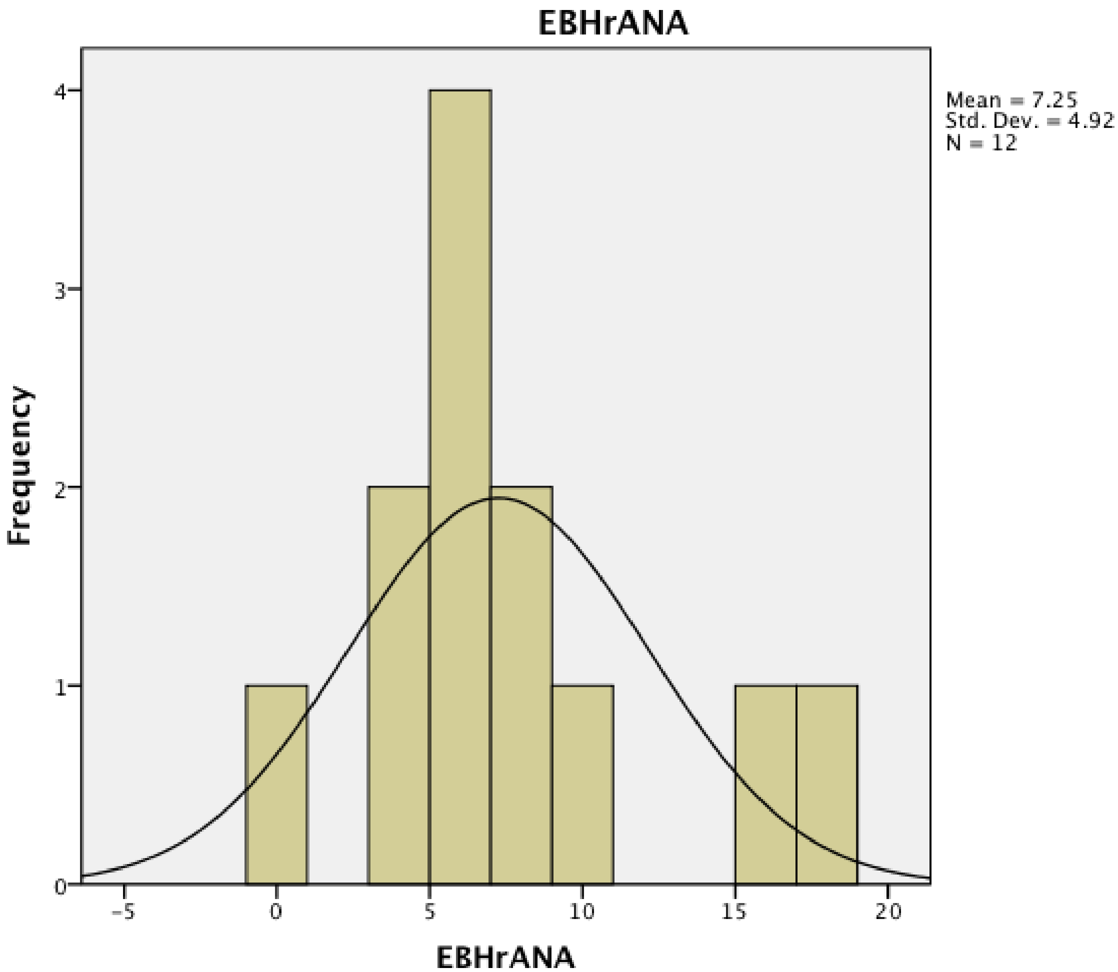
Frequency Statistics for Percent Body Fat (N=12)



Appendix E: The *y axis* represents the number of participants and the *x axis* represents body fat percentage.

APPENDIX F

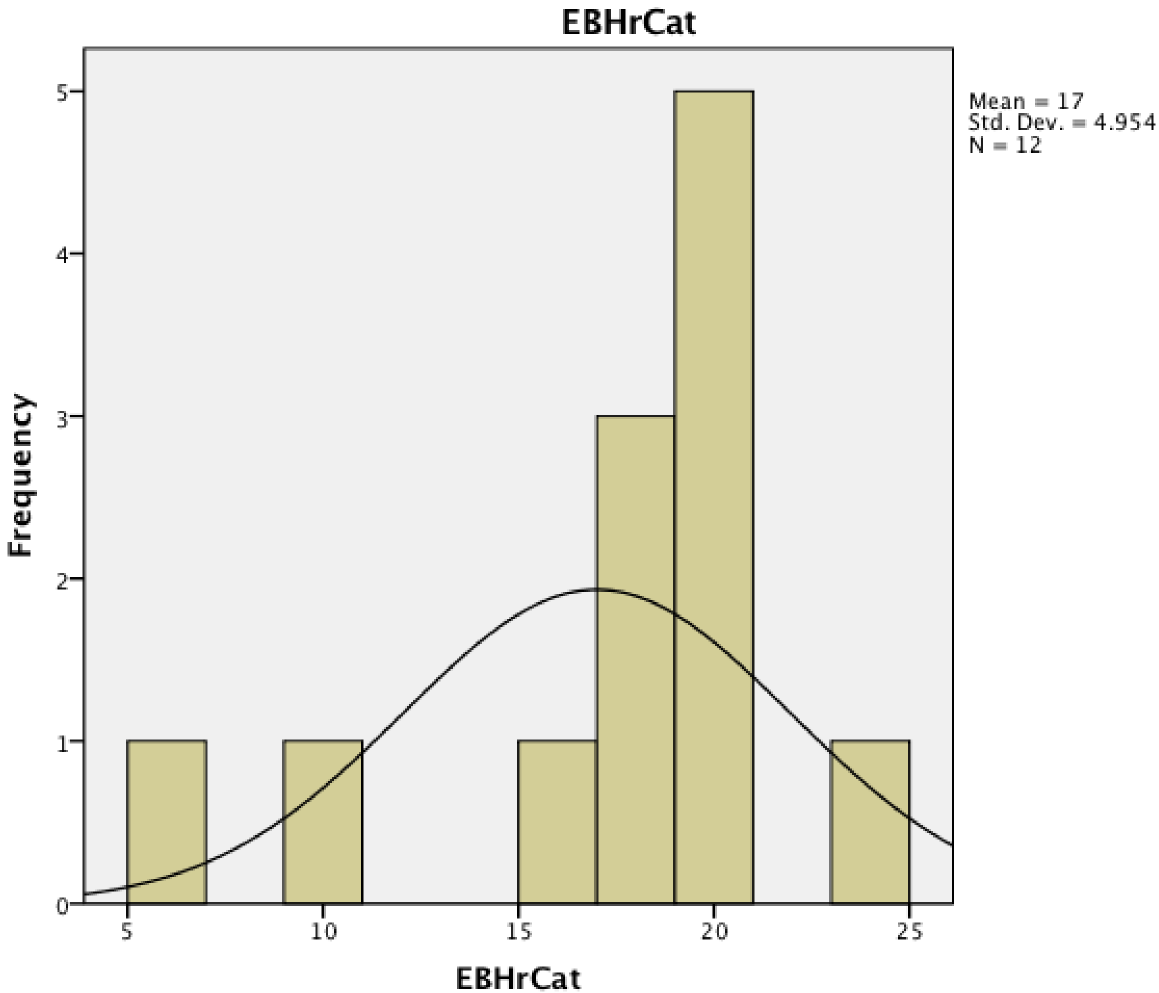
Frequency Statistics for Hours Spent in an Anabolic State (N=12)



Appendix F: The *y axis* represents the number of participants and the *x axis* represents the number of hours in an anabolic state over a 24 hour period. An anabolic state is defined as the calories over 0 in a participant's energy balance.

APPENDIX G

Frequency Statistics for Hours Spent in a Catabolic State (N=12)



Appendix G: The *y axis* represents the number of participants and the *x axis* represents the number of hours in a catabolic state over a 24 hour period. A catabolic state is defined as the calories less than 0 in a participant's energy balance.

APPENDIX H

Spearman's Correlations: Protein Intake and Body Composition (N=12)

		BMI	BF (%)	FFM per kg	FFM to Ht Ratio
Protein (g)	R	**-0.594**	0.088	-0.063	**-0.839**
	P	0.042	0.786	0.846	0.001
Protein(g)/Protein Recommended(g)	R	**-0.648**	-0.012	0.032	**-0.897**
	P	0.023	0.97	0.923	0
Protein(g)/Eating Opportunities	R	-0.21	0.151	-0.112	**-0.601**
	P	0.513	0.64	0.729	0.039
# Times Eat > 25g	R	-0.467	-0.134	0.175	**-0.663**
	P	0.126	0.678	0.586	0.019
Eating Opps w 25g Pro/ProtIS	R	**-0.757**	-0.415	0.431	**-0.855**
	P	0.004	0.18	0.162	0
% Protein	R	-0.559	0.035	0	**-0.818**
	P	0.059	0.914	1	0.001
Protein per kg	R	**-0.648**	-0.012	0.032	**-0.897**
	P	0.023	0.97	0.923	0

Appendix H:
Numbers in bold contain a p value less than or equal to 0.05
Protein (g) – total protein intake in grams
Protein(g)/Protein Recommended(g) – total protein intake divided by 1.5 grams
Protein(g)/Eating Opportunities – total protein intake divided by total number of eating opportunities
Times Eat > 25g – number of times athlete consumed 25 grams of protein or more
Protein Index Score – 1.5 grams times body weight (kg) divided by 25 grams of protein
Eating Opps w 25g Pro/ProtIS – eating opportunities with 35 grams of protein or more divided by the protein index score
% Protein – percentage of protein from total calories
Protein per kg – protein in grams per kg of bodyweight

APPENDIX I

Spearman's Correlations:
Energy Balance with Protein Intake and Body Composition (N=12)

		BMI	BF (%)	FFM per kg	FFM to HT Ratio
Avg EB at 25g Protein	R	0.084	0	-0.049	-0.259
	P	0.795	1	0.88	0.417
Avg EB/P All Day	R	-0.154	-0.018	-0.014	-0.301
	P	0.633	0.957	0.966	0.342
Avg EB/P 6am-12pm	R	0.133	-0.179	0.147	0.021
	P	0.681	0.578	0.649	0.948
Avg EB/P 12pm-6pm	R	-0.217	-0.474	0.441	-0.126
	P	0.499	0.12	0.152	0.697
Avg EB/P 6pm-12am	R	-0.14	0.123	-0.161	-0.476
	P	0.665	0.704	0.618	0.118
Tot EB/Protein	R	-0.189	-0.144	0.105	-0.35
	P	0.557	0.656	0.746	0.265
Tot EB of Pro EO	R	-0.098	-0.091	0.049	-0.287
	P	0.762	0.778	0.88	0.366

Appendix I:
Numbers in bold contain a p value less than or equal to 0.05
Avg EB at 25 g protein – the average energy balance across all eating opportunities with 25 grams of protein
Avg EB/P All Day – average total EB divided by average total protein intake over the course of 24 hours
Avg EB/P 6am-12pm - average EB divided by average protein intake from 6am to 12pm
Avg EB/P 12pm-6pm - average EB divided by average protein intake from 3pm to 6pm
Avg EB/P 6pm-12am - average EB divided by average protein intake from 6pm to 12am
Tot EB/Protein – total energy balance divided by total protein intake
Tot EB of Pro EO – total energy balance from eating opportunities with protein

APPENDIX J

Spearman's Correlations: Three Zone Protein Intake and Body Composition (N=12)

		Protein(g) Intake: 6am-12pm	Protein(g) Intake: 12pm-6pm	Protein(g) Intake: 6pm-12am
24-Hour EB	R	0.028	0.343	-0.21
	P	0.931	0.276	0.513
Eat Opportunities	R	-0.111	0.082	-0.243
	P	0.731	0.799	0.446
Fat Mass_kg	R	0.133	-0.105	-0.091
	P	0.681	0.746	0.779
FFM_kg	R	-0.056	**-0.629**	-0.077
	P	0.863	0.028	0.812
FFM per kg	R	-0.112	0.084	0.007
	P	0.729	0.795	0.983
FFM to Ht Ratio	R	-0.287	**-0.615**	0.021
	P	0.366	0.033	0.948

Appendix J:
Numbers in bold contain a p value less than or equal to 0.05
Fat Mass_kg – fat mass in kg
FFM_kg – fat free mass in kg
FFM per kg - amount of FFM per kg of body weight
FFM to Ht ratio – amount of FFM per cm of height
Eating Opportunities – number of times athlete consumed calories
24 Hour EB – net kcal at the end of the day (energy consumed less energy expended)

APPENDIX K

Spearman's Correlations: Six Zone Protein Intake and Body Composition (N=12)

		Protein(g) Intake: 6am-9am	Protein(g) Intake: 9am-12pm	Protein(g) Intake: 12pm-3pm	Protein(g) Intake: 3pm-6pm	Protein(g) Intake: 6pm-9pm	Protein(g) Intake: 9pm-12am
Fat Mass_kg	R	-0.21	0.305	-0.331	0.151	-0.039	-0.317
	P	0.513	0.336	0.293	0.64	0.905	0.316
FFM_kg	R	**-0.692**	**0.718**	-0.507	-0.07	-0.091	0.176
	P	0.013	0.009	0.092	0.829	0.778	0.584
FFM per kg	R	0.168	-0.268	0.239	-0.077	-0.049	0.211
	P	0.602	0.399	0.454	0.812	0.88	0.51
FFM to Ht Ratio	R	**-0.629**	0.486	-0.556	-0.06	0.014	0.028
	P	0.028	0.109	0.06	0.854	0.966	0.931
Eating Opportunities	R	-0.258	0.445	0.31	0.05	-0.24	0.551
	P	0.419	0.147	0.327	0.877	0.452	0.063
24-Hour EB	R	0.119	0.007	0.296	0.354	-0.263	0.528
	P	0.713	0.982	0.351	0.259	0.409	0.078

Appendix K:
Numbers in bold contain a p value less than or equal to 0.05
Fat Mass_kg – fat mass in kg
FFM_kg – fat free mass in kg
FFM per kg - amount of FFM per kg of body weight
FFM to Ht ratio – amount of FFM per cm of height
Eating Opportunities – number of times athlete consumed calories
24 Hour EB – net kcal at the end of the day (energy consumed less energy expended)

APPENDIX L

NutriTiming® Data Entry Form

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

NutriTiming Activity Factor Scale

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

Begin Hour	End Hour	Activity Factor	Activity Description	Food/Drink Description	Food/Drink Amount
				****Begin Example****	
12am	7am	1.0	Sleep		
7am	8am	1.5	Nothing Special	Whole Wheat Waffles (Frozen-Kellogg)	3
				Maple Syrup	2 Tablespoons
				1 % Milk	1 Cup
				Orange Juice (from concentrate)	1.5 Cups
				Coffee	2 Cups
				1 % Milk for Coffee	2 Tablespoons
10am	11am	5.0	Jog 30 minutes	Gatorade	16 Ounces
12noon	1pm	1.5	Nothing Special	Medium size beef sandwich with white	1 Sandwich
				bread, mayonnaise, lettuce, and tomato.	
				Coffee	2 Cups
				Artificial Coffee Creamer	2 Packets
				Apple Pie	1 Slice (small)
5pm	6pm	4.0	Walk 1 hour	Water	16 ounces
7pm	8pm	1.5	Nothing Special	Lasagna with ground beef and cheese	Large Plate
				Lettuce Salad with Tomatoes and Cucumbers	Medium Size Salad
				Blue Cheese Salad Dressing	1 Tablespoon
				Red Wine	1 Medium Glass
10pm	11pm	1.5	Nothing Special	Popcorn (air popped; no butter)	100 Calorie Pack

NutriTiming® Data Entry Form

Name:______________________________ Age: ____Years Date of Birth:____/____/________
MM DD YYYY

Gender: M or F Height: ____Feet ___Inches Weight: ____ lbs.

Date Analyzed: ___/____/____ Time last meal eaten before date analyzed: ____________
M DD YYYY

Begin Hour	End Hour	Activity Factor	Activity Descriptions	Food/Drink Descriptions	Food/Drink Amounts

APPENDIX M

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999
Phone: 404/413-3500
Fax: 404/413-3504

In Person: Dahlberg Hall
30 Courtland St, Suite 217
April 16, 2014

Principal Investigator: Dan Benardot

Study Department: GSU - Nutrition

Study Title: Relationship Between Diet and Body Composition in Collegiate
Sand Volleyball Players.

Review Type: Expedited Continuing Review Category 7

IRB Number: H13383

Reference Number: 326989

Approval Date: 04/18/2014

Expiration Date: 04/17/2015

The Georgia State University Institutional Review Board (IRB) reviewed and approved the above referenced study in accordance with 45 CFR 46.111. The IRB has reviewed and approved the research protocol and any informed consent forms, recruitment materials, and other research materials that are marked as approved in the application for data analysis and follow-up only. The approval period is listed above. Research that has been approved by the IRB may be subject to further appropriate review and approval or disapproval by officials of the Institution.

Federal regulations require researchers to follow specific procedures in a timely manner. For the protection of all concerned, the IRB calls your attention to the following obligations that you have as Principal Investigator of this study.

1. For any changes to the study (except to protect the safety of participants), an Amendment Application must be submitted to the IRB. The Amendment Application must be reviewed and approved before any changes can take place

2. Any unanticipated/adverse events or problems occurring as a result of participation in this study must be reported immediately to the IRB using the Unanticipated/Adverse Event Form.

3. Principal investigators are responsible for ensuring that informed consent is properly documented in accordance with 45 CFR 46.116.

4. For any research that is conducted beyond the approval period, a Renewal Application must be submitted at least 30 days prior to the expiration date. The Renewal Application must be approved by the IRB before the expiration date else automatic termination of this study will occur. If the study expires, all research activities associated with the study must cease and a new application must be approved before any work can continue.

5. When the study is completed, a Study Closure Report must be submitted to the IRB.

All of the above referenced forms are available online at http://protocol.gsu.edu. Please do not hesitate to contact the Office of Research Integrity (404-413-3500) if you have any questions or concerns.

Sincerely,

Shelia L. White

Shelia L. White, IRB Member

Federal Wide Assurance Number: 00000129