# RELATIONSHIP BETWEEN TOTAL PROTEIN INTAKE AND ESSENTIAL AMINO ACID THRESHOLD FREQUENCY ON MEASURES OF MUSCLE MASS IN ADULTS BETWEEN 50-80 YEARS OF AGE

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RELATIONSHIP BETWEEN TOTAL PROTEIN INTAKE AND ESSENTIAL AMINO ACID THRESHOLD FREQUENCY ON MEASURES OF MUSCLE MASS AND FUNCTION IN ADULTS BETWEEN 50-80 YEARS OF AGE

The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Education with a major in Exercise Science.

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# ABSTRACT

Sarcopenia, loss of skeletal muscle in older adults, can lead to serious health consequences. Several non-pharmacological strategies have been suggested to prevent sarcopenia, one of which is increased protein consumption above the current RDA and/or reaching a certain threshold (≈10 grams of essential amino acids) of quality protein at each meal. This study examined the association between the amount of skeletal muscle mass in older active adults with regard to their total protein intake and essential amino acid threshold frequency (EAATF) (number of meals per day that provided at least 10 grams of essential amino acids). Fifty-one healthy male (n=22) and female (n=29) active older adults participated in this cross-sectional study. Average daily total protein (grams) and EAATF were determined from a seven-day dietary record. Dietary records and subsequent amino acid profiling were analyzed using a computer software program. Daily food records were averaged across seven-days to give an average representation of total protein intake and EAATF. Total and appendicular lean mass (aLM) were determined by Dual Energy X-Ray Absorptiometry (DXA). Relative lean mass (RLM, total lean mass/height$^2$) and appendicular lean mass index (aLM/height$^2$) were also calculated. Data were analyzed using Pearson Partial Correlation Coefficients controlling for body weight and physical activity level with an alpha level of 0.05. EAATF (1.1 $\pm$ 0.6 times/day) was positively associated with RLM ($r = 0.621$, $p < 0.001$) as well as aLM index ($r = 0.583$, $p < 0.001$). Total protein intake (91.1 $\pm$ 29.1) was also positively associated with RLM ($r = 0.582$, $p < 0.001$) and aLM index ($r = 0.598$, $p < 0.001$). Our data suggest that, not only total protein intake, but also consuming a certain threshold of quality protein with each meal throughout a day may be important for maintaining muscle mass during aging.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

# Chapter 1

## INTRODUCTION

Throughout the aging process, there is a shift in body composition where a decrease in skeletal muscle mass is accompanied by an increase in fat mass (Fielding et al., 2011). Without a significant amount of lean mass, activities of daily living become cumbersome and can lead to serious injury, which reduces the likelihood of maintaining independence in old age. The term sarcopenia refers to a significant loss of skeletal muscle mass with a reduction in physical function and muscle strength (Cruz-Jentoft et al., 2010; Zhong, Chen, & Thompson, 2007). For one to be considered sarcopenic, his or her relative amount of skeletal muscle mass must fall two standard deviations below the average young, healthy adult (Baumgartner et al., 1998). Based on this definition for sarcopenia, between 5 and 13 percent of older adults, between the ages of 60 and 70, are believed to be sarcopenic (Morley, 2008). The number of older adults affected by sarcopenia increases to about 50 percent for individuals who live to 80 years and older (Baumgartner et al., 1998; Morley, 2008).

A significant loss in skeletal muscle mass, strength and physical function can increase the risk of many health-related problems for older adults. These losses contribute to a decrease in posture, locomotion and balance, which can ultimately affect an older adult's ability to perform activities of daily living required to maintain physical independence (Brooks, Fahey, & Baldwin, 2005). A loss in mobility can also increase the risk of muscle injury or a fall (Brooks, Fahey, & Baldwin, 2005). A fall for older adults can lead to serious consequences such as bone fractures which may lead to an increase in morbidity and/or mortality (Robbins et al., 1989). Discovering practical and lifestyle-related factors that could assist in maintaining muscle mass and strength

could be one approach to decreasing the risks for immobility in older adults (Goodpaster et al., 2006).

Ingestion of dietary protein above the current Recommended Dietary Allowance (RDA) has been associated with maintenance of skeletal muscle mass in older adults (Campbell, Trappe, Wolfe, & Evans, 2001; Houston et al., 2008; Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009a). However, the current RDA may be too low for some older adults to support protein turnover and aid in the maintenance of muscle mass. (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2005). The current RDA of 0.8 grams of protein per kilogram of body weight (Institute of Medicine, 2005; Joint & Organization, 2007; Paddon-Jones & Rasmussen, 2009) is the same for every adult, regardless of age, sex or activity level (Joint & Organization, 2007). Furthermore, the RDA does not include recommendations regarding the type/quality of protein and/or the distribution of protein over a 24 hour period. Recent evidence suggests that both of these factors may be important for maintaining muscle health. For example, essential amino acids (EAAs) are responsible for the acute increase in muscle protein synthesis, which is maximized in older adults at a dose of approximately 10-12 grams (25-30 grams of a quality protein source) (Symons et al., 2009a; Wolfe, 2002). EAAs are proteins that cannot be produced by the body but instead need to be gained through dietary ingestion. EAAs come primarily from quality protein sources including dairy products, eggs, meat, and soy (Phillips, Moore, & Tang, 2007). This has led to recent studies recommending ingestion of 25-30 grams of a quality protein source at each meal, on about a 3 to 4 meal feeding schedule as a strategy to minimize muscle loss (Calvani et al., 2013; Dideriksen, Reitelseder, & Holm, 2013; Paddon-Jones & Rasmussen, 2009; Pennings et al., 2012; Symons, Sheffield-Moore, Wolfe, & Paddon-Jones, 2009b; Yang et al., 2012). While a range has been established, a set amount of total protein to be consumed has

not been agreed upon. The most important aspect to protein consumption is the amount of EAAs needed at each meal. Some researchers agree, the amount of EAAs consumed in one sitting, may need to be closer to 10-12 grams (Casperson, Sheffield-Moore, Hewlings, & Paddon-Jones, 2012; Dideriksen et al., 2013; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004).

### 1.1 Statement of the problem

A decline in skeletal muscle mass with age is common. Research shows that resistance exercises can help limit or prevent much of this loss if continued over a lifetime. One of the problems with resistance exercise involves the addition of activities to a person's daily life. This can be hard for adults to incorporate into a busy schedule. Also, many older adults have not participated in resistance exercise before and the idea of starting a routine could seem daunting. Therefore, researchers have looked at other methods to counter the loss of skeletal muscle mass in older adults that might be easier to incorporate into activities of daily living. One such recommendation is an increase in the consumption of dietary protein or consuming at least 10-12 grams of EAAs at each meal, throughout the day (Casperson, Sheffield-Moore, Hewling, & Paddon-Jones, 2012; Dideriksen et al., 2013; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004). While evidence suggests that increasing total protein intake is beneficial to maintaining muscle mass, few studies have been conducted that specifically sought to examine the consumption of 10-12g EAAs at each meal throughout a day.

### 1.2 Statement of purpose

The purpose of this study was to determine the association between measures of dietary protein intake and relative lean mass in older adults after statistically controlling for confounding variables such as physical activity level and body weight. Specifically, dietary protein intake was

examined at both the average daily total protein intake level as well as the average number of meals consumed containing at least 10 grams of EAAs over a seven day period.

### 1.3 Significance of study

Helping older adults maintain skeletal muscle mass is important to remaining independent. Meeting a certain EAA threshold at each meal could be a way to counter this skeletal muscle loss and help older adults maintain their level of physical function.

This is one of the few studies to specifically investigate consuming 10g EAAs at each meal and its association with the amount of relative skeletal muscle mass. Consuming higher levels of EAAs comes from an increase in quality protein. The quality of the protein being consumed could be a better predictor of skeletal muscle mass maintenance, which could narrow this life style change so it is more effective for older adults.

### 1.4 Variables

#### 1.4.1 Independent

- Total dietary protein intake and EAATF were the independent variables in this study.

#### 1.4.2 Dependent

- Relative lean body mass and appendicular lean body mass were the dependent variables in this study.

#### 1.4.3 Control

- One of the variables that was controlled in this study was age. All participants were between the ages of 50 to 80 years.
- Physical activity level was controlled for during statistical analysis by use of the Godin Leisure Time Exercise Questionnaire (Godin, Jobin, & Bouillon, 1985).
- Weight was controlled for during statistical analysis.

### 1.5 Hypothesis

There will be a significant relationship between the amount of relative skeletal muscle mass and the EAATF, after statistically controlling for body weight and physical activity level. will be greater in participants that consume higher levels of dietary protein and have a higher EAATF.

### 1.6 Assumptions

It was assumed that participants were truthful when filling out the pre-screening, health and physical activity questionnaires. Participants were expected to follow the instructions the morning before testing. It was also assumed that the participant filled out the dietary records truthfully and accurately and that the information filled out represents the normal diet of the participant.

### 1.7 Limitations

There are certain limitations to this study that need to be taken into account. Below is a list of such limitations.

- When interpreting the data reported on the dietary records, some participants were less specific than others. This led to the author assuming what was meant and trying to remain consistent with what was used for other participants.
- The dietary analysis software used did not have amino acid values for all the foods listed. Some of the foods had to be chosen based on what was the closest to that actual food or meals had to be pieced together to get all of the values needed. Different software, with more values included, could have helped to expand the options that the author had to choose from.
- Physical activity levels were self-reported and could have been incorrectly estimated.

- The research was conducted in a cross-sectional design which cannot explain a lifetime of dietary and physical activity habits.

**1.8 Delimitations**

- The participants used in the study were recruited from Wichita State University and the city of Wichita and surrounding areas. Resistance exercise training is known to help maintain or increase muscle mass and therefore, individuals with a history of resistance training were not included in this study.
- A population of younger adults was not studied in comparison to the age range determined. The author decided to focus on the age range of 50-80 to see if there were differences in skeletal muscle mass associated with increased levels of dietary protein intake.

**1.9 Definitions**

- Essential Amino Acids (EAAs) - Amino acids that the body needs to grow skeletal muscle but must be obtained through diet because they cannot be synthesized by the body.
- Quality Protein Source - Ratio of essential amino acids to dietary protein in grams (Loenneke et al., 2010; Loenneke et al., 2012)
- Sarcopenia – When an individual has skeletal muscle mass two standard deviations below the average for a young, normal population (Baumgartner et al., 1998). Sarcopenia is defined by how a loss of skeletal muscle mass affects volume of muscle, strength of muscle, and physical function (Cruz-Jentoft et al., 2010).
- Recommended Dietary Allowance (RDA) – The amount of a certain nutrient an individual needs to consume daily to meet their need based on age. This amount is

established by the Food and Nutrition Board of the National Academy of Science (Institute of Medicine, 2005).

- Total Lean Mass – The amount of non-bone body mass that does not include fat mass.
- Appendicular Lean Mass (aLM) – The sum of lean mass in the arms and legs.
- Relative Lean Mass (RLM) – The total amount of lean mass divided by weight to make it relative.
- Appendicular Lean Mass Index (aLM index) – The sum of lean mass in the arms and legs divided by weight to make it relative.
- Essential Amino Acids Threshold Frequency- Consuming at least a total of 10 grams of EAAs during a meal.
- Relative Total Protein Intake – Protein in grams per body mass in kilograms.

# Chapter 2

# REVIEW OF LITERATURE

## 2.1 Skeletal Muscle Structure and Function

There are approximately 660 skeletal muscles that make up the human body which comprise approximately 45 percent of a human's total body mass (Brooks, Fahey, & Baldwin, 2005). Making up such a large portion of the body, maintaining a healthy level of muscle mass is important to maintaining physical function over the life span. An important function of muscle is to generate the force necessary for the body to perform everyday activities that allow for physical independence. Furthermore, the muscles also help to maintain a state of bioenergetic homeostasis during both exercise and at rest (Brooks et al., 2005).

Skeletal muscles range in size and function but all have the same basic structural components. They are surrounded by a connective tissue called the epimysium, which provides a tough coat that separates the muscle from other muscles (MacIntosh, 2006). The skeletal muscle is broken down into bundles, called muscle fascicles, which are surrounded by a membrane called perimysium (Brooks et al., 2005). This membrane has two functions. First, it divides the muscle into bundles (MacIntosh, 2006). Second, the perimysium allows a pathway for the major nerves and blood vessels to run (MacIntosh, 2006). The muscle fascicles can further be broken down into muscle fibers, which are covered by a membrane called endomysium (MacIntosh, 2006). Finally, the muscle fiber can be broken down into even smaller sections called a myofibril, which are small sections of the single muscle fiber that are cylindrical in shape (MacIntosh, 2006).

The myofibrils are separated by the sarcoplasmic reticulum, which stores calcium to be used during muscle contraction (MacIntosh, 2006). They are also made up of a couple protective

layers and then the sarcomeres. The basement membrane is a glycoprotein layer (MacIntosh, 2006) that surrounds the interior of the muscle fiber (Brooks et al., 2005). The plasma membrane is the cell's boundary (Brooks et al., 2005). The basement membrane and plasma membrane make up the sarcolemma (MacIntosh, 2006). The plasma membrane is selective when it comes to letting different ions, solutes and substrates cross it (Brooks et al., 2005).

Below the plasma membrane is the sarcomere, which is the structure in the muscle where contraction occurs and is composed of thick and thin protein filaments called myosin and actin, respectively. Actin has regulatory proteins attached that control contraction that are known as tropomyosin and troponin (Brooks et al., 2005). Myosin is half of the protein content in the myofibril while actin makes up one fifth and the protein regulators, tropomyosin and troponin, make up an even smaller portion (Brooks et al., 2005).

Contraction of the muscle can be explained by the cross-bridge theory (Brooks et al., 2005). Myosin attaches to actin in an attempt to pull the two sides of opposing actin in the sarcomere towards each other (MacIntosh, 2006), with each of these cross-bridge cycles occurring independently of one another (Brooks et al., 2005). Myosin is the filament that moves during contraction (Sheetz & Spudich, 1983). There are proteins that stop myosin from attaching to actin all the time, otherwise the muscle would be in constant contraction. Tropomyosin is the protein that attaches to the binding sites on actin so myosin is not allowed to attach (Brooks et al., 2005). For contraction to occur, troponin must move tropomyosin off of these active binding sites which can only be achieved if calcium is allowed to flood the sarcomere (Brooks et al., 2005) because these ions activate protein kinase by attaching to special binding proteins (MacIntosh, 2006). It has been shown in frogs that calcium is important to contraction because muscle fibers did not contract in a potassium rich solution unless calcium was present (Frank,

1958). It has also been shown that when calcium is directed into the muscle fibers, localized contractions take place (Heilbrunn & Wiercinski, 1947). This process begins from the sodium potassium ion pump. This pump uses adenosine triphosphate (ATP) to push sodium out of the cell and allows potassium to enter at a three to two ratio, respectively (MacIntosh, 2006). Once this occurs, the cell becomes negatively charged and creates the circumstances for the whole contraction process to begin (MacIntosh, 2006).

**2.2 Skeletal Muscle Protein Turnover**

Protein turnover is the body's process of creating new proteins and breaking down old proteins. This process is continuous and simultaneous, with both synthesis and degradation occurring at the same time; these processes cause muscle proteins to undergo constant change and remodeling (Norton & Layman, 2006). The level of synthesis or degradation can be altered by different factors which lead to periods of either muscle growth or rebuilding/wasting.

Protein synthesis is a complex process that involves ribonucleic acid (RNA) (mostly messenger RNA) and the activation of various intracellular signaling pathways. There are three components that affect signaling and can either promote or hinder protein synthesis. First is the energy status of the muscle cell (Weinert, 2009). ATP is the main energy source of muscle, and when this energy source gets low, the rate of protein synthesis is reduced (Weinert, 2009). Adenosine monophosphate activated protein kinase (AMPK) is activated when levels of ATP are decreased (Weinert, 2009). This enzyme phosphorylates TSC2, which turns off one of the mammalian target of rapamycin (mTOR) signaling (Weinert, 2009).

The second component is insulin. Insulin secretion plays an indirect role in skeletal muscle protein synthesis by permitting glucose to enter the cell (Weinert, 2009). Insulin accomplishes this movement of glucose by activating the phosphoinositol-3-kinase (PI3K)

pathway, which translocates skeletal muscle's glucose transport protein (GLUT4) to the sarcolemma (Weinert, 2009). Within the PI3K pathway, mTOR is stimulated by the activation of the protein Akt (Weinert, 2009). This protein also phosphorylates and inactivates glycogen synthase kinase (GSK-3), allowing the activation of eukaryotic initiation factor 2B (eIF2B) (Weinert, 2009). The activation of both mTOR and eIF2B stimulate protein synthesis (Weinert, 2009). Insulin also has an effect on muscle protein synthesis by aiding the entry of amino acids into the cells (Weinert, 2009). Elevated insulin levels do not have much of an effect on the rate of protein synthesis in the absence of amino acids (Biolo, Fleming, & Wolfe, 1995).

The third component is amino acids and in particular the branched chain amino acid leucine (Weinert, 2009). Leucine concentrations have a stimulatory effect on mTOR activity (Norton & Layman, 2006). This influences the skeletal muscle's rate of protein translation and its translation capacity through mTOR activity on eIF4E and rpS6 (Norton & Layman, 2006); both eIF4F and rpS6 are major initiation factors in the process of protein synthesis (Norton & Layman, 2006). Anthony et al. (2000), has also shown that there is an increase in eIF4E availability for the formation of the active eIF4f complex through the oral administration of leucine.

Several factors that regulate the process of protein synthesis: exercise, nutrition and hormones. Exercise has been shown to increase muscle protein synthesis at a level that can be as great as two times that of resting values (Biolo, Maggi, Williams, Tipton, & Wolfe, 1995). Also, the greatest increases in protein synthesis are seen during the recovery phase of exercise versus the actual activity (Chesley, MacDougall, Tarnopolsky, Atkinson, & Smith, 1992). Resistance exercise increases muscle protein synthesis (Biolo et al., 1995; Norton & Layman, 2006) but does not affect AMPK (Norton & Layman, 2006). This type of exercise does not impair muscle

protein synthesis. Muscle protein degradation is not inhibited by exercise, which leads to a negative balance of protein turnover, but this value is closer to zero than it is at rest (Biolo et al., 1995). Therefore, exercise is more important for increasing the rate of protein synthesis than it is for degradation (Biolo et al., 1995). If essential amino acids are consumed following exercise, it can increase muscle protein synthesis, which allows muscle protein turnover to enter a net positive balance resulting in more muscle being formed than broken down. Adding these essential amino acids through nutrition is the most effective way to maximize the muscle protein synthetic response (Biolo et al., 1995; Biolo, Tipton, Klein, & Wolfe, 1997; Børsheim, Tipton, Wolf, & Wolfe, 2002). The combination of exercise, followed by ingestion of essential amino acids, is ideal. Post exercise has been shown to increase the transport rates of leucine, lysine, and alanine (Biolo et al., 1995). Leucine, in particular, is released more from the visceral tissue to the skeletal muscle after exercise (Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974).

Nutritional intake has an effect on the rate of muscle protein synthesis. The intake of essential amino acids and leucine has the most influence on muscle protein synthesis (Sugden & Fuller, 1991; Weinert, 2009). Some researchers believe the optimal proportions of essential amino acids are not known (Børsheim et al., 2002), while others believe 25g of a quality source of protein (milk products, meat, eggs or soy) that contain 10g of essential amino acids should be enough to maximally stimulate muscle protein synthesis after exercise (Phillips et al., 2007). Some sources of protein are more effective than others with whey protein having greater intracellular signaling than soy (Anthony et al., 2007) and greater intracellular levels of leucine than caesin or a control (Tipton et al., 2004). There are some discrepancies, with one study's results showing caesin having greater levels of phenylalanine uptake than whey protein (Tipton et al., 2004). The time frame for when these essential amino acids need to be ingested does not

seem to matter; for example, leucine has been shown to have a complete recovery effect of muscle protein synthesis within the first hour of ingestion after exhaustive exercise (Norton & Layman, 2006). Also, the increase in protein synthesis is the same whether it is one hour after exercise or three hours (Rasmussen, Tipton, Miller, Wolf, & Wolfe, 2000). Other dietary foods, such as carbohydrates, did not have an effect on the rate of protein synthesis (Anthony, Anthony, Kimball, Vary, & Jefferson, 2000; Børsheim et al., 2002; Norton & Layman, 2006). Nonessential amino acids did not have a significant effect on muscle protein synthesis (Børsheim et al., 2002; Norton & Layman, 2006; Rasmussen et al., 2000).

Insulin plays an important role in protein turnover. Insulin deficiency during fasting has been shown to inhibit protein synthesis (Sugden & Fuller, 1991). However, following ingestion of a protein-rich drink, blood insulin concentration increased significantly, which did not occur with a placebo group (Rasmussen et al., 2000). An adequate supply of amino acids is necessary for insulin to have any significant effect on rates of protein synthesis (Anthony et al., 2000; Rasmussen et al., 2000) but leucine does not need increased insulin concentrations to be effective (Rasmussen et al., 2000).

Protein degradation is the breakdown of muscle protein to free up individual amino acids, which can then be used to rebuild new muscle proteins. Depending on the type of exercise, there may be a small increase, usually about half of the increase that occurs with protein synthesis (Biolo et al., 1995; Weinert, 2009). Exercise stimulates muscle protein degradation to breakdown damaged muscle tissue which can then be replenished (Norton & Layman, 2006; Weinert, 2009).

In the process of exercise, degradation has more to do with rebuilding, while during fasting, degradation has more to do with muscle wasting. Ingestion of amino acids is shown to decrease muscle degradation rates in animals (Sugden & Fuller, 1991), while fasting increases

the rate of degradation (Li & Goldberg, 1976; Li & Wassner, 1984; Lowell, Ruderman, & Goodman, 1986; Preedy, Smith, & Sugden, 1986). Ingestion of amino acids does not seem to have a large effect on muscle degradation in humans (Rasmussen et al., 2000). Insulin is the one regulator of degradation that has the largest effect and has a greater affect inhibiting degradation than stimulating synthesis (Gelfand & Barrett, 1987; Tessari et al., 1987), but the mechanisms for insulin's inhibitory effect on protein degradation are not fully understood (Sugden & Fuller, 1991). There is also some evidence that shows that the removal of calcium from the cell may decrease protein degradation (Baracos, Greenberg, & Goldberg, 1986; Hasselgren, Säljö, & Seeman, 1986; Kameyama & Etlinger, 1979; Rodemann, Waxman, & Goldberg, 1982).

### 2.3 Changes in Skeletal Muscle with Aging

Minimizing the detrimental effects of aging has been an increasing area of research. One of the major areas that aging effects in the human body is the skeletal muscular system, which can lead to a decrease in the ability to perform daily activities that come with a decrease in posture and locomotion (Brooks, Fahey, & Baldwin, 2005). This immobility can come with any impairment to the skeletal muscles (Brooks & Faulkner, 1994) and loss in mobility increases the older adult's risk for muscle injuries or falls (Brooks, Fahey, & Baldwin, 2005). Half of the people over the age of 65 are estimated to experience a fall at least once a year (Campbell, Reinken, Allan, & Martinez, 1981; Tinetti, 1986). A fall in this age range can lead to an increase in morbidity and mortality (Robbins et al., 1989). Older adults who have a history of frequent falls have a significant decrease in muscle strength in power in the leg muscles that are associated with balance (Whipple, Wolfson, & Amerman, 1987). This impairment usually comes in the form of loss of skeletal muscle, which leads to reduced muscle strength (Evans & Lexell, 1995). Finding a method to maintain muscle strength, to decrease immobility, can counter these

problems and this comes from identifying what contributes to the loss of muscle strength in older adults (Goodpaster et al., 2006).

The composition of the human body changes through the aging process. In general, there is an increase in fat mass as human's reach midlife (Hughes, Frontera, Roubenoff, Evans, & Singh, 2002). Men have a significant increase in fat mass when they approximately reach the age of 50 compared to younger adults while women have this significant increase a little earlier in life at about the age of 40 (Lynch et al., 1999). This increase is associated with the time that women experience menopause; as studies have shown that post-menopausal women have significantly more fat mass than premenopausal women (Hughes et al., 2002). The change in fat mass represents approximately a 7.5 percent increase per decade of life (Hughes et al., 2002).

The functions and structures of skeletal muscle changes as well (Lexell, Taylor, & Sjöström, 1988). From mid-20s to approximately age 50, there is about a 10 percent decrease in muscle area with about a 50 percent decrease in muscle area from 50-80 (Lexell et al., 1988). Loss of skeletal muscle mass was significant in both older men and women (Frontera, Hughes, Lutz, & Evans, 1991; Hughes et al., 2001) but men have a greater percent change in muscle mass than women (Hughes et al., 2001; Janssen, Heymsfield, Wang, & Ross, 2000). For every kilogram of body weight that is lost or gained there is a loss or gain of fat free mass that is equivalent to 0.32 kg for men and 0.22 kg for women (Hughes et al., 2002). For men, the significant age decreases in muscle mass was in lower body and not the upper body (Janssen et al., 2000) and this loss of lean mass was greater than what is observed in women (Goodpaster et al., 2006). For women, the significant age decreases in muscle mass were in both the lower and upper body (Janssen et al., 2000).

Muscle mass values decrease as the human body ages. These decreases are about 25 to 30 percent less for a 65 year old compared to the peak values observed for those 25-30 (Brooks, Fahey, & Baldwin, 2005). Some believe that this loss in muscle mass can be as great as 40 percent between the second through seventh decade of life (Rogers & Evans, 1993). The loss of muscle mass is believed to be from either a loss of muscle fibers, atrophy of muscle fibers or a combination of both of these (Brooks, Fahey, & Baldwin, 2005). The general consensus is that type II fibers atrophy the most with age (Lexell et al., 1988; MacIntosh, 2006; Rogers & Evans, 1993) and there is an average 39 percent reduction in fibers from the ages of 20-80 years (Lexell et al., 1988). A study done by Frontera et al. (2000) concluded that a reduction in type I fibers may be present. This research group observed a significant decrease in capillary-to-fiber ratio in their longitudinal study (Lexell et al., 1988). However, others have shown no significant reduction in type I fibers with age (Lexell et al., 1988). There seems to be a shift between appearance and make-up of the fibers in the muscle as aging occurs. From 20-50 years of age, the appearance of the fibers in the muscle are normal (Lexell et al., 1988); as there are no abnormalities within the fibers (Lexell et al., 1988). By the age range of 70 to 80, there were variations in fiber shape (Lexell et al., 1988) and the area of the muscle that is composed of muscle fibers had changed between these two age groups as well. For the younger age group, approximately 70 percent of the muscle was composed of muscle fiber, while the muscles in the older age range were approximately 50 percent composed of muscle fibers (Lexell et al., 1988). There also seems to be a decrease in the number of sarcomeres in the fibers (Hooper, 1981) which decreases the length of the fibers at rest (MacIntosh, 2006).

When it comes to a decreases of muscle strength with age, there are several factors that need to be taken into account. Older adults can recruit muscle neurons as effectively as younger

adults (MacIntosh, 2006), which means this is not a reason for the observed decrease in muscle strength with aging. Body composition, in particular loss of muscle mass, and muscle fiber atrophy/loss are the two areas that are focused on when studying muscle strength. There is also a loss of contractile proteins (Frontera et al., 1991), with a slower turnover of myosin in particular, (MacIntosh, 2006) that could be a reason for decrease in muscle strength.

The decrease in muscle mass can account for about a five percent decrease in muscle strength (Goodpaster et al., 2006; Hughes et al., 2001). However, when weight or lean mass is gained, there is not necessarily an increase in muscle strength (Goodpaster et al., 2006). Some believe that between the second and seventh decades of life there is approximately a 30 percent decrease in muscle strength (Rogers & Evans, 1993), but there are contradictions on what the average annual decline of strength is for adults. Frontera et al. (2000) found a rate of 1.4 to 2.5 percent depending on the muscle group (Frontera et al., 2000). Yet, Goodpaster et al. (2006) found that white males had a rate of decline of 3.42 percent, black male 4.12 percent, white female 2.65 percent, and black female 2.97 percent (Goodpaster et al., 2006) and men typically lost more leg muscle mass with age than women (Goodpaster et al., 2006).

Lower body muscles around the knee are the area where the most muscle strength is lost, but the amount of loss is not entirely known. For the knee extensors and flexors, there is a 23.7 to 29.8 percent decrease in muscle strength with age (Frontera et al., 2000). Some studies show that this rate is similar for men and women (Hughes et al., 2001). Another study examined knee extensor and flexor strength by men and women and obtained different results. For older men, the percent decline was between 20.0 and 22.0 percent while older women were between 17.6 and 15.5 percent (Frontera et al., 1991). For elbow strength, the percent decrease is greater in men than in women (Hughes et al., 2001). Arm and leg peak torque was significantly lower in

older adults when compared to young adults (Lynch et al., 1999) and was significant for both concentric and eccentric peak torque (Lynch et al., 1999).

Looking at these studies, there are some limitations that should be noted. Looking at change in lower body muscle, longitudinal studies show a greater decrease than cross-section studies, which can be as much as a 60 percent difference (Hughes et al., 2001). Also, for most of these studies it can be hard to control for variables such as a person's nutrition, physical activity level, disease, etc. (Lexell et al., 1988).

**2.4 Sarcopenia**

As aging occurs, there are body composition changes that are consistent. Two of these changes are an increase in fat mass and reduction in lean mass (Fielding et al., 2011). A reduction in muscle mass can lead to serious problems in older populations, such as, the ability of an older adult to maintain an independent lifestyle and an increased risk for fall. When depletion of muscle mass is below two standard deviations of the mean for young, normal adults it is considered serious (Baumgartner et al., 1998). This condition is termed sarcopenia; sarco is used to refer to the word flesh while penia is used to refer to a reduction in amount or need of something (Evans, 1995).

Traditionally, most of the  focus has been on the amount of muscle lost when determining sarcopenia, but changes in functional capability also need to be considered (Cruz-Jentoft et al., 2010). Most definitions of sarcopenia include multiple components: a decline in muscle mass, a reduction in muscle strength, and a reduction in physical function (Cruz-Jentoft et al., 2010; Zhong et al., 2007). The one limitation with this definition is that it has not been researched across different racial and ethnic groups (Van Kan, 2009). When looking at how much lean mass is lost, sarcopenia is defined as a 3-8 percent reduction every decade after the age of 30 (Paddon-

Jones & Rasmussen, 2009). This rate can be accelerated by disease, disuse and anorexia (Narici & Maffulli, 2010). Others use lean muscle mass below the 20th percentile for values of healthy, young adults as a diagnosis for sarcopenia (Castillo et al., 2003). This definition makes sarcopenia a good predictor of disability or falls later in life (Castillo et al., 2003; Fielding et al., 2011). For people in the age range of 60 to 70 years, it is believed that 5-13 percent have sarcopenia (Morley, 2008). This percentage jumps to about 50% for older adults over the age of 80 (Baumgartner et al., 1998; Morley, 2008). Also, women seem to be at higher risk for developing sarcopenia than men (Narici & Maffulli, 2010). There are several factors that contribute to sarcopenia, which include: nutrition, physical activity, hormones, genetic heritability, insulin resistance, atherosclerosis and proinflammatory cytokines (Cooper et al., 2012).

Within the condition of sarcopenia there are several different types or level of severity. The main one that is talked about in literature is obese sarcopenia, which occurs when lean mass is lost but fat mass is actually constant or increased (Prado et al., 2008; Zamboni, Mazzali, Fantin, Rossi, & Di Francesco, 2008). This was actually found to be a better predictor of falls or physical disabilities than either obesity or sarcopenia alone (Baumgartner et al., 2004). People in this category struggle because they have to use more force to move the excess body fat that they have while having lower muscle mass available to perform daily activities (Narici & Maffulli, 2010). There is also primary and secondary sarcopenia. Primary sarcopenia is due solely to the aging process (Cruz-Jentoft et al., 2010), while secondary sarcopenia is when circumstances other than just aging cause a loss in lean mass, muscle strength and decrease in physical function (Cruz-Jentoft et al., 2010). On top of these, sarcopenia can be broken down into three categories. The first is pre-sarcopenia, which is when only muscle mass is affected but not muscle strength

or physical function (Cruz-Jentoft et al., 2010). Sarcopenia is the next phase, which incorporates low muscle mass with either low muscle strength or a decrease in physical function (Cruz-Jentoft et al., 2010). Finally, severe sarcopenia is a combination of all three of these characteristics: low muscle mass, low muscle strength and a decrease in physical function (Cruz-Jentoft et al., 2010).

There are several different types of assessments that are used to determine if someone has sarcopenia. For determining lean mass, there are a couple of different methods and most indirectly measure lean mass through body imaging, which assume that skeletal muscle remains a constant percentage of fat free mass, which is incorrect (Heymsfield & Waki, 1991). To avoid this, appendicular skeletal mass that has been corrected for height can be used (Fielding et al., 2011). Dual energy X-ray absorptiometry (DXA), computed tomography (CT) and magnetic resonance imaging (MRI) can be used as indirect ways to determine body composition (Cruz-Jentoft et al., 2010). CT and MRI are the gold standards for estimating muscle mass, but DXA is the preferred method used (Cruz-Jentoft et al., 2010) and bioimpedance analysis can be used if a more portable method is needed (Cruz-Jentoft et al., 2010). A method that is less commonly used for estimating skeletal muscle is total or partial body potassium per fat-free soft tissue (Cruz-Jentoft et al., 2010). Finally, anthropometric measurements can be used but are not recommended since they are accompanied by increase in error (Cruz-Jentoft et al., 2010).

Muscle strength does not have as many options as techniques for measuring muscle mass. One technique used is grip strength, which is a good measure for a couple of reasons. First, it is a simple measurement (Cruz-Jentoft et al., 2010). Second, it correlates with leg strength (Cruz-Jentoft et al., 2010). Another technique would be to measure leg strength directly through knee flexion and extension (Cruz-Jentoft et al., 2010). While this measurement is accurate, it needs special equipment, which is not easily portable or cheap to perform (Cruz-Jentoft et al., 2010).

The final way to measure muscle strength is to use peak expiratory flow (Cruz-Jentoft et al., 2010); this method measures respiratory muscle strength but cannot be recommended at this time for lack of research (Cruz-Jentoft et al., 2010).

The last area to test for sarcopenia is physical function. Short physical performance battery is used as a measure of physical performance and measuring gait speed is an example of this method (Cruz-Jentoft et al., 2010). Another example is the Timed Get-up and Go Test, which involves the subject starting in chair (Cruz-Jentoft et al., 2010). The subject has to stand up, walk a distance, then return to the chair and sit down (Cruz-Jentoft et al., 2010).

### 2.5 Current Protein Requirements for Older Adults

The current recommendation for adult dietary protein intake is 0.8 grams of protein per kilogram of body weight each day (Joint & Organization, 2007; Paddon-Jones & Rasmussen, 2009) and is the same for every adult regardless of age or sex (Joint & Organization, 2007). Short duration nitrogen studies were done in young adults by the Institute of Medicine to determine the daily dietary recommendation for adults (Rand, Pellett, & Young, 2003; Trumbo, Schlicker, Yates, & Poos, 2002) with the purpose of this recommendation being to prevent the dietary protein deficiency in 97% of the population (Bauer et al., 2013). Studies have been done to examine if this amount of protein is in fact adequate for older adults with many groups, such as the PROT-AGE working group, believing this recommendation is actually too low for older individuals (Bauer et al., 2013). This can be illustrated by the fact that skeletal muscle makes up 45 percent of the total body weight in young adults but drops to 27 percent of total body weight in older adults over the age of 70 years (Chernoff, 2004). Others argue, that while there is a difference in lean body mass among groups, like men, women, younger and older adults, body weight offsets these differences (Trumbo et al., 2002).

Nitrogen studies are conducted by documenting the intake of nitrogen from protein, then allowing at least five days for the individual to adjust to the intake of nitrogen, followed by determining the amount of nitrogen excreted (Bauer et al., 2013). The problem with this type of study is determining if adaptation or accommodation occurred. Adaption is a metabolic change that happens when a change in protein intake occurs to reach a steady-state but without a compromise or loss of physical function (Campbell et al., 2001). Adaption is the desired response from these studies to show that protein intake is adequate. Accommodation is more of a survival response where metabolic changes compromise physical function to reach a new steady state when protein intake is decreased (Campbell et al., 2001). The easiest way to determine this is by looking at nitrogen excretion (Campbell et al., 2001). For accommodation to have occurred nitrogen excretion would have had to decreased over long periods of time (Campbell et al., 2001).

One study found that the RDA's recommendation for protein intake was adequate for older adults by showing changes in nitrogen excretion that were consistent with changes in mid-thigh skeletal muscle area (Campbell et al., 2001). However, this study also supports the idea that with higher protein intake, lean body mass can be better maintained in older individuals (Campbell et al., 2001). Other studies are in agreement that ingesting more dietary protein than the recommended amount can lead to good health, maintenance of functionality, and promote recovery from illness in older individuals (Chernoff, 2004; Gaffney-Stomberg, Insogna, Rodriguez, & Kerstetter, 2009; Kurpad & Vaz, 2000; Morse, Haub, Evans, & Campbell, 2001). For example, older women had fewer health problems when they consumed 1.2 grams of protein per kilogram of body weight every day when compared to older women who consumed 0.8 grams of protein (Morse et al., 2001) and other studies have seen this same effect (Vellas et al.,

1997). Also, increasing dietary protein above the recommended amount has been shown to optimize muscle mass and strength (Gaffney-Stomberg et al., 2009). For example, Morse et al. (2001) estimated that a protein intake of 0.90 gram per every kilogram of body weight a day was needed (Morse et al., 2001).

Another concern is that older adults are not consuming even enough protein to meet the current recommendation (Fulgoni, 2008; Rousset, Patureau Mirand, Brandolini, Martin, & Boirie, 2003; Vikstedt et al., 2011), which seems to be a trend, especially among older Americans (Fulgoni, 2008). There does seem to be some inconsistency among older women with one study showing that both young and old women had inadequate intake of dietary protein (Fulgoni, 2008), while another showed that older women had higher intake of dietary protein than younger women (Rousset et al., 2003). This particular study, also showed that older men had lower intake of dietary protein than younger men (Rousset et al., 2003). Still a third study, showed that both older men and women, who were residents of a service house, had inadequate levels of dietary protein intake (Vikstedt et al., 2011). Some believe that protein may be difficult for older adults to consume for a couple of reasons: difficulty chewing or tearing protein rich foods, the cost of protein, intolerance to certain foods, or fear of consuming too much cholesterol or fat (Chernoff, 2004).

### 2.6 Protein Intake and Skeletal Muscle Mass in Older Adults

Protein intake is an important aspect for all adults. Weight loss studies have shown the many benefits from high protein diets, an example being a decrease in fat mass (Johnston, Tjonn, & Swan, 2004; Loenneke et al., 2010; Paddon-Jones et al., 2008; Pasiakos et al., 2013). These results were also reproduced in several long-term studies (Due, Toubro, Skov, & Astrup, 2004; Skov, Toubro, Rønn, Holm, & Astrup, 1999) but the participants had to be in an energy deficit

state (Paddon-Jones et al., 2008; Pasiakos et al., 2013). High protein diets have been seen to increase satiety which may be why they are more effective for weight loss (Johnston et al., 2004; Paddon-Jones et al., 2008). These effects have been reported more in high protein / low fat diets compared to high carbohydrate / low fat diets, although the outcome on body composition was about the same and weight loss was maintained for both of the above mentioned groups (Johnston et al., 2004).

While losing fat mass is important, an individual also needs to be able to maintain fat-free mass. This is important for being able to function, especially later in life. There is a positive association between lean mass and quality protein intake (Hulmi, Lockwood, & Stout, 2010; Loenneke et al., 2010) with high protein diets increasing the maintenance of fat-free mass (Loenneke et al., 2010; Pasiakos et al., 2013). High protein groups also show hypertrophy in both type I and type II muscle fibers (Hulmi et al., 2010) and some show that fat-free mass is spared when higher levels of protein are consumed (Pasiakos et al., 2013). One study showed an opposite effect, where the high protein group lost just as much fat-free mass as the high carbohydrate group (Johnston et al., 2004); this decrease was about four percent of the participant's fat-free mass (Johnston et al., 2004). There was an increase until the consumption of 1.6 grams protein per kilogram body weight per day, but did not increase any more at 2.4 grams protein per kilograms of body weight per day (Pasiakos et al., 2013). Therefore, there seems to be a threshold where more protein does not have an added effect on the maintenance of lean mass.

The added benefit in maintaining muscle mass from high protein diets are that these types of diets may be easier for people to maintain. Studies have reported that compliance was better for high protein groups (Johnston et al., 2004) & 2) with many participants reporting not eating

additional food items when on the high protein diet (Johnston et al., 2004). Also, these types are easier to maintain in the long run because it is an adaption to the individuals lifestyle that is more feasible (Paddon-Jones et al., 2008). There are added benefits with being able to maintain a diet that promotes weight loss. One of these is a decrease in risk for diseases like diabetes or cardiovascular (Rodriguez & Garlick, 2008).

Diets high in protein have been reported to positively affect the adult population in general, but how do these diets affect older adults in particular? Older adults who do not have an adequate intake of protein have been shown to have a greater loss of skeletal muscle cross-sectional area (Campbell et al., 2001), a reduction in muscle repair, muscle wasting, and reduced protein synthesis (Thalacker-Mercer, Fleet, Craig, Carnell, & Campbell, 2007) as well as reductions in lean muscle mass, body cell mass and skeletal muscle mass (Carmen Castaneda, Charnley, Evans, & Crim, 1995; Carmen Castaneda, Dolnikowski, Dallal, Evans, & Crim, 1995; C Castaneda, Gordon, Fielding, Evans, & Crim, 1999). Ingestion of quality protein above the RDA has been reported to be essential for older adults to maintain muscle mass and remain functional (Symons et al., 2009a). However, the amount of protein consumed with each meal does not have to be high, because a moderate size portion (113 g) was seen to have the same effects on muscle protein synthesis rates as a high portion (340 g) (Symons et al., 2009a). Higher protein diets have also been shown to increase skeletal muscle mass and body cell mass in a group of older women who consumed 0.92 grams of protein per kilogram of body weight per day for a nine week period (Campbell et al., 2001). In older individuals, whose muscle mass was reduced, the consumption of amino acids from protein only have been seen to stimulate muscle protein anabolism (Volpi, Ferrando, Yeckel, Tipton, & Wolfe, 1998). Protein consumption was also the only macronutrient seen to have an inverse relationship with a difference in waist

circumference in older adults (Halkjær, Tjønneland, Thomsen, Overvad, & Sørensen, 2006), but this relationship was only seen with animal protein and not vegetable protein (Halkjær et al., 2006). Most studies show positive effects from protein consumption, like the studies mentioned above. However, not all studies are in agreement on these beneficial effects. For example, one study showed no beneficial effect from a high protein diet on muscle protein synthesis (Walrand et al., 2008).

### 2.7 Adverse Effects of High Protein Diets

There has been some concern as to the potential deleterious effects of high protein diets. From a health stand point, the most commonly purported are a decrease in bone mineral density (increased risk of osteoporosis) and impaired kidney function. However, more recent work in the area of dietary protein intake and bone health actually point to the contrary, reporting that individuals who consume low protein diets are at risk for impaired bone health and function (Kerstetter, O'Brien, & Insogna, 2003). With regard to impaired kidney function, the Institutes of Medicine's standpoint based on current research evidence suggests that the level of dietary protein intake is not associated with impaired kidney function with ageing (Institute of Medicine, 2005). However, it is well known and accepted that individuals with pre-existing renal disease should not consume a diet high in protein. (Workeneh & Mitch, 2013).

### 2.8 Proposed Protein Feeding for Older Adults

Studies suggest that there needs to be an increase the amount of protein consumption with age to help prevent skeletal muscle loss (Cuthbertson et al., 2005; Dideriksen et al., 2013; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004; Pennings et al., 2012; Symons et al., 2009b; Yang et al., 2012) and it has been suggested that the most effective way to accomplish this is to balance out total consumption among 3 to 4 meal (Cuthbertson et al., 2005;

Dideriksen et al., 2013; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004; Pennings et al., 2012; Symons et al., 2009b; Yang et al., 2012). A decrease in stimulation in muscle protein synthesis in older adults compared to young adults has been reported, which has led to researchers suggesting older adults may need to increase their consumption of EAAs per meal (Katsanos et al., 2005). EAAs come primarily from quality protein sources which can be defined as the ratio of EAAs to dietary protein in grams (Loenneke et al., 2010; Loenneke et al., 2012).

While there are currently recommendations for the amount of protein needed in a day, there are no recommendations for the type of protein or an amount of protein needed on a meal to meal basis (Loenneke et al., 2010; Loenneke et al., 2012). However, there is a threshold amount of EAAs that can be consumed, since after a certain point, excess amino acids have no effect on stimulating muscle protein synthesis (Layman, 2009). Based on these data, several researchers have been advocating that protein intake should be recommended, not only by total protein intake per day, but also for a recommendation to consume at least 10 grams of EAA's (25-30 grams of quality protein) per meal (Paddon-Jones, 2009).

For younger adults, differences in muscle protein synthesis can be seen between ingestion of 10 grams of quality protein versus 5 grams of quality protein, but after about 20 grams of quality protein there is no increase in muscle protein synthesis (Moore et al., 2009). For older adults, some believe that 20 grams of protein is the amount that needs to ingested at each meal (Dideriksen et al., 2013; Yang et al., 2012) while others think this number is a little higher and should be about 30 grams of protein (Paddon-Jones & Rasmussen, 2009; Symons et al., 2009b). One study even showed increases in skeletal muscle protein synthesis at about 35 grams of protein for each meal (Pennings et al., 2012). There can even be a greater increase if the older adult participates in resistance exercise with a dose of 40 grams of whey protein being shown to

have a greater increase on skeletal muscle protein synthesis than any of the lower doses (Yang et al., 2012). The combination of resistance exercise and protein feeding results in rates of skeletal muscle protein synthesis much higher that protein feeding alone (Yang et al., 2012). The main recommendation that most researchers agree on, is that the amount of EAAs that need to ingested at each meal should be somewhere between 10 and 15 grams (Cuthbertson et al., 2005; Dideriksen et al., 2013; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004). However, few longitudinal or intervention studies have been investigated to determine the benefits of consuming a certain threshold of EAA's with each meal.

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# Chapter 3

# METHODS

## 3.1 Participants

Seventy-one apparently healthy and active older adults between the ages of 50-80 were recruited for this study. Recruiting took place by distributing email, word of mouth and delivering flyers around the university campus and city of Wichita and surrounding areas. Upon responding to the initial recruitment advertisement, participants were pre-screened for inclusion/exclusion by phone or email (Appendix A). After meeting the initial criteria, participants were scheduled for their one-time visit to the Human Performance Laboratory to fill out an Informed Consent form (Appendix B), and questionnaires used to further determine study eligibility (Appendix C & D), to undergo testing, and to be educated on how to properly track and document seven days' worth of their dietary consumption. All methods and procedures were approved by the Wichita State University Institutional Review Board (IRB No. 2967). Subject inclusion and exclusion criteria were as follows:

**Inclusion Criteria**

1. Adults between the ages of 50 and 80 years of age.
2. Free of any overt medical condition such as cancer, heart disease, diabetes, etc.
3. Cyclists had to cycle at least 3 times a week with an average of 60 miles per week. They also had to participate in the activity for at least 4 years.
4. Runners had to run at least 3 times a week with an average of 25 miles per week. They also had to participate in the activity for at least 4 years.

**Exclusion Criteria**

1. Having any overt medical condition such as diabetes, heart disease, cancer, etc.

2. Regularly taking anti-inflammatory medication.
3. Regularly taking steroidal medication.
4. Smoked in the last year.
5. Been instructed by a physician not to perform physical activity.

## 3.2 Procedures

### 3.2.1 Questionnaires

Once the participants made it through the pre-screening process, an appointment was scheduled for testing. The participants were called 48 hours before their day of testing to remind them of their appointment. They were also given directions to the institute and instructions for the morning before testing.

Upon arrival to the laboratory, participants filled out a health history and status questionnaire and physical activity readiness questionnaire (Thomas, Reading, & Shephard, 1992) to insure that they met all criteria and testing would not be harmful to them. They also filled out the Godin Leisure-Time Exercise Questionnaire (Godin et al., 1985) as an estimate of physical activity level.

### 3.2.2 Anthropometric Measures

After paperwork was filled out, height and weight were obtained for all participants. Shoes and clothing other than shorts and a t-shirt were removed to obtain the most accurate measurements possible. Height was measured using a calibrated standiometer and weight was measured using a calibrated clinical digital scale.

### 3.2.3 Body Composition

Subjects underwent dual energy x-ray absorptiometry scans (DXA) (Discovery A, Hologic Inc., Bedford, MA, USA) of the whole body to determine percentage body fat (%BF),

relative bone free lean body mass (RLM) [total bone free lean body mass (kg)/height$^2$(m)], and appendicular lean tissue mass (aLM) for calculation of aLM index [aLM (kg)/ height$^2$(m)]. Quality assurance testing (QA) and calibration was performed the morning of data collection days to ensure that the DXA was operating properly. Participants were not allowed to wear metal because it would affect the results for the DXA. Other guidelines the participant was required to follow were to stay hydrated, do not eat four hours before testing, and to not perform physical activity the morning before testing. All of these factors would also have an effect on body composition measures. Test-retest reliability using intraclass correlation coefficient ($ICC_{3,1}$), standard error of the measure (SEM), and minimal difference to be considered real were previously determined from 17 subjects scanned twice 24 hours apart for %BF (0.99, 0.49 %, and 0.95%), total bone free lean body mass (0.99, 0.42 kg, and 1.16 kg) and aLT (0.99, 0.21 kg, and 0.58 kg).

### 3.2.4 Dietary Intake

All participants were educated on tracking and documenting their dietary intake and sent home with a seven-day diet log, serving size guide and stamped pre-addressed envelope. They were asked to fill out a total of seven consecutive days (two weekend days and five week days) of their dietary intake. All participants were asked to record any nutrient intake throughout the day under the appropriate meal as well as recording the portion size. All diet logs were analyzed for total kilocalories, grams (g) of protein, carbohydrate, and fat using a computer software program (Nurtibase, V.11, Cybersoft Inc., Phoenix, AZ) and then averaged over the 7 days to calculate the average daily intake of each of these variables. In addition, amino acid profiling for each meal was determined and used to calculate the number of times (meals) per day that an individual consumed at least 10 grams of essential amino acids in one sitting. If a participant

consumed at least 10 grams of EAAs in one meal, this was considered meeting the EAAFT. The total number of meals that included at least 10 grams of EAAs were added through the day and the EAATF was then averaged across the seven days. Relative total protein intake was also calculated using the participant's average total protein intake for the week in grams and dividing it by the participant's body mass in kilograms.

### 3.3 Statistical Analysis

All data analyses were performed using SPSS for Windows version 20.0 (IBM, Seattle, WA). Data are presented as mean ± standard deviation (SD). All dependent variables were evaluated for normality of distribution using the Kolmogorov-Smirnov procedure. Subsequently, all dependent variables were normally distributed. Sex comparisons of the main dependent variables were completed using an independent samples t-test. Pearson Partial Correlation Coefficients and the adjusted Coefficient of Determination, controlling for body mass and physical activity level, were run to determine the association between average daily protein intake variables and relative indices of lean mass. An alpha level of $p \leq 0.05$ was considered statistically significant.

# Chapter 4

# RESULTS

## 4.1 Participants

The study originally enrolled 71 participants. However, due to participants failing to return food logs or filling out incomplete information, the final number of participants that were included in the analysis was 51. The sample was comprised of 22 men and 29 women which is equal to 43 percent and 57 percent, respectively. Participant characteristics are presented in table 4.1. Men were significantly taller by an average of 20 cm ($p < 0.001$) and heavier by an average of 10.7 kg ($p = 0.013$). However, average body mass index ($p = 0.628$) was not significantly different between sex. Based on results from the Godin Leisure Time Questionnaire, physical activity level was not significantly different between men and women ($p = 0.114$). Results from body composition showed a significant difference between men and women. Women had significantly lower total lean mass ($p < 0.001$), appendicular lean mass ($p < 0.001$), relative lean mass ($p < 0.001$), and aLM index ($p < 0.001$). Baumgarter et al. (1998), gives an aLM index of 7.26 $kg/m^2$ for men and 5.45 $kg.m^2$ as thresholds for sarcopenia. Based on these thresholds one male and one female met this classification. The one body composition measure that was higher in women than men was percent body fat ($p < 0.001$).

TABLE 4.1

PARTICIPANT DESCRIPTIVE AND PHYSICAL CHARACTERISTICS

| Variables | Total (n=51) | Men (n=22) | Women (n=29) | P-Value |
|---|---|---|---|---|
| Age (yr) | 60.2 ± 7.3 | 61.5 ± 7.1 | 59.2 ± 7.3 | 0.268 |
| Height (m) | 1.7 ± 9.6 | 1.8 ± 7.3 | 1.6 ± 5.6 | 0.001* |
| Body Mass (kg) | 73.8 ± 15.7 | 79.9 ± 13.9 | 69.2 ± 15.5 | 0.013 |
| Body Mass Index ($kg/m^2$) | 25.7 ± 4.7 | 25.3 ± 3.6 | 26.0 ± 5.4 | 0.628 |
| Body Fat (%) | 26.0 ± 8.7 | 19.0 ± 4.1 | 31.4 ± 7.3 | 0.001* |
| Total Lean Body Mass (kg) | 51.6 ± 11.5 | 61.4 ± 8.9 | 44.2 ± 6.7 | 0.001* |
| Relative Lean Body Mass ($kg/m^2$) | 17.9 ± 2.6 | 19.6 ± 2.0 | 16.6 ± 2.2 | 0.001* |
| aLM (kg) | 22.6 ± 5.6 | 27.6 ± 4.1 | 18.8 ± 3.2 | 0.001* |
| aLM Index ($kg/m^2$) | 7.8 ± 1.3 | 8.8 ± 0.9 | 7.0 ± 1.0 | 0.001* |
| PA Scores | 47.9 ± 28.7 | 55.2 ± 26.8 | 42.3 ± 29.3 | 0.114 |

Values are expressed as means ± standard deviation. PA: physical activity, aLM: appendicular lean mass.
*Significant Value

### 4.2 Dietary Intake

Table 4.2 displays the averages for dietary intake for the total number of participants, men and women. There was no significant difference in relative total protein intake ($p = 0.145$) between sex. There was a significant difference in caloric intake ($p = 0.029$), total protein intake ($p < 0.001$), and EAA threshold frequency ($p = 0.002$) between sex. Based on the RDA for protein intake, 6% of the participants from this study were not consuming the amount of protein that is recommended daily.

TABLE 4.2

DIETARY PROTEIN INTAKE AND CALORIC CONSUMPTION

| Variable | Total (n=51) | Men (n=22) | Women (n=29) | Mean Difference | P-Value |
|---|---|---|---|---|---|
| Caloric Intake (kcal/day) | 2000.0 ± 523.3 | 2181.7 ± 571.9 | 1862.1 ± 445.2 | 319.6 | 0.029 |
| Total Protein (g/day) | 91.1 ± 29.1 | 107.2 ± 28.7 | 78.8 ± 23.0 | 28.4 | 0.001* |
| Relative Total Protein (g/day) | 1.3 ± 0.5 | 1.4 ± 0.4 | 1.2 ± 0.4 | 0.2 | 0.145 |
| EAATF (times/day) | 1.1 ± 0.6 | 1.4 ± 0.5 | 0.9 ± 0.5 | 0.5 | 0.002* |

Values expressed as means ± standard deviation. EAATF: Essential Amino Acid Threshold Frequency.
*Significant Value

After statistically controlling for physical activity level and body weight, EAATF (1.1 ± 0.6) was significantly and positively associated with RLM ($r = 0.621$, $p < 0.001$) (Figure 4.1) as well as aLM index ($r = 0.583$, $p < 0.001$) (Figure 4.2). Total protein intake (91.1 ± 29.1) was also significantly and positively associated with RLM ($r = 0.582$, $p < 0.001$) (Figure 4.3) and aLM index ($r = 0.598$, $p < 0.001$) (Figure 4.4). Total relative protein intake (1.3 ± 0.5) was not significantly associated with RLM ($r = -0.038$) (Figure 4.5) or aLM index ($r = 0.040$) (Figure 4.6).

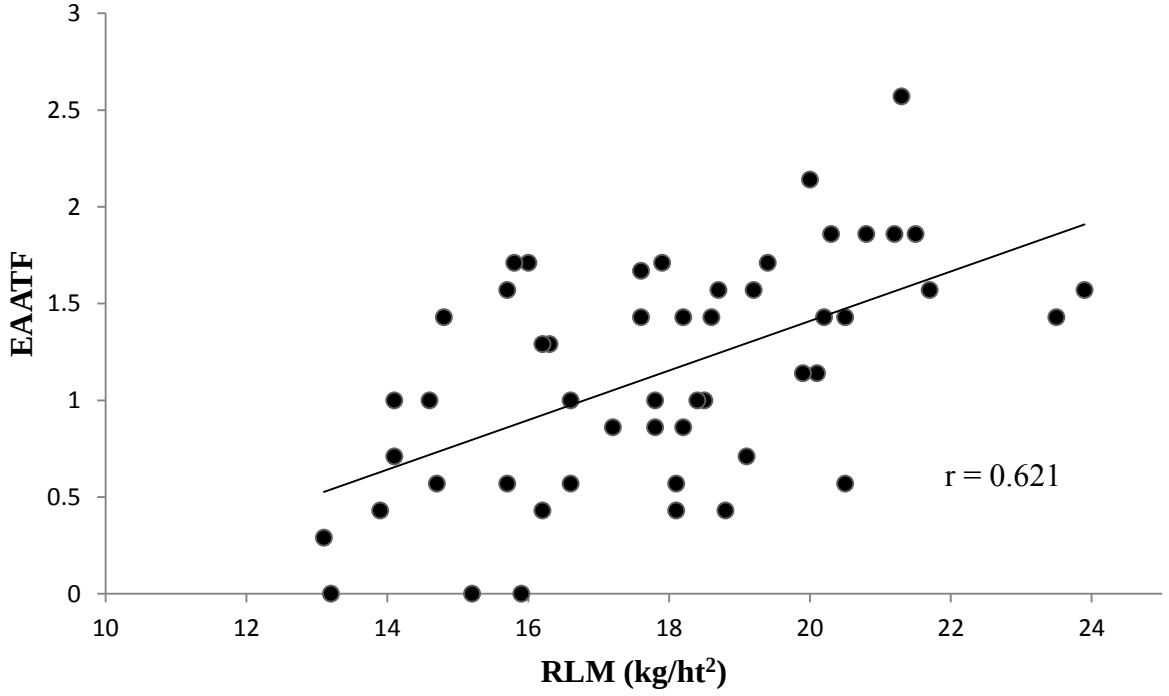


Figure 4.1 Comparison of essential amino acid threshold frequency and relative lean mass. Physical activity level and body weight were controlled for. Line of best fit was added.

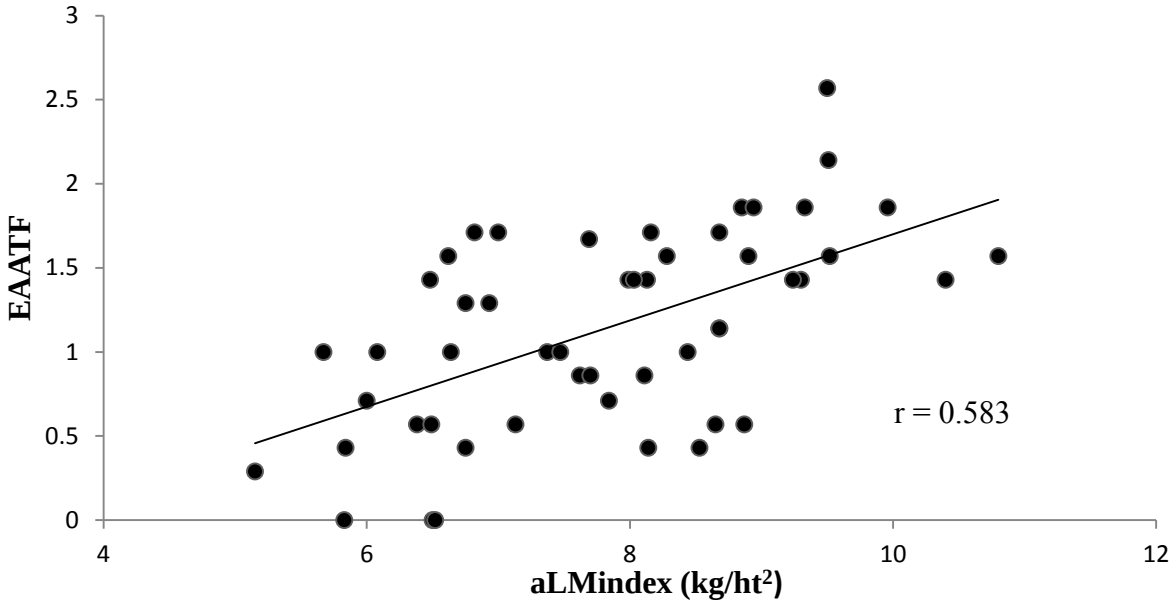


Figure 4.2 Comparison of essential amino acid threshold frequency and appendicular lean mass index. Physical activity level and body weight were controlled for. Line of best fit was added.

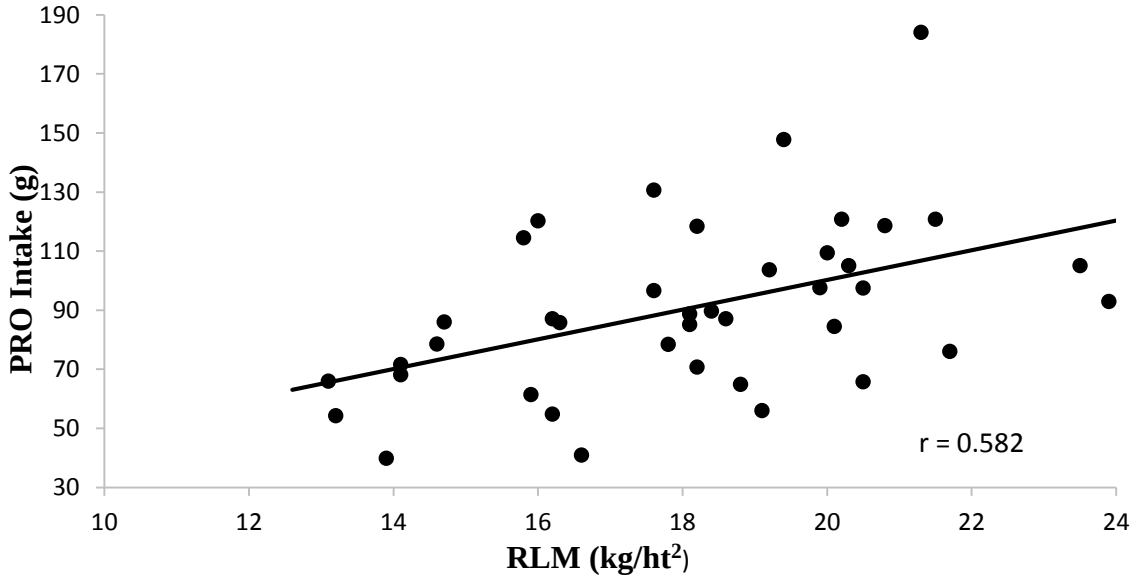


Figure 4.3 Comparison of total protein intake and relative lean mass. Physical activity level and body weight were controlled for. Line of best fit was added.

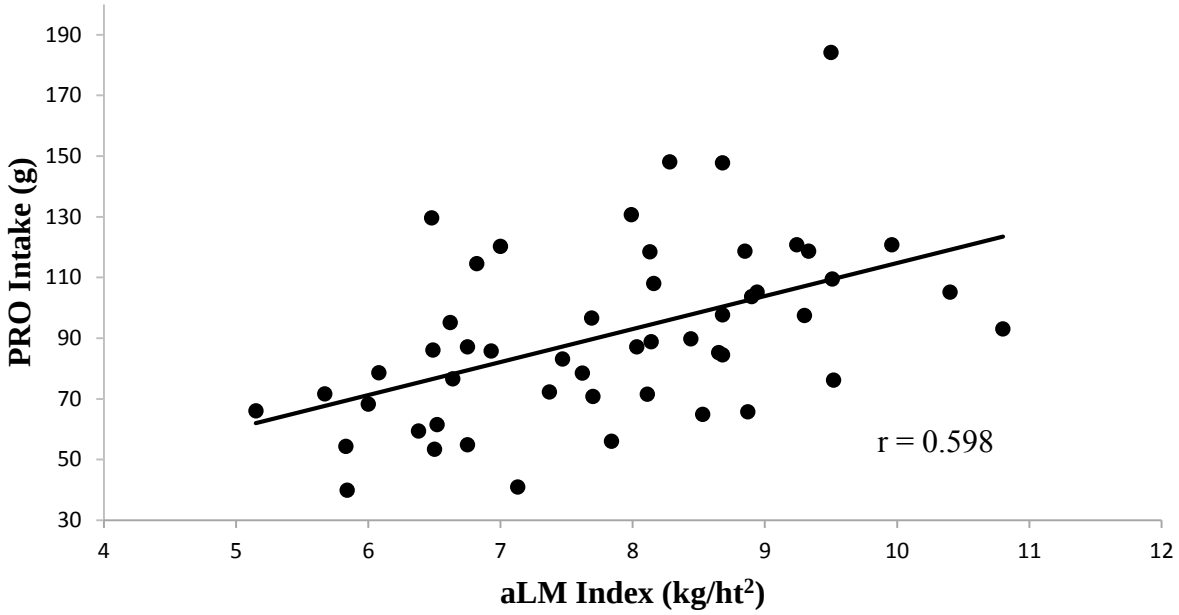


Figure 4.4 Comparison of total protein intake and appendicular lean mass index. Physical activity level and body weight were controlled for. Line of best fit was added.

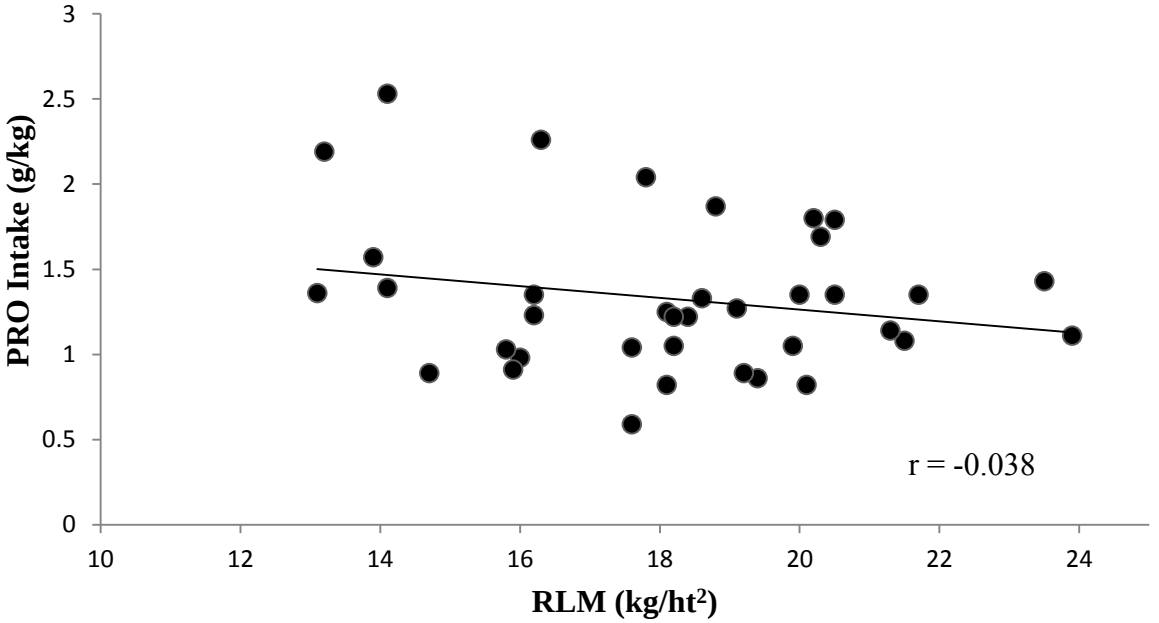


Figure 4.5 Comparison of relative protein intake and relative lean mass. Physical activity level and body weight were controlled for. Line of best fit was added.

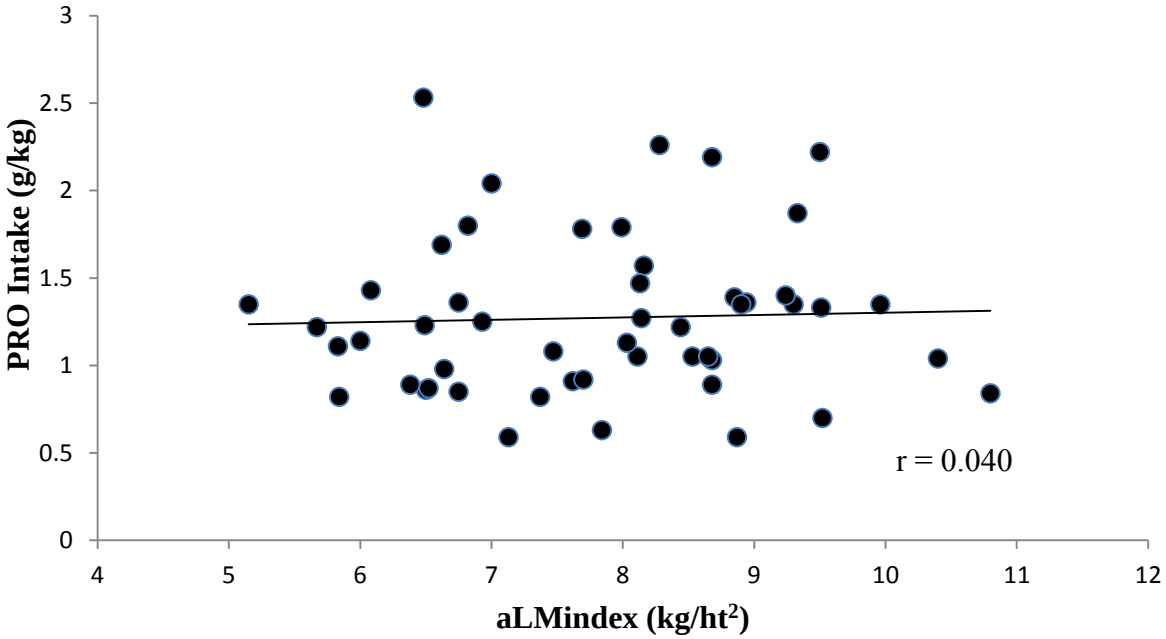


Figure 4.6 Comparison of relative protein intake and appendicular lean mass index. Physical activity level and body weight were controlled for. Line of best fit was added.

# Chapter 5

## DISCUSSION

The main objectives of this study was twofold. First to examine the relationship of total daily protein intake to relative lean mass and appendicular lean mass index levels. Second, to examine the relationship between the essential amino acid threshold frequency and relative lean mass and appendicular lean mass index. While there are many studies that examine an increase in dietary protein consumption and distribution of protein throughout each meal, these usually focus on protein consumed in grams. They do not examine the influence of reaching a certain threshold of EAAs tat each meal. This study is unique in that dietary records were analyzed using essential amino acid profiles for each meal. Participants also underwent a DXA scan to measure levels of relative body lean mass and appendicular lean mass index.

Our findings show a positive association between EAAFT compared to RLM and aLM index. The positive correlation between skeletal muscle mass and reaching a certain threshold (10 grams EAAs) of protein intake per meal is in agreement with other studies (Cuthbertson et al., 2005; Dideriksen et al., 2013; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004). A study similar to this one examined protein distribution per meal throughout the day and found that most adults consumed enough protein during the day to meet the RDA but they did not meet the 30 grams of protein per meal (Valenzuela et al., 2013). Appendicular skeletal mass differed between the groups that met the daily distribution and the group that did not (Valenzuela et al., 2013). Paddon-Jones & Rasmussen (2009) also studied the relationship between protein distribution throughout the day and lean body mass. Their consensus was that consuming a certain amount of protein per meal (25-30 grams) was more important than total daily protein consumption (Paddon-Jones & Rasmussen, 2009). The major difference between these studies

and ours is that they used a total amount of protein per meal where we used an amount of EAAs as a threshold for meeting protein needs per meal.

A positive correlation was also found with total protein consumed compared to RLM and aLM index. These findings are also in support of other studies performed (Cuthbertson et al., 2005; Dideriksen et al., 2013; Houston et al., 2008; Paddon-Jones & Rasmussen, 2009; Paddon-Jones et al., 2004; Pennings et al., 2012; Symons et al., 2009b; Yang et al., 2012). For instance, the ABC study found that adults who consumed protein in the highest quintile lost the least amount of lean mass and appendicular lean mass over a 3 year period (Houston et al., 2008). Another study found that older adults in the lower quintile for protein consumption were more likely to be frail (Bartali et al., 2006). The difference between these studies and this one is they only examined total daily protein intake and do not look at daily distribution.

These findings are important because maintaining skeletal muscle mass with age is important to remaining independent and reducing risk for fall. There is a consistent decline in skeletal muscle mass with age as total body weight goes from 45 percent to 27 percent from young adults to older adults over the age of 70, respectively (Chernoff, 2004). It is believed that somewhere between 5 to 13 percent of adults ages 60 to 70 are effected by sarcopenia (Morley, 2008) while 50 percent of adults above the age of 80 are effected by sarcopenia (Baumgartner et al., 1998; Morley, 2008). Currently, it is common for most older adults to only reach this threshold at dinner time, which may be one reason for loss of muscle mass over time (Figure 5.1) (Layman, 2009). Increased quality protein intake distributed evenly throughout 3 or 4 meals per day could be a viable solution for older adults to help maintain muscle mass and minimize loss of muscle function during aging (Figure 5.2) (Layman, 2009).

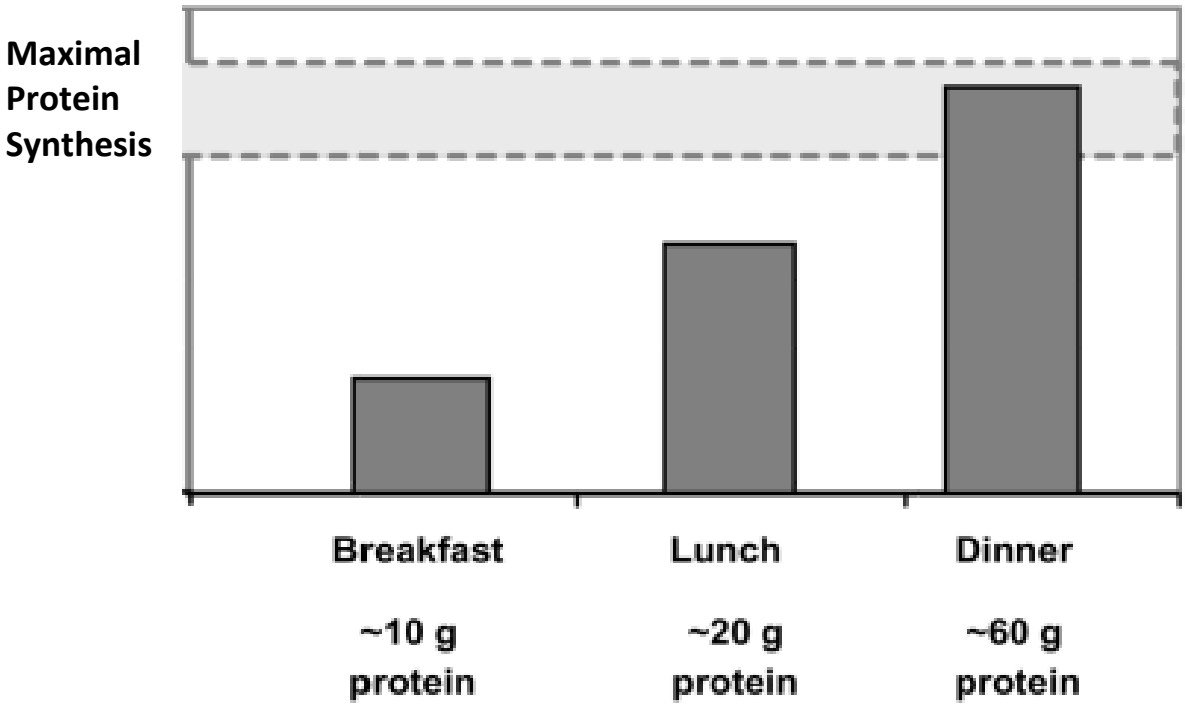


FIGURE 5.1 Current trends in PRO intake throughout the day (Layman, 2009)

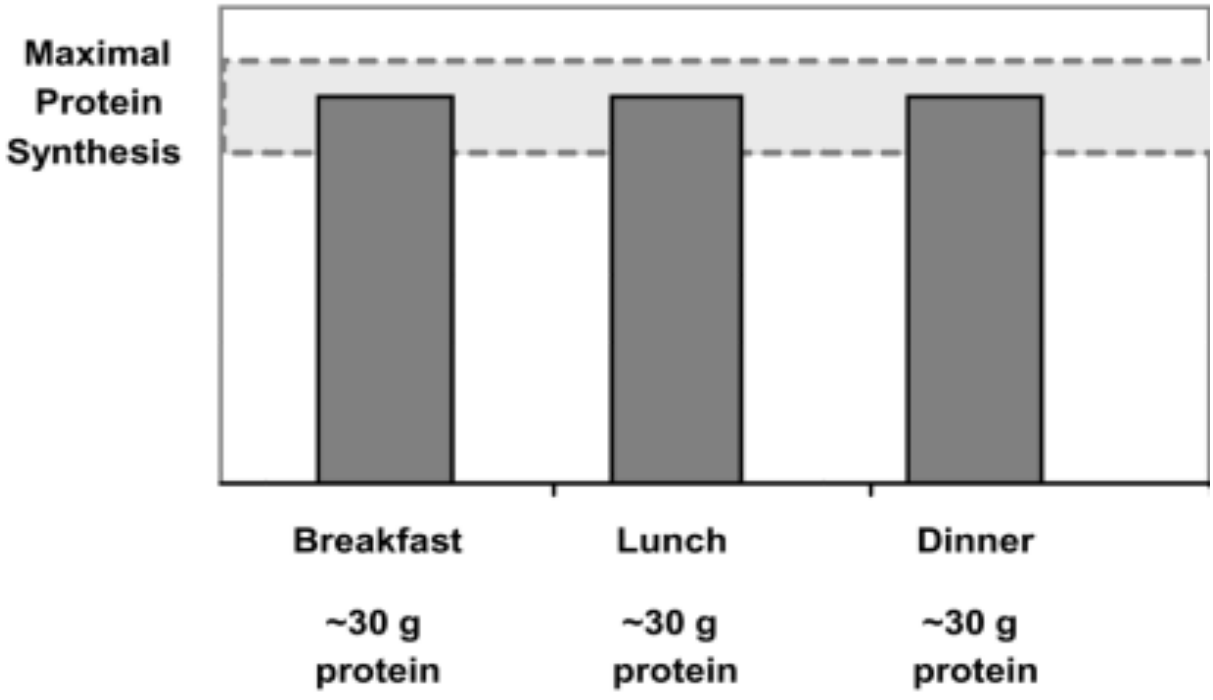


FIGURE 5.2 Proposed PRO intake throughout the day (Layman, 2009)

The results of this study suggest that increased protein consumption above the current RDA may help older adults maintain skeletal muscle mass. Consuming quality protein, which consists of dairy products / eggs / meat / soy, during each meal of the day to meet the EAA threshold frequency may be a solution for preventing sarcopenia. This type of lifestyle change, with the addition of staying physically active, would be beneficial for aging adults to maintain physical independence and a higher quality of life.

It is a limitation that the design of this study is cross-sectional and does not reflect a life time of dietary and physical activity habit. Future studies in this area may include more of a longitudinal component to control for flaws in cross-sectional designs. Also, information in dietary records was self-reported. There is a difference in the level of specificity between participants. This could have led to inaccurately estimating amount and types of foods the participant consumed. The dietary analysis software also had flaws in that it did not have all the foods that participants listed and some of the foods that the software did have did not include amino acid profiling. This led to trying to match those foods with the closest food possible to try and obtain accurate results. Different software may be better for analysis in future studies. Physical activity levels were also self-reported and could have overestimated.

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# APPENDIX

## APPENDIX A

Pre-screening Checklist

1. Do you have any injuries, joint, or orthopedic conditions that would limit you from doing a strength or physical function test?
    a. Yes No
2. Have you ever been diagnosed with diabetes, heart disease, cancer, or any other pertinent medical condition?
    a. Yes No
3. Do you use any prescription anti-inflammatory or steroidal medication regularly?
    a. Yes No
4. Do you use any form of androgen, testosterone, or hormone replacement therapy?
    a. Yes No
5. Have you ever been told by your physician that you shouldn't to do exercise?
    a. Yes No
6. Do you currently smoke or smoked in the past year?
    a. Yes No

Master's athletes specific:

1. How long have you been training (years, months etc.):_____
2. How many days per week do you cycle/run:_____
3. On average, how many miles do you run/cycle per week: _____
4. Do you compete in running/cycling events? Yes No

# APPENDIX B

Wichita State University
Institutional Review Board Approval
9/4/13 - 8/25/14

WICHITA STATE UNIVERSITY
COLLEGE OF EDUCATION
*Department of Human Performance Studies*

## Informed Consent Form

### Exercise and Nutritional Parameters Related to Sarcopenia and Osteoporosis Status

**PURPOSE:** You are being invited to participate in a research study that is investigating certain exercise and nutritional parameters and how they relate to bone and muscle health in older adults. This research study will be conducted at Wichita State University in the Human Performance Laboratory. You are being asked to participate because you are between the ages of 18-30 or 50-75 years and you may or may not exercise regularly and participate in competitive events. Approximately 120 people will be enrolled.

*You do not have to participate in this research study. It is important that before you make a decision to participate, you read the rest of this form. You should ask as many questions as needed to understand what will happen to you if you participate in this study.*

**PROCEDURES:** If you decide to participate in this study, you will be asked to sign this consent form that allows us to gather information from you and perform the procedures described below. You will be asked to fill out four questionnaires: a health status and history questionnaire, a physical activity readiness questionnaire, a 7-day leisurely exercise recall questionnaire, and a 7-day food record that you will complete at home and return upon completion (for individuals 50-75 years only). The health history questionnaire and physical activity readiness questionnaire will be used to determine whether or not you can participate in the study. The 7-day leisurely exercise recall questionnaire will be used to determine your level of weekly physical activity. The 7-day food record is used to determine nutritional parameters that we are investigating. Following completion of the questionnaires, you will have your height and weight, and circumferences of your arms, legs, waist and hips assessed. Next, you will be asked to undergo a dual energy x-ray (DXA) scan which measures your bone health as well as body composition. For this test, you will lie down on the bed of the DXA machine for approximately 10 minutes while the scan arm passes over you several times taking images of your skeleton. The DXA scanner emits a low radiation dose that presents minimal exposure to you about one-tenth of what you would get in a chest x-ray. Therefore, if you are female and between the ages of 18-30 years, you will be asked to provide a small urine sample which will be used to make sure you are not pregnant. Following the DXA scan, you will have your muscle and fat thickness assessed at several sites on your leg, arm, back, and torso using a hand-held ultrasound. This procedure is similar to what a woman who is pregnant would have to capture an image of the growing fetus. Following the ultrasound testing, you will be asked to undergo several physical function tests such as a timed walking test as well as a maximal strength of the upper leg test. The maximal leg strength test is performed in a machine that you will sit in and you will contract your leg muscles as hard as you can. These tests will take about 15 minutes to complete. In total, your visit to the laboratory will take approximately 60-90 minutes to complete.

**DISCOMFORT/RISKS:** You will be exposed to a very small amount of radiation during the DXA scan. The radiation exposure is small compared with other commonly accepted medical procedures such as chest x-rays, lumbar spine x-rays, and dental bite wings. In fact, patient dose is even smaller than exposure to natural background radiation. The amount of radiation that you will receive from this procedure is equivalent to a uniform whole-body exposure of 0.1 mrem, with the exposure being 2.4-4.8 mrem per hip (femur) and spine scan. The typical radiation exposure from a normal chest x-ray is 30 mrem. Although you will have a small amount of exposure, the risk from radiation exposure of this magnitude is too small to be measured

directly and is considered to be negligible when compared with other everyday risk. We also want to make sure that the amount of radiation that you have received in the past year is within safe limits, so if you have had an x-ray, let us know. If you have recently undergone CT (Computerized Tomography), PET, fluoroscopic, or nuclear medicine studies within the past year, you cannot have a DXA scan. If you are currently pregnant, or receive a positive pregnancy test prior to assessment, you cannot have a DXA scan. Other changes during DXA assessment may include but are not limited to motion sickness (lightheadedness, nausea) due to the mechanical movement of the DXA machine or muscle discomfort due to body position. The Radiation Safety Officer (Dr. Glendon Miller, 978-3347) of Wichita State University can provide you with more information about radiation exposure if you are interested. In addition to the discomfort/risks associated with the DXA assessment, you may also experience some discomfort during the maximal strength assessments including local muscle fatigue or muscle strain. All the researchers are trained in maximal strength assessments which will minimize the discomfort that you may experience. Furthermore, you will go through a proper muscular warm-up prior to having the strength assessment which will help to minimize any type of muscle strain.

**BENEFITS:** The DXA assessment will provide information pertaining to your bone health and body composition. The information received is not intended to diagnose osteopenia, osteoporosis, and/or obesity. It is suggested that you share the information obtained from you DXA assessment with your primary care physician. Upon your request, you will be given an additional copy of all assessment information for the purpose of physician consultation. The information gained in this study will be particularly beneficial to health care professionals and to the general population to have a better understanding of how physical activity and nutrition relate to bone and muscle health over the lifespan.

**CONFIDENTIALITY:** Any information obtained in this study in which you can be identified will remain confidential and will be disclosed only with your permission.

**COMPENSATION OR TREATMENT:** Wichita State University does not provide medical treatment or other forms of reimbursement to persons injured as a result of or in connection with participation in research activities conducted by Wichita State University or its faculty, staff, or students. If you believe that you have been injured as a result of participating in the research covered by this consent form, you can contact the Office of Research and Technology Transfer, Wichita State University, Wichita, KS 67260-0007, telephone (316) 978-3285.

**REFUSAL/WITHDRAWAL:** Participation in this study is entirely voluntary. Your decision whether or not to participate will not affect your future relations with Wichita State University. If you agree to participate in this study, you are free to withdraw from the study at any time without penalty.

**CONTACT:** If you have any questions about this research, you can contact me: Dr. Kaelin Young, office #106A, Heskett Center, telephone (316) 978-3343. If you have questions pertaining to your rights as a research subject, or about research-related injury, you can contact the Office of Research and Technology Transfer at Wichita State University, Wichita, KS 67260-0007, telephone (316) 978-3285.

You are under no obligation to participate in this study. Your signature indicates that you have read the information provided above and have voluntarily decided to participate. You will be given a copy of this consent form to keep.

_____ _____
Signature of Subject Date

_____ _____
Witness Signature Date

## APPENDIX C

# *Human Performance Laboratory*

# *WSU Department of Human Performance Studies*

# *Health Status Questionnaire*

**Instructions** Complete each question accurately. All information provided is confidential. Your information will not be used for anything that is not study-related.

**Part 1. Information about the individual**

1._____

Date

2. _____

Legal name

3._____

Mailing address

_____

Phone # (Best # to be contacted) Email

4.Gender (circle one): Female Male

5. Year of birth: _____ Age _____

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

What activities below best describe your physical job duties?

Sitting at desk Lifting or carrying loads Standing Walking Driving

**Part 2. Medical history**

7. Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8. Date of: Last medical physical exam: _____ (Year) Last physical fitness test: _____ (Year)

9. Circle operations you have had:

| | | | | | |
|---|---|---|---|---|---|
| Back | Heart | Kidney | Eyes | Joint | Neck |
| Ears | Hernia | Lung | Other _____ | | |

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

| | | |
|---|---|---|
| Alcoholism | Diabetes | Kidney problem |
| Anemia, sickle cell | Emphysema | Mental illness |
| Anemia, other | Epilepsy | Neck strain |
| Asthma | Eye problems | Obesity |
| Back strain | Gout | Osteoporosis |
| Bleeding trait | Hearing loss | Phlebitis |
| Bronchitis, chronic | Heart problems | Rheumatoid arthritis |
| Cancer | High blood pressure | Stroke |
| Cirrhosis, liver | Hypoglycemia | Thyroid problem |
| Concussion | Hyperlipidemia | Ulcer |
| Congenital defect | Infectious mononucleosis | Other _____ |

11. Circle all medicine taken in last 6 months:

| | | |
|---|---|---|
| Blood thinner | Epilepsy medication | Nitroglycerin |
| Diabetic pill | Heart-rhythm medication | Estrogen/Birth control |
| Digitalis | High-blood-pressure medication | Thyroid |
| Diuretic | Insulin | Corticosteroids |
| Asthma | Testosterone/Anabolic Steroids | Other _____ |

12. Any of these health symptoms that occur frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

1 = Never/Almost Never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood
1 2 3 4 5

b. Abdominal pain
1 2 3 4 5

c. Low back pain
1 2 3 4 5

d. Leg pain
1 2 3 4 5

e. Arm or shoulder pain
1 2 3 4 5

f. Chest pain
1 2 3 4 5

g. Swollen joints
1 2 3 4 5

h. Feel faint
1 2 3 4 5

I. Dizziness
1 2 3 4 5

j. Breathless with slight or mild exertion
1 2 3 4 5

13. Do any of the following apply:

- A sudden death in your biological father or brother, or mother or sister prior to age 55 or 65, respectively? Yes
  No
- Current smoker or have you quit smoking within the past 6 months? Yes
  No
- Do you take hypertensive medication or have a confirmed systolic or diastolic blood pressure $\geq$140 or 90 mmHg, respectively? Yes
  No
- Take lipid lowering medication or have high blood cholesterol? Yes
  No
- You have a confirmed fasting blood glucose of $\geq$100 mg/dL? Yes
  No
- Have you recently been diagnosed as clinically obese (BMI > 30)? Yes
  No
- Are you sedentary? Yes
  No
- Diagnosed Crohn's or Inflammatory Bowel Disease Yes No
- Past fracture of a hip, pelvis, or femur Yes No
- Major Surgery within the last 6 months Yes No
- Been diagnosed with varicose veins Yes No

– Family history of Deep Vein Thrombosis or Pulmonary Embolism Yes

No

**Part 3. Health-related behavior**

14. (RF) Do you now smoke or chew tobacco? Yes No

15. If you are a smoker, indicate number smoked per day:

| | | | | |
|---|---|---|---|---|
| Cigarettes: | 40 or more | 20-39 | 10-19 | 1-9 |
| Cigars or pipes only: | 5 or more or any inhaled | | Less than 5, none inhaled | |

16. Weight now: _____lb. One year ago: _____lb.. Age 21: _____lb.

17. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

18. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

19. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 20 and 21) 2. No (stop)

20. How many days per week (circle)?

1 2 3 4 5 6 7

21. Circle the type (s) of exercise that best describes what you participate in.

A. Aerobic Exercise (walking/jogging/Cycling etc.) B. Weight-lifting C. Sport-specific activities D. Yoga/Stretching

22. If you circled "C" above, please list the types of sport-specific activities:_____

23. If you are a competitive or collegiate-athlete, Please list the total # of years you have been competing in your sport.

# of Years Competing:_____

24. On average, how many minutes do you spend exercising each day: _____ minutes

**APPENDIX D**

# Physical Activity Readiness Questionnaire (PAR-Q)

NAME:_____ DATE:_____

HEIGHT:_____ cm WEIGHT:_____ kg AGE:_____

**PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)**

| | Questions | Yes | No |
|---|---|---|---|
| 1 | Has your doctor ever said that you have a heart condition and that you should only perform physical activity recommended by a doctor? | | |
| 2 | Do you feel pain in your chest when you perform physical activity? | | |
| 3 | In the past month, have you had chest pain when you were not performing any physical activity? | | |
| 4 | Do you lose your balance because of dizziness or do you ever lose consciousness? | | |
| 5 | Do you have a bone or joint problem that could be made worse by a change in your physical activity? | | |
| 6 | Is your doctor currently prescribing any medication for your blood pressure or for a heart condition? | | |
| 7 | Do you know of any other reason why you should not engage in physical activity? | | |

*If you have answered "Yes" to one or more of the above questions, consult your physician before engaging in physical activity. Tell your physician which questions you answered "Yes" to. After a medical evaluation, seek advice from your physician on what type of activity is suitable for your current condition.*