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COMPARISON OF MODELS IN DETERMINING SARCOPENIA STATUS IN OLDER ADULTS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

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ABSTRACT

COMPARISON OF MODELS IN DETERMINING SARCOPENIA STATUS IN OLDER ADULTS

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Sarcopenia is defined as the age-related loss of muscle mass and function. The purposes of this study were to examine the consistency among the four different sarcopenia classification models and explore new variables to improve sarcopenia classification, to determine the effects of aging on body composition, functionality, muscle quality, handgrip strength, and skeletal muscle index (SMI) and determine the relationships among muscle mass, functionality, mobility, muscle quality, handgrip strength, and SMI. Ninety-one women (age = 68.5±7.9 yrs; height = 162.1±6.5 cm; weight = 64.7±11.1 kg) and 76 men (age = 70.7±6.2 yrs; height = 176.1±6.6 cm; weight = 82.8±10.6 kg) volunteered to participate in one of two separate studies: a two-phase clinical trial (phase one = A08, n=53; phase two = A09, n=54) sponsored by Abbott Nutrition conducted in 2008 and 2009 entitled "Evaluation of AN777 in Elderly Subjects," and a clinical trial (G10, n=60) sponsored by General Nutrition Corporation conducted in 2010 entitled "Effects of Whey Protein Supplementation on

body Composition, Muscular Strength, and Mobility in Older Adults." Participants completed body composition, handgrip strength, functionality and mobility, and bench press and leg press 1-repetition maximum (1-RM) strength assessments. A full body dual-energy x-ray absorptiometry (DEXA) scan was completed to assess total body lean mass (LM), total body fat mass (FM), and appendicular lean mass (ALM). Additional calculations included estimated total body skeletal muscle (TBSM), non-skeletal muscle lean mass, and SMI (ALM/ht^2). Handgrip strength was measured as the average of the two highest of three trials using a hand-held digital or hydraulic handgrip dynamometer with their dominant hand. The timed get-up-and-go (TGUG) was performed on a measured and marked 3-meter course using an armless wooden chair and a digital stopwatch. Bench press and leg press strength were assessed using a five-repetition maximum (5-RM) protocol on a standard Olympic bench and 45° hip sled, respectively, 5-RM was then used to estimate 1-RM strength. Participants were classified as sarcopenic or non-sarcopenic using four different cut-off value criteria established by Baumgartner et al. (1998), Delmonico et al. (2007), and two methods by Newman et al. (2003): (a) ALM/ht^2 and (b) residuals method. Handgrip muscle quality (HGMQ), upper- and lower-body muscle quality (UMQ and LMQ, respectively) were also calculated as maximal strength divided by dominant arm muscle mass, total arm muscle mass, or total leg muscle mass, respectively. Fourteen separate two-way analyses of variance (ANOVA) (gender [men vs. women] x age [50s vs. 60s vs. 70s vs. 80s]) were used to analyze LM, FM, ALM, TBSM, handgrip strength, TGUG, SMI, SMI residuals, non-skeletal muscle lean mass, bench press and leg press 1-RM, HGMQ, UMQ, and LMQ. Independent *t*-tests were used to analyze

gender differences amongst all variables and one-way ANOVAs used to analyze differences between age groups (50s: n=20, 60s: n=63, 70s: n=60, and 80s: n=11). In addition, Kendall's W and chi-squared tests were performed along with binary logistic regression to identify the best cut-off values and models in classification of sarcopenia. PASW version 18.0 was used for all statistical analysis (Chicago, Illinois, United States). An alpha of $p \leq 0.05$ was used to determine statistical significance for all analyses. Independent *t*-tests indicated that participants were significantly younger in G10 than A08 or A09 ($p<0.05$) and men were younger than the women in G10 ($p<0.05$). Men were taller, weighed more, and had lower body fat percentages than women in all studies ($p<0.05$), with no differences between studies. Using the Baumgartner et al. (1998), Newman et al. (a) (2003), and Delmonico et al. (2007) cut-off values to classify sarcopenia, sarcopenic individuals were significantly older than non-sarcopenic individuals ($p<0.05$). However, there were no age-related differences when using the Newman et al. (b) cut-off values ($p>0.05$). There were no gender- or age-related differences for TGUG ($p>0.05$). There was a significant interaction for handgrip strength ($p<0.05$). Men in their 50s, 60s, and 70s had greater handgrip strength than women ($p<0.05$), men in their 50s, 60s, and 70s had greater handgrip strength than those in their 80s, and women in their 50s and 60s had greater handgrip strength than those in their 70s and 80s ($p<0.05$). Men had greater values for ALM, TBSM, LM, non-skeletal muscle LM, bench press and leg press 1-RM, HGMQ, UMQ, LMQ, SMI, or SMI residuals ($p<0.05$) than women. Men and women in their 50s, 60s, and 70s had significantly greater LM, TBSM, ALM, and LB 1-RM than those in their 80s ($p<0.05$). Non-skeletal LM was greater for individuals in their 60s

than in their 80s (p<0.05). Upper-body 1-RM was greater for individuals in their 60s than those in their 80s (p<0.05). LMQ was greater for individuals in their 50s than those in their 80s (p<0.05), and SMI was greater for individuals in their 60s than those in their 80s (p<0.05). There were low to moderate positive correlations among UMQ and LMQ (r=0.39) and leg press 1-RM and handgrip strength (r=0.47) in women, UMQ and leg press 1-RM (r=0.48), LMQ and HGMQ (r=0.31), HGMQ and bench press 1-RM (r=0.37), handgrip strength and upper-body 1-RM (r=0.67), and handgrip strength and leg press 1-RM (r=0.57) in men. Men and women had low to moderate positive correlations among LMQ and handgrip strength (r=0.43 and r=0.32, respectively) and bench press 1-RM (r=0.58 and r=0.49, respectively). There were low to moderate negative correlations among UMQ and TGUG (r= -0.27), age and LMQ (r= -0.35), HGMQ (r= -0.34), and leg press 1-RM (r= -0.46) in women, and age and handgrip strength (r= -0.30 and r= -0.54, respectively) and bench press 1-RM (r= -0.37 and r= -0.28, respectively) in men and women. In men and women, SMI was positively correlated with bench press 1-RM (r=0.59 and r=0.53, respectively), leg press 1-RM (r=0.65 and r=0.61, respectively), handgrip strength (r=0.52 and r=0.37, respectively), LM (r=0.72 and r=0.70, respectively), ALST (r=0.83 and r=0.82, respectively), LMQ (r=0.43 and r=0.36, respectively), and TBSM (r=0.83 and r=0.82, respectively). In women, SMI was positively correlated with FM (r=0.29) and negatively correlated with age (r= -0.37) and negative correlated with TGUG (r= -0.42) in men. The prevalence of sarcopenia ranged from 31-44% in women and was 13% in men based off of the four different cut-off values. To identify which of the four cut-off values would be the most appropriate to adapt as the standard in classifying

sarcopenia, Kendall's W and chi-squared tests were performed. The highest agreement in distributions was among Newman et al. (a) (2003) and Delmonico et al. (2007), with 100% agreement (r=1.00, p<0.001), followed by Baumgartner et al. (r=0.760, p<0.001). Exploratory binary logistic regression was calculated to determine if sarcopenia status (sarcopenic vs. non-sarcopenic) could be determined with theory-based predictors (age, gender, LM, handgrip strength, and TGUG). The best predicted probability estimates were derived with Newman et al. (a) (2003) or Delmonico et al. (2007) as the dependent variable in classifying sarcopenia using gender and lean mass as the predicting variables. The results of the present study confirm previous findings that functional strength and muscle quality were negatively correlated with age and that LM and functional strength decreased in the 7th and 8th decades of life. Previous studies have used cut-off values established by Baumgartner et al. (1998), however, using ALM/m^2 and cut-off values established by Newman et al. (a) (2003) or Delmonico et al. (2007) may be more appropriate in classifying sarcopenia. A larger epidemiological database needs to be established in order to generalize the proper cut-off values to the entire elderly population.

CHAPTER I

INTRODUCTION

Aging presents a multitude of potential complications ranging from diabetes and cardiovascular disease, to reduction in bone mineral density and loss of muscle mass among others. The reduction of muscle mass as a result of age was first scientifically investigated in the early 1930s by Macdonald and Critchley.[14] The term sarcopenia is derived from the Greek roots *sarc* and *penia* meaning *flesh* and *loss*, respectively, and was originally defined by Rosenberg as the age-related loss of muscle mass.[60] Reduction in muscle mass plays a role in the loss of function, and subsequently, changes in quality of life. For example, Grimby et al.[29] reported that 78-81 year old men and women had on average 10-30% lower muscle fiber area and a much higher risk of falls and disability.[41,45] Recently, the European Working Group on Sarcopenia in Older People (EWGSOP) developed a working definition of sarcopenia, which indicated that the individual must exhibit low muscle mass accompanied by either low muscle strength and/or low physical performance.[15]

Measurements of total-body and appendicular muscle mass have been compared using bioelectrical impedance analysis (BIA),[38,58] dual-energy x-ray absorptiometry (DEXA),[2,28,43,68] magnetic resonance imaging (MRI),[40,43,48] and axial computed tomography (CT)[28,48,68] scans. Although MRI and CT scans are the most accurate way to assess muscle mass, these methods are not cost-effective and require trained personnel to conduct and interpret the scan. Skeletal muscle index (SMI) is a commonly used method in identifying relative muscle mass. SMI can be calculated from appendicular skeletal muscle mass (the sum of fat-free mass of the arms and legs,

expressed as ALM, ALST, or ASM) using DEXA and/or BIA.[2,17,25,39,44,57] This index is calculated the same way as body mass index (BMI), but specific to muscle mass rather than body mass. However, the cut-off values used to determine what is categorized as normal or sarcopenic based on a criterion method has yet to be validated. In attempt to establish universal criteria for sarcopenia classification, Baumgartner et al.[2] suggested reference cut-off values based on the average of 229 young men and women from the Rosetta Study[25] and cross-referenced in 301 elderly men and women from the New Mexico Aging Process Study.[2,3] Similar to osteoporosis classifications, individuals in the reference group were considered sarcopenic if their SMI was greater than two standard deviations below the young adult average. For Baumgartner et al.,[2] SMI was calculated as ASM divided by height in meters squared, which set the cut-off values at less than 7.26 kg/m^2 for men and 5.45 kg/m^2 in women. However, many studies published since Baumgartner et al.[2] have established additional reference cut-off values to classify sarcopenia based on different populations. Although there are inconsistencies in cut-off values, most previous studies have used ASM/m^2 to estimate SMI.[2,17,37,39,57]

As an assessment of functional strength, handgrip strength is traditionally used to provide insight regarding upper-body muscle loss with aging.[24] A loss in handgrip strength can impair the ability to complete activities of daily living, such as opening jars or carrying groceries or laundry.[63] Likewise, impaired gait speed or balance instability could result in difficulties completing daily tasks. One study by Kallman et al.[42] investigated hand grip strength across all ages (20-100 years) and over a 9-year follow-up period. The authors reported that only a fraction of the participants lost a

significant amount of handgrip strength over the course of the study. Furthermore, handgrip strength had a greater correlation with age than muscle mass. Kallman et al.[42] examined the decrease in handgrip strength across age and longitudinally and reported that handgrip strength declined from age 40 on as did muscle mass.[42] Most studies have evaluated handgrip strength and age cross-sectionally and have reported a decrease in handgrip strength as age increased.[5,19,42,47,63] However, decrements in handgrip strength can only be partially associated with muscle mass, therefore, handgrip strength alone cannot accurately reflect age-related changes in muscle mass. The addition of lower body mobility and strength assessments in older individuals may help in accurately identifying sarcopenic individuals, such as the short physical performance battery (SPPB), isometric leg extensor and flexor strength, dynamic strength (i.e., leg press), and timed get-up-and-go (TGUG). Lauretani et al.[47] reported muscle power to be the best determining factor in poor mobility in the elderly, and the least sensitive was calf muscle cross-sectional area. The SPPB includes several tests including repeated chair raises, gait speed test, and standing balance tests.[47] An alternative is the TGUG, where in one timed series, an individual must rise from a chair, walk 3-meters, turn around an obstacle, and return to the seated position, which assesses balance, gait speed, and leg strength all in one measurement. A score greater than 9 seconds is considered impaired in older adults.[7]

Yet another alternative to muscle mass, strength, and function is to assess muscle quality. Ivey et al.[36] recently investigated the effects of short-term (9-week) strength training and detraining (31 weeks) on muscular strength, muscle mass, and muscle quality in young and old men and women. The authors reported that all groups

significantly improved 1-RM, muscle volume, and muscle quality, but young women expressed the greatest increase in muscle quality compared to all other groups. Following 31 weeks of detraining, all groups except older women maintained the improvements in muscle quality. The authors suggested there were non-muscle mass factors contributing to the strength gains in all groups, which may have been related to neural adaptations. Muscle quality was calculated by the authors as the dominant quadriceps one-repetition maximum (1-RM) divided by the quadriceps muscle volume of the dominant leg as assessed by MRI. Alternatively, several studies have reported muscle quality as isometric,[53,56] isokinetic,[28,49,56] or dynamic[36] maximal strength using various exercises relative to muscle mass either estimated from anthropometric assessment, estimated from single cross-sectional images from MRI or CT,[49] or actual muscle volume from multi-slice MRI or CT.[11]

Currently, the consistency among methods for classifying individuals as sarcopenic is unknown. There is a need to identify which model or combination of models would most accurately classify sarcopenia in the older adult population. Because previously established cut-off values have been developed on independent homogeneous populations, more specific criteria is needed to establish a valid and reliable model for the diagnosis of sarcopenia Variables to consider for such a model might include muscle quality, muscle strength, functionality assessments (i.e., handgrip strength and timed get-up-and-go), and body composition. The incorporation of new criteria may be necessary to generalize the overall model.

Purposes of the Study

1. The primary purpose of this study was to examine the consistency among the four different sarcopenia classification models and explore new variables to improve sarcopenia classification.
2. The secondary purpose of this study was to examine the effects of aging on body composition, muscle strength, functionality, and muscle quality.
3. The tertiary purpose of this study was to examine the relationships among age, muscle mass, and functionality in determining sarcopenia status.

Research Questions

The research questions for this study are:

1. Do muscle quality, functionality, muscle mass, and strength change similarly across age?
2. How many individual subjects are consistently classified as sarcopenic with all 4 models? How many individuals are classified as sarcopenic with any 2 or 3 of the 4 models?
3. What is the consistency among the 4 different ways to classify sarcopenia?
4. Do muscle quality and/or functionality improve consistency of classification?
5. What are the relationships among age, muscle quality, SMI, and functionality?
6. What are the common traits among all the subjects that are consistently classified?

Hypotheses

1. It was hypothesized that muscle quality, functionality, muscle mass, and strength decrease with age.
2. It was hypothesized that similar proportions of individuals are classified by any two SMI methods, however, not always the same two methods.
3. It was hypothesized that the proportion of individuals classified as sarcopenic would be greater using the Baumgartner et al. method than the other three methods.
4. It was hypothesized that the addition of muscle quality and functional capacity to low muscle mass will more accurately classify sarcopenic individuals.
5. It was hypothesized that there are positive correlations among muscle quality and SMI and negative correlations among SMI and functionality, and functionality and SMI.
6. It was hypothesized that individuals classified as sarcopenic by any method also had low handgrip strength, slow get-up-and-go times, and/or low bench and leg press 1-RM strength.

Study Variables

Independent Variables

- Gender (male vs. female)
- Age (50s vs. 60s vs. 70s vs. 80s)
- Sarcopenia Status (sarcopenic vs. non-sarcopenic)

Dependent Variables

- Age—chronological age of participant.

- Lean mass (LM)—total body adipose-free lean mass estimated from total body DEXA scan.
- Fat mass (FM)—total body fat mass estimated from total body DEXA scan.
- Appendicular lean mass (ALM), appendicular lean soft tissue (ALST), or appendicular skeletal mass (ASM)—the sum of the left and right arm and leg lean mass, as determined by DEXA.
- Total body skeletal muscle (TBSM)—total body adipose-free lean tissue estimated using the equation developed by Kim et al.[44]

  TBSM = (1.13 x ALST) – (0.02 x age) + (0.61 x sex) + 0.97, where male=1 and female =0
- Handgrip strength—maximal amount of force the dominant hand can produce isometrically. The average two highest of three attempts was considered handgrip strength.
- Timed get-up-and-go (TGUG)—timed assessment of mobility of functionality
- Skeletal muscle index (SMI)—the amount of total body skeletal muscle mass (kg) relative to height (m) squared.
- SMI residuals—difference between estimated ASM and actual ASM from DEXA. Estimated ASM was calculated using gender-specific equations.[57]

  Men: predicted ALM (kg) = -22.48 + 24.14 x height (m) + 0.21 x fat mass (kg)

  Women: predicted ALM (kg) = -13.19 + 14.75 x height (m) + 0.23 x fat mass (kg)
- Non-skeletal muscle lean mass—Bone- and adipose-free lean tissue calculated as total body lean mass – ALST.

- Upper-body 1-repetition maximum (1-RM)—maximal bench press weight estimated from 5-RM bench press.
- Lower-body 1-RM—maximal leg press weight estimated from 5-RM leg press.
- Handgrip muscle quality (HGMQ)—dominant hand handgrip strength relative to dominant arm lean mass.
- Upper-body muscle quality (UMQ)—relative upper body strength, expressed as upper body 1-RM divided by total arm lean mass
- Lower-body muscle quality (LMQ)—relative lower body strength, expressed as lower body 1-RM divided by total leg lean mass

Delimitations

The delimitations of this study are:

1. One hundred sixty seven men and women 55-90 participated in one of three studies conducted between 2008 and 2011.
2. All participants were required to complete a health history questionnaire and sign a written statement of informed consent prior to any testing.
3. All participants were non-diabetics, free of cancer, any kidney or liver disease.
4. No participants had taken any protein, weight loss supplements, calcium or vitamin D within 2 months of participation.
5. No participants had any type of surgery within 1 month of study participation.
6. All participants had a BMI 18.5-30 kg/m^2.

7. No participants had the presence of uncontrolled blood pressure or cardiovascular disease, no presence of arthritis that may inhibit handgrip measurement.
8. Participants recruited were not actively participating in a structured exercise program.
9. Variables were measured using:
    a. Dual-energy x-ray absorptiometry (DEXA) measuring body composition.
    b. Handgrip dynamometry measuring dominant handgrip strength.
    c. Timed get-up-and-go with a digital stopwatch on a 3-meter course to measure gait speed, balance, and stability.
    d. 5-repetition maximum bench and leg press to measure upper and lower body absolute strength.

Limitations

The limitations of this study are:

1. Only a small sample of the elderly population from the Oklahoma City metropolitan area volunteered to participate and results may not accurately represent the entire elderly population.
2. Not a truly random sample was taken due to local recruitment (local churches, Huston Huffman Center, campus faculty, etc.). Additionally, the study sample may not represent a completely random sample as all participants are volunteers.

3. The variables were measured by different investigators from one study to the next, therefore, there may be intra-tester error in the measurements taken.
4. A different handgrip dynamometer was used in A08 and A09 than was used in G10.
5. Maximal effort was required for testing sessions and slight discomfort from exertion may have prevented true maximal effort.
6. Only participants in A09 and G10 (n=114) completed 5-RM bench press and leg press strength assessments. Whereas all other measurements were completed on all 167 participants.
7. Upper-body muscle quality was calculated as bench press 1-RM divided by total arm lean mass, however, the primary mover of bench press is the pectoralis major and axial muscle mass was not included in the calculation. Therefore, UMQ as calculated in the present study does not accurately reflect the quality of all muscles involved in the movement.

Assumptions

The assumptions of this study are:

Theoretical Assumptions

1. Accurate health history will be provided.
2. Maximal exertion will be put forth during testing measurements.
3. Equipment is calibrated and working properly.

Statistical Assumptions

1. The populations from which the samples are drawn are normally distributed.

2. The sample will be randomly selected.
3. The variability of the samples in the experiment are exactly or nearly equal (Homogeneity of Variance).
4. Independence of observations; there is no correlation, dependence, or association between groups (all groups are independent of one another).
5. Data are based on a parametric, interval or ratio measurement scale for all parametric tests.
6. Logistic regression outcome must be discrete.
7. Logistic regression: ratio of cases to variables, for every variable, there is a sufficient number of cases.
8. Logistic regression: absence of multicollinearity
9. Logistic regression: independence of errors, between-subjects design not within-subjects.

Operational Definitions

Functionality—the ability to perform activities of daily living without difficulty, including walking, carrying groceries, opening containers, standing from a seated position, etc.

Elderly—adults aged 65 years or older.

Comorbidity—the occurrence of a disease or illness in the presence of another disease or illness.

Sarcopenia—the age-related reduction in muscle mass and function.

Skeletal Muscle Index—calculated as appendicular skeletal muscle divided by height in meters squared.

Five-Repetition Maximum (5-RM)—A measure of absolute strength, the amount of weight an individual can lift no more than five times in good form.

Muscle Quality—muscular strength relative to the amount of muscle mass used.

Abbreviations

HT – Height (cm)

BM – Body mass (kg)

CT – Computed tomography

MRI – Magnetic resonance imaging

pQCT – peripheral quantitative computed tomography

BIA – bioelectrical impedance analysis

DEXA – dual-energy x-ray absorptiometry

FM – fat mass

FFM – fat-free mass

ASM – appendicular skeletal muscle

ALM – appendicular lean mass

ALST – appendicular lean soft tissue

TBSM – total body skeletal muscle

TBMM – total body muscle mass

RSMI – relative skeletal muscle index

SMI – skeletal muscle index

MQ – muscle quality

5-RM – five-repetition maximum

1-RM – one-repetition maximum

CHAPTER II

REVIEW OF LITERATURE

Recently, the European Working Group on Sarcopenia in Older People (EWGSOP) convened and established recommendations for identifying and possibly diagnosing sarcopenia. The parameters include low muscle mass, plus either low muscle strength or low physical performance.[15] The primary index used in classifying sarcopenia is low muscle mass, which can be measured by magnetic resonance imaging (MRI), dual-energy x-ray absorptiometry (DEXA), or bioelectrical impedance analysis (BIA). Several researchers have identified different ways to evaluate muscle mass across the age spans with various assessment tools.[2,30,34,37,38,44,57] For example, skeletal muscle index (SMI) is defined as appendicular muscle mass divided by height in meters squared (kg/m^2), [2] which is the same conceptual formula as the body mass index (BMI). However, body composition of body mass is not accounted for by BMI, whereas SMI considers muscle mass in the arms and legs, which is particularly important for elderly adults as sarcopenia diminishes mobility and functionality facilitated by appendicular muscle. When attempting to use SMI as a tool for diagnosing sarcopenia, cut points have been established in several populations,[2,17,57] however, there is no universally adopted cut points, best for identifying sarcopenia. Furthermore, there is no criterion method for how to obtain or calculate SMI, and it is not entirely clear-if SMI is the most appropriate screening tool for sarcopenia. Therefore, the general purpose of this review is to identify any apparent differences in body composition, and functional performance among gender and measurement issues in an aging population.

Differences in Skeletal Muscle Mass Across Age and Gender

Peak muscle mass is often achieved in the second or third decade if life, maintained through the fourth decade, and diminished thereafter - beginning in the fifth decade.[21,42] Subsequently, a similar pattern exists for the development and eventual loss of muscle strength over the age span.[28,33,42] Of concern is the age-related reduction in muscle mass is proportionally greater for larger muscle groups than smaller muscles.[40] Furthermore, men tend to experience a greater reduction in muscle mass than women with advanced age, perhaps related to having more to start with.[40] The goal of this literature review section is to explore the age-related changes in skeletal muscle mass, how these patterns compare between genders, and hypotheses developed to explain sarcopenia.

Baumgartner, Waters, Gallagher, Morley, and Garry, 1999[4]

It is evident that men and women lose muscle mass as they age. The purpose of this study was to identify any factors that may influence the reduction on skeletal muscle mass in an older population. A sample of 121 males and 180 females 65-97 years old was collected from the New Mexico Aging Process Study (NMAPS). Dietary intake, sex hormones, physical activity questionnaire, grip strength, and a DEXA were recorded for all participants after IRB approval and consent. Partial correlations and stepwise regression was run on the data to determine relationships among variables and the strongest predictors of the reduction in muscle mass with age. The authors reported that both genders experience a reduction of muscle mass and

corrected muscle strength with advancing age and that grip strength decreased with age, independent of muscle mass. Physical activity levels ranged from low to moderate across genders. There were positive associations with physical activity and muscle mass and negative correlations with fat mass and physical activity. In men, free-testosterone, physical activity, cardiovascular disease, and IGF-1 were significantly associated with muscle mass, whereas, only total fat mass and physical activity were associated with muscle mass in women. After controlling for these factors, age is not a significant contributor to muscle mass. The authors concluded that there is a multifactorial effect on muscle loss and strength loss in healthy men and women. Muscle loss is related to lack of physical activity in both genders, and hormone status is an important factor in maintaining muscle mass in men only.

Kim, Wang, Heymsfield, Baumgartner, and Gallagher, 2002[44]

The purpose of this study was to develop an equation for predicting TBSM from DEXA, compared to MRI as the actual SM measure. Four hundred fourteen men and women of diverse ethnicities ≥18 years old were recruited to participate in this study. About two-thirds of the participants were included as the model development group, while the others were to be utilized as the model validation group. Height and weight were measured before each participant underwent a full body DEXA scan. Using the scan analysis, appendicular lean soft tissue (ALST) was calculated from the sum of all lean tissue in the right and left arms and legs. ALST was then used as a variable in multiple regression along with age, race, and gender as predictors of TBSM. Actual TBSM was determined from ~40 axial slices from MRI. Between

group differences were tested using Student's t-tests and Pearson's correlations were used to identify any relationships between ALST from DEXA and TBSM from MRI. The authors reported that there was no significant difference for BMI between men and women in the model development group, however, the men were taller, weighed more, and were younger. All men had a lower percent fat and greater TBSM. Women in the model validation group were younger and taller, had greater ALST and TBSM than the women of the model development group. Upon model development, ALST was the strongest predictor of TBSM, with $r^2 = 0.96$ and standard error of 1.63 kg. Two additional models incorporating age and sex as predictors of TBSM were included and stayed in the final model, and when race was included, it did not contribute significantly to the model. The final prediction model was TBSM = (1.13 x ALST) – (0.02 x age) + (0.61 x sex) + 0.97. The prediction model for TBSM was not significantly different from actual measured TBSM. The authors concluded that TBSM can be accurately predicted using a single DEXA scan, age, and gender in a diverse population of individuals, and because age and gender are factors of the equation, it can be used across all populations.

Narici, Maganaris, Reeves, and Capodagilo, 2003[55]

The purpose of this cross-sectional study was to determine if changes in muscle architecture influence the prevalence of sarcopenia in addition to the reduction in muscle mass and to address the functional significance of the changes in muscle architecture. Sixteen men 70-81 years old and 14 men 27-42 years old volunteered to participate in this study. All participants were healthy, recreationally active, and free

of injury. Computerized tomography was completed on all participants to determine anatomical cross-sectional area (ACSA) from the largest of a sequence of slices completed beginning at the knee space. The maximal contour of the medial gastrocnemius was selected and used as ACSA, and medial gastrocnemius muscle volume was calculated from all sequential slices using a spline algorithm to account for missing slices and the sum of all of the determined areas and multiplied by slice thickness (10mm). Muscle architecture was determined at a set joint angle of 115°, and an ultrasound was conducted to measure resting fascicle length and pennation angle at the midbelly of the dominant medial gastrocnemius. Physiological cross-sectional area (PCSA) was determined as the ratio of volume and fascicle length. Any age-related differences were determined using paired-samples t-tests and Pearson's product moment correlation from linear regression was used to compare the associations among variables. The results indicated that the younger men had significantly higher maximal ACSA, muscle volume, PCSA, fascicle length, and pennation angle. There was a significant correlation between pennation angle and maximal ACSA. Although expected, there were no significant differences in the ratio of ACSA to PCSA between the older and younger men. The authors concluded that aging significantly affects muscle architecture and these alterations may have negative effects on muscle function with advanced age.

Kim, Heshka, Gallagher, Kotler, Mayer, Albu, Shen, Freda, and Heymsfield, 2004[43]

The purpose of this study was to develop and validate models in prediction of adipose-free total-body skeletal muscle with MRI and DEXA. The study involved two

phases, one included participants receiving a full body MRI for adipose-free total-body skeletal muscle and a DEXA scan for appendicular lean soft tissue (ALST). A multi-slice MRI was performed to complete the total-body skeletal muscle as inter- and intramuscular adipose-free skeletal muscle and a full body DEXA scan was analyzed for right and left arm and leg fat-free and bone-free lean mass, identified as ALST. Adipose-free total-body skeletal muscle was predicted using a model with ALST determined by DEXA as the predicting variable. The second phase was to cross-validate the prediction models in subjects with different body compositions. The model development group consisted of healthy men and women over the age of 18 with a BMI under 35 kg/m^2 and the model validation group consisted of anorexic females, recreationally active men and women, and men and women before acromegaly treatment. The phase one prediction equations were developed using linear regression with race and gender as fixed factors and MRI-derived adipose-free total-body skeletal muscle as the dependent variable. The best fit model was determined as the one with the lowest standard error and appropriate independent variables such as ALST, age, body weight and body fat where the adjusted R^2 was maximized without violating the multicollinearity assumption. The second phase was the model validation with a diverse sample of individuals. The difference between the actual measured skeletal muscle and the value from the predicted equation was compared using student t-tests. Individuals in either the model development or model validation group were 18-88 years old of varying ethnicities. ALST calculated from the DEXA scan was the strongest predictor in explaining MRI-derived adipose-free total-body skeletal muscle (model 1), followed by a minimal influence from age

(model 2), gender and race (model 3), and body weight and body fat did not add to the models therefore were not included. All of the models were validated with a diverse group of individuals classified as athletic and acromegalic, but did not accurately predict skeletal muscle in anorexic females. Model 1 was, however, validated in anorexics with a BMI >16 kg/m^2. The authors could conclude that a full body DEXA scan, along with age, gender, and race can accurately predict MRI-derived adipose-free total body skeletal muscle as an alternative, low cost, and quick method in determining skeletal muscle.

Cuthbertson, Smith, Babraj, Leese, Waddell, Atherton, Wackerhage, Taylor, and Rennie, 2005[16]

The purpose of this study was to determine if older men had a reduced anabolic response to different amounts of essential amino acids (EAAs). Twenty-four older and 20 younger men were assessed for BMI and skeletal muscle mass prior to muscle biopsies and determination of fractional synthesis rate of the vastus lateralis. All participants were divided into groups of four and consumed 0, 2.5, 5, 10, 20, or 40g of EAAs in water. Fractional synthesis rate was assessed over 3 hours and muscle biopsies were taken before and after the 3 hour period. There were no observed differences in basal muscle protein synthesis, however, the older individuals displayed a reduced anabolic response to the EAA ingestion. RNA:protein and MPS:RNA ratios were reduced in the older individuals after 10g of EEA, as were the responses of the anabolic signaling pathway. The authors concluded that older individuals had a reduced capacity of MPS, and in general, EAAs can stimulate MPS independent of an

insulin response. Therefore, older individuals may not benefit from a high protein diet or supplementation, notwithstanding the nutrient availability of such.

Delmonico, Kostek, Johns, Hurley, and Conway, 2008[18]

The purpose of this study was to determine how DEXA compares to computerized tomography (CT) in tracking changes in thigh muscle mass after strength training in 50 healthy adults 50-83 years old. All participants underwent a full body DEXA scan, used to determine body composition and thigh muscle volume for bone and adipose-free muscle mass (considered fat free mass) before and after a 10 week strength training program. A peripheral CT scan was completed on both thighs to determine the muscle volume before and after the strength training program. The muscle volume on the untrained leg served as a control to factor biological variation. All muscle mass was outlined, excluding bone and adipose tissue, and was noted as FFM. One-repetition maximum strength was determined for the knee extensors using a Keiser leg extension machine. All strength training sessions were performed on the same equipment with the right leg, three times per week for 10 weeks. Paired sample t-tests were conducted to determine any differences between the trained and untrained legs' strength and muscle mass and Bland and Altman plots were created with the differences between DEXA and CT plotted against the average of each method. The authors reported that men and women improved their 1-RM strength after the strength training program in both the trained and untrained legs. DEXA and CT indicated an increase in thigh CSA in all participants and both legs, but the increase in thigh FFM was much greater in the trained leg. Men and women exhibited similar percent

changes in thigh FFM using DEXA and CT, as well as no percent change differences in trained and untrained legs when comparing either method. There was a strong correlation between DEXA and CT thigh FFM before and after the strength training program. The slopes of the percent change lines measured by CT vs. DEXA were not significantly different, but the DEXA was shown to underestimate by 0.25% for every 1% change in muscle mass. In some cases CT indicated an increase in thigh FFM, whereas DEXA indicated a decrease. Overall, thigh FFM was overestimated by DEXA before and after the 10 week strength training program. The authors concluded that DEXA may not be sensitive enough to detect small changes in muscle mass after a training intervention.

Development of Sarcopenia Cut-off Values

In 1990 and again in 1997, Heymsfield et al.[32] and Gallagher et al.,[25] respectively, suggested using the sum of total arm and leg lean mass from a single DEXA scan to estimate appendicular skeletal muscle mass as opposed to total body potassium due to the utility and availability of DEXAs for clinical assessment of muscle mass. Skeletal muscle mass decreases with age and has been linked to functional impairment in older individuals.[33,42] In attempt to generalize ASM across men and women of varying heights, ASM was divided by height squared, similar to BMI.[2] Therefore, the purpose of this review section is to assess the various studies that use ASM/ht^2 in different populations for establishing sarcopenia cut points.

Baumgartner, Koehler, Gallagher, Romero, Heymsfield, Ross, Garry, and Lindeman, 1998[2]

There were multiple purposes of this study, to determine a method to estimate relative skeletal muscle mass, to estimate prevalence of sarcopenia in elderly men and women, and finally to determine any relationships among sarcopenia, health behaviors, physical impairment, mobility, and comorbidities. Several datasets were used from the New Mexico Elder Health Survey including healthy men and women, Hispanic and non-Hispanic whites where sub-samples underwent a full body DEXA to determine body composition. Data from two other studies were used as reference data for the analyses, one included 301 elderly men and women, and the other included 229 men and women 18-40 years old used to define the cut points for sarcopenia. The survey included medical histories and questionnaires including record of dietary intake, mental status, behavior, and attitude assessment. Other health parameters such as glucose tolerance, electrocardiograms, and clinical and biochemical nutrient chemical analyses were assessed. Anthropometric measures were assessed including height, weight, hip and waist circumference, and triceps and subscapular skinfolds on the right side. Grip strength was measured on the dominant hand three times and the average of the two highest was used for analysis. From the DEXA scan, appendicular skeletal muscle mass (ASM) and body composition were determined as suggested by Heymsfield[31]. A subsample of the individuals was divided into two groups, one as an equation development group and the other ad an equation validation group. An equation to predict ASM was determined from gender, anthropometric, handgrip strength, and body composition data and an equation to predict percent fat was

developed using gender and anthropometric data. The best predictive equations were ASM = (0.2487 x weight) + (0.0483 x height) – (0.1584 x hip circ.) + (0.0732 x grip strength) + (2.5843 x sex) + 5.8828 and percent body fat = (0.2034 x waist circ.) + (0.2288 x hip circ.) + (3.6827 x ln(triceps skinfold)) – (10.9814 x sex) – 14.3341. These prediction equations were validated against the actual, measured values obtained by the DEXA and further tested with an independent sample from the Aging Process Study. The agreement of the predicted and actual values was determined by regression and tested to see if the slope and intercept were significantly different than 1 and 0, respectively. Additionally, the fit of the predicted equations was determined using the residuals, as the difference between the actual and predicted value, against age and ethnicity. Sarcopenia was identified as a measure of relative muscle mass and calculated using ASM (kg)/height^2 (m^2), similar to that of BMI, which takes into account height differences across gender. The cut points to identify sarcopenia were set at less than 2 standard deviations below the average for the younger adults' reference data previously mentioned. The authors reported that the predicted percent body fat and predicted ASM were highly correlated with their respective estimates from the DEXA. The predictive equations were found to overestimate muscle mass at the higher levels. Predicted body fat was within ±4% and muscle mass within ±1.7kg. The authors could not determine any alternative factors affecting the equations related to body composition and muscle mass. Muscle mass was lower in the elderly men and women when compared to the younger men and women. The prevalence of sarcopenia was 60% in individuals over 80 years old and only 13.5-24% in individuals under 70 years old. The incidence of sarcopenia was also greater in Hispanics than

non-Hispanics. The authors concluded that older individuals have reduced muscle mass and incidence of sarcopenia increases with age.

Newman, Kupelian, Visser, Simonsick, Goodpaster, Nevitt, Kritchevsky, Tylavsky, Rubin, and Harris, 2003[57]

The purpose of this study was to compare two different models that identify sarcopenia in relation to lower extremity function. The Health Aging and Body Composition (Health ABC) Study was conducted in 2 cities that recruited 2,984 70-79 year old adults. A DEXA was performed on all participants and then classified using one of two methods. One method was aLM adjusted to height squared as established by Baumgartner et al.[2] and the other was based on residuals of predicted aLM using height and fat mass. For both methods, the lowest 20% of the distribution of residuals was used to classify individuals as sarcopenic and not sarcopenic. A predicted aLM was determined differently for men and women, the predicted aLM was calculated for males using the equation (aLM (kg) = -22.48 + 24.14 x height (m) + 0.21 x fat mass (kg)) and for females using (aLM (kg) = -13.19 + 14.75 x height (m) + 0.23 x fat mass (kg)). Gait speed, balance and chair stands were performed to determine lower extremity function. Additional confounding variables such as obesity, race, age, alcohol or tobacco use, and physical activity were also recorded. The authors reported that fat mass and percent body fat were higher in women than men. Men also had higher values of lean body mass. Within each gender, black men and women had higher values for aLM/ht^2 than their white counterparts. Black women also had higher values of lean mass and higher BMI and physical activity than white women. The

lowest 20% of the population was considered sarcopenic regardless of the method used based on the arbitrary cut point of 20%. Not everyone that was classified by both methods, where 202 men and 155 women were classified by both, but 85 men and 155 women were only classified by one method. Of the individuals classified by the aLM/ht^2 method, less than 9% were overweight and obese, however, for the residual method, 26.9% men and 42.7% women were overweight and classified as sarcopenic. There were no age differences in those classified as sarcopenic by either method. In men and women, BMI was higher in those classified as sarcopenic by the residuals method than the aLM/ht^2 method. More black men were classified as sarcopenic by the aLM/ht^2 method and there were no racial differences in women. Conversely, the proportion of women with lower extremity limitation was higher when classified by the residual method and there was no difference in men. Overall, regardless of which method classified an individual as sarcopenic, they typically had reduced lower extremity function. The authors suggest the inclusion of height and fat mass in determining the prevalence of sarcopenia in overweight individuals.

Tankó, Movsesyan, Mouritzen, Christiansen, and Svendsen, 2002[66]

In women, the incidence of sarcopenia is one of the leading causes of disability and mortality. The purpose of this study was to identify any hormone and age-related variations in total body muscle mass and appendicular muscle mass. 754 healthy women 18-85 volunteered to participate in different studies in a local area and were included in the present analysis after meeting specific criteria. Height, weight, and a DEXA scan were collected on each individual. Appendicular lean tissue mass was

determined as the sum of bone mineral-free and adipose-free lean mass from each arm and leg, and total lean tissue mass was all bone mineral-free and adipose-free lean tissue. ALM and TLM were reported individually, and relative to height in meters squared. The results of this study indicated there was a strong negative correlation between age and ALM or TLM. The incidence of sarcopenia was identified using criteria similar to that established by Baumgartner et al.[2] was identified using 216 women 18-39 years old and resulted in a gradual increase in those affected from the fourth decade of life and every decade thereafter. The authors gathered that, although apparently healthy, aging women are progressively stricken by the onset of sarcopenia with advancing age.

Delmonico, Harris, Lee, Visser, Nevitt, Kritchevsky, Tylavsky, and Newman, 2007[17]

The purpose of this study was to compare two methods used to classify individuals as sarcopenic to predict functional impairment in men and women. Two thousand nine hundred seventy six men and women 70-79 years old participated in body composition and physical function testing for the Health Aging and Body Composition Study. After completion of a DEXA scan, aLM was calculated using the aLM/ht^2 model as established by Baumgartner et al.[2] and then calculated in an equation that incorporated height and fat mass. The predicted aLM was calculated using different equations for males using (aLM (kg) = -22.59 + 24.21 x height (m) + 0.21 x fat mass (kg)) and for females using (aLM (kg) = -13.21 + 14.76 x height (m) + 0.23 x fat mass (kg)). The difference between the actual aLM compared to the predicted aLM was considered the residual aLM. Similar to Newman et al.,[57]

sarcopenia was defined as the lowest 20% of the study's sample from either model. Participants were asked if they had persistent lower extremity limitation, defined as difficulty walking one quarter mile or climbing stairs without rest over two 6-month periods. Participants were asked of this self-report over a 5-year follow-up. Actual physical performance was also assessed using functionality tests such as sit-and-stand tests, gait speed, and standing balance, all of which are included in the short physical performance battery (SPPB). Physical activity status was also recorded and used as an estimation of caloric expenditure. Additionally, men and women were analyzed separately due to apparent differences in skeletal muscle mass and lower extremity performance was used as a covariate based on its association with the incidence of sarcopenia. The authors reported that the women had lower total body mass, LM, and aLM, with higher total fat mass and percent body fat than the males. There were also differences among white and black men for total body mass, aLM, fat mass, and percent body fat and the incidence of sarcopenia was lower among the black men and women using the residuals method. Black women also had significantly higher total body mass, BMI, LBM, aLM, fat mass and percent body fat than white women. Women that were classified as sarcopenic based on the residuals method had a higher incidence of lower extremity limitation, however, men and women classified as sarcopenic using the aLM/m^2 method had a reduced incidence of lower extremity limitation than their non-sarcopenic counterparts. Men classified as sarcopenic using either method had reduced lower extremity performance scores than non-sarcopenic men. Lower extremity performance was lower for sarcopenic women identified using the residuals method, but only after adjusting for confounding variables such as age,

race, and alcohol consumption. The authors concluded that the residuals method identified low muscle mass more accurately than the aLM/m^2 method, especially in women. Additionally, if an individual had reduced muscle mass, especially relative to fat mass and height, then their incidence of disability would be greater due to the individual's inability to carry their body weight and maintain functional movement.

Iannuzzi-Sucich, Prestwood, and Kenny, 2002[34]

The this study wanted to confirm the incidence rate of sarcopenia based on the cut points of less than 2 standard deviations below the gender-specific young adult average for skeletal muscle mass as established by Baumgartner and colleagues[2]. Baseline data was compiled from four different longitudinal studies involving older men and women. Participants underwent a DEXA scan, the SPPB, and different questionnaires regarding physical activity and quality of life. ASM was determined and expressed relative to height^2, and TSM was also calculated and expressed relative to height^2. Prevalence of sarcopenia in the present group of older adults was 22.6% and 26.8% for women and men, respectively. The best predictors of ASM were BMI for women, and BMI, mean power, and bio-available testosterone in men. The authors concluded that any intervention that may influence any of the predictors may be necessary in the reduction of the incidence of sarcopenia in this particular population.

Alternative Methods to Determine Sarcopenia

DEXA has become a popular clinical means to assess bone density and skeletal muscle mass. Using the DEXA imaging software to establish regions of interest (ROI)

for segmenting and compartmentalizing the appendicular versus axial skeleton also provides density-based values for axial and appendicular muscle mass.[32] Segmenting the muscle mass of the left arm, right arm, left leg, and right leg may help predict the incidence of sarcopenia, because the appendicular musculature is largely responsible for mobility and functionality. Another, simple but less common method for determining segmental muscle mass comes from the use of bioelectrical impedance analysis (BIA).[37-39] Using BIA, intra and extracellular water and adipose are factored into determining TBSM, and introduce less error into determination of such. Several studies have determined alternative definitions and cut points of sarcopenia than those determined using DEXA.[38,48,58] Therefore, the purpose of this review section is to assess the studies that utilize BIA in determining sarcopenia cut points in older individuals.

Janssen, Heymsfield, Baumgartner, and Ross, 2000[38]

The purposes of this study were to develop an equation to predict total body skeletal muscle mass using BIA and to cross-validate the developed equation. Height, weight, and actual total body muscle mass (TBSM) were determined in 388 men and women across two different laboratories using magnetic resonance imaging (MRI), and then all participants underwent BIA testing where all resistance measurements were adjusted for height. Each Laboratory developed an equation to predict TBSM from BIA. After each equation was developed, the data were pooled to generate a final regression equation of [TBSM = (height(cm)^2 / $R(\Omega)$ x 0.401) + (gender x 3.825) + (age x -0.071) + 5.102], where gender: men=1 and women=0. The r^2 of the equation

was 0.86 and standard error was 9%. The equation was developed using only Caucasian data and can be applied to African Americans and Hispanics, however, under estimated TBSM in Asians. The average difference between the TBSM from BIA and MRI were not significantly different, however, there is a significant positive correlation between the difference in the MRI and predicted BIA TBSM. The authors reported that the greater the actual TBSM, the more BIA over-predicted TBSM, and those with lower actual TBSM, the more the BIA prediction underestimated TBSM. Additional confounding variables such as adipose tissue and fat-free lean mass did not add significantly to the prediction model, therefore they were not included. The authors concluded that the equation developed is valid to use in predicting TBSM in healthy adults 18-86 years old.

Janssen, Heymsfield, and Ross, 2002[39]

The purpose of this study was to identify the prevalence of sarcopenia in older adults and to identify the relationship of sarcopenia with functional impairment. Fourteen thousand eight hundred eighteen adults 18 and older participated in this study and underwent assessment of height, weight, and BIA. Six thousand four hundred fourteen men and women 18-39 years old were used as a reference group to determine the normal and sarcopenic cut-offs, then skeletal muscle mass was determined on 4,502 men and women 60 years and older. They also reported functional impairment (the inability to walk one quarter mile or climb 10 stairs) and physical disability (inability or difficulty performing activities of daily living and ability to lift or carry 10 pounds). Whole body BIA was measured with electrodes on the right wrist and ankle,

where skeletal muscle mass was calculated using an equation by Janssen et al.[38] Skeletal muscle mass was predicted with [(height^2/BIA resistance x 0.401) + (gender x 3.825) + (age x -0.071)] + 5.102 = SM (kg). The prediction of SM was highly correlated (0.93) with measured muscle mass from MRI and the standard error at 9%. Absolute skeletal muscle mass was converted to a percentage of total body mass [(SM/BM)*100]. Sarcopenia was classified using the younger adults' gender-specific means and -1 standard deviations below the young adult mean was considered class I sarcopenia and -2 standard deviations below the young adult mean was considered class II sarcopenic. Additional confounders that were factored into analysis include age, race, healthy behaviors, comorbidity, and BMI. The cut-offs for class I sarcopenia were set as 37-31% for men and 28-22% for women, class II was set at <31% for men and <22% for women. The prevalence of sarcopenia increased from the third to the sixth decade and then plateaued. The authors reported there were a greater percentage of sarcopenic women than sarcopenic men over the age of 60. The incidence of physical disability and functional impairment was also greater among class I and class II sarcopenic men and women. The authors concluded that reduced skeletal muscle mass is significantly associated with functional impairment and physical disability in older Americans, especially women.

Janssen, Baumgartner, Ross, Rosenberg, and Roubenoff, 2004[37]

The purpose of this study was to determine skeletal muscle cut points for identifying risk of disability in older adults. Four thousand four hundred forty nine individuals 60 years and older participated in the Third National Health and Nutrition

Examination Survey (NHANES III). Total body muscle mass was determined relative to height using BIA and physical disability was assessed from a standard questionnaire. Muscle mass was determined using an equation previously developed by Janssen et al.[38] and considered skeletal muscle index (SMI). Polynomial regression was run with lines fit to the pattern of percent of individuals disabled against SMI (kg/m^2). Cut points were determined and identified as low-, moderate-, and high-risk disability at specific SMIs. The cut points were determined independently of gender where there is the lowest incidence of disability and the highest SMI. The likelihood ratio or positive and negative results based on frequency distribution of individuals with disability were used to determine the upper and lower limits of disability risk characterized by SMI for both men and women. The cut points established for women were ≤ 6.75 kg/m^2 and ≤ 5.75 kg/m^2 for moderate- and high-risk, and for men the cut points were identified at ≤ 10.75 kg/m^2 and ≤ 8.5 kg/m^2. The authors reported that women with physical disability increased from 10.8 % and 14.1% in women with low- and moderate-risk SMI, respectively, to 25.8% in those with a high-risk SMI, and a similar, but reduced pattern was observed in men with 2.8%, 8.1%, and 14.8% in low-, moderate-, and high-risk SMI individuals, respectively. The SMI cut points used to predict physical disability were also used to predict functional limitation (climb 10 stairs or carry 10 pounds) where increased limitation was found to appear at lower SMIs. The authors concluded that these cut points used to determine disability risk will be useful in determining morbidity risk among individuals with and without sarcopenia.

Muscle Quality

Reduced muscle mass can impair overall functionality and mobility associated with healthy aging.[51] Understanding the implications of sarcopenia is vital in determining mortality risk and disability.[61] Remaining functional and having the ability to maintain quality of life by completing activities of daily living are important for healthy living. The term "muscle quality" typically calculates strength relative to muscle mass.[49] The importance of muscle quality stems from the disassociation between muscle strength and muscle mass in the elderly.[9,49,53,67] That it, a loss in muscle mass may not always be matched by a concomitant loss in muscle strength or vice versa. Therefore, monitoring muscle quality may not only account for sarcopenia, but also monitor the risk for functional impairment.[37] Therefore, the purpose of this review section is to introduce the utility of muscle quality as relative muscular strength and the effects with aging or exercise interventions in maintaining or improving muscle quality.

Lynch, Metter, Lindle, Fozard, Tobin, Roy, Fleg, and Hurley, 1999[49]

The purpose of this study was twofold, to identify differences in muscle quality of the arms and legs across age and secondly, to determine if either gender or muscle group affects the relationship between MQ and age. Seven hundred three subjects 19-93 years old volunteered to participate in the Baltimore Longitudinal Study on Aging, however, only 502 individuals underwent the body composition testing. Height, weight, and body composition from a full-body DEXA scan were assessed on all participants. Concentric and eccentric peak torque was also measured on the dominant

arm flexors and the leg flexors. Arm and Leg muscle quality was calculated as concentric or eccentric peak torque divided by the muscle mass of the respective limb. One way ANOVAs were run to determine differences across age for all variables and multiple regression analyses were performed on all peak torque, muscle quality, and arm-leg differences for peak torque and muscle quality by age and gender. In the event of an age by gender interaction, men and women were analyzed separately. Men were reported as taller and heavier, with more muscle mass and less body fat than their female counterparts. Body mass was significantly lower for the oldest decade of men when compared to the youngest men and there was no trend for body mass in women across age. Men had reduced arm and leg muscle mass from their 60s and older, whereas women had reduced arm muscle mass from their 60s and older but leg muscle mass was reduced from their 40s and older. Arm and leg eccentric and concentric peak torque decreased as age increased for men and women. Arm muscle quality decreased between men and women as age increased. Leg muscle quality was higher in men than women and was sustained until the 5^{th} decade, then accelerated after then in both men and women. Alternatively, arm muscle quality was higher than leg muscle quality in men and women and the rate of decline in arm and leg muscle quality was the same, where the decline in leg muscle quality was greater than arm muscle quality in women. The authors concluded that muscle quality is influenced by gender and age, with differences among muscle quality of different body parts and muscular contractions.

Tracy, Ivey, Hurlbut, Martel, Lemmer, Siegel, Metter, Fozard, Fleg, and Hurley, 1999[67]

Upper and lower body muscle quality declines with age in both men and women. The purpose of this study was to examine the effect of unilateral lower body strength training in men and women 65-75 years old. Twelve men and 11 women volunteered to participate in the program. All participants were sedentary who had not worked out in 6 months or more. All participants underwent a full-body DEXA scan to determine bone-free lean body mass. Strength was assessed on an isokinetic dynamometer for peak torque and force production. A 1-repetition maximum was also assessed using a Keiser knee extension machine for familiarity on the equipment used for the training program and to determine the training load. All participants trained their dominant knee extensors for approximately 9 weeks, completing 5 sets of varying repetitions with varying rest periods. Participant's thighs were scanned using an MRI to determine muscle cross-sectional area before and after training. The quadriceps muscle was selected as the region of interest for each successive slice and summed to determine muscle volume and calculated volume was divided by isometric and 1-RM values to represent muscle quality. As expected, men were taller, weighed more with higher fat free mass, and had less body fat. Men alone had a slight increase in body mass after training, where neither gender had experienced changes in percent fat or fat free mass. The strength training program resulted in an increase in strength for both groups and men had a greater absolute increase than women, but the relative strength gains were similar for men and women. Strength gains were significantly higher in the trained than the untrained leg. Isometric peak force increased in the trained leg in men

only. Additionally, there was no significant increase in the untrained leg isometric peak force in both men and women. Isometric peak torque increased in the trained leg of the men but not women, but not significantly different from the untrained leg in both genders. There were no changes in isometric peak torque at the faster speed during isokinetic strength testing. Quadriceps muscle cross-sectional area increased in the trained leg of both genders. The men demonstrated greater absolute increases in quadriceps muscle cross-sectional area. There was also a slight increase in the untrained leg's quadriceps muscle cross-sectional area in men but not women. The trained leg had greater increases in muscle cross-sectional area than the untrained leg for both genders. Muscle quality was significantly improved in the trained and untrained leg of men and women when expressed as 1-RM per unit of muscle volume and there was no difference in genders. The increase in muscle quality was greater in the trained leg then the untrained leg of men but not women. When expressed at peak force per unit of muscle volume, there was no significant change from the training program for either leg or gender. The authors indicated that the cross-education of the untrained leg in men and women could be due to neural or paracrine factors. Additionally, older men and women can exhibit improvements in trained and cross-educated legs' muscle quality when expressed as 1-RM per unit of muscle volume, and that men had greater absolute increases in 1-RM strength after a 9 week strength training program.

Inaba, Kurajoh, Okuno, Imanishi, Yamada, Mori, Ishimura, Yamakawa, and Nishizawa, 2010[35]

In a clinical population, maintenance of muscle mass to retain physical function is imperative. The purpose of this study was to examine if a lower creatinine level in diabetic hemodialysis patients is related to lower muscle mass or muscle quality. Three hundred ten individuals undergoing hemodialysis, with and without diabetes mellitus participated in this study with their age ranging from 26 to 89 years old. Blood sampling was performed before the Monday hemodialysis session and frozen until needed for analysis. Handgrip strength was determined using the non-dominant hand's highest of three trials. Each participant underwent a full-body DEXA scan. Each scan was analyzed for fat free mass by subtracting fat mass from total dry weight. Body mass and lean mass were expressed relative to height and reported as BMI and LMI. ALMI was also calculated using the sum of both arm's lean mass. Muscle quality was calculated as handgrip strength divided by total body LMI. There were no differences between diabetic and non-diabetic for age, gender, weight, BMI, whole body LMI, or ALMI. Hand grip strength was significantly lower in diabetic patients. The ratios of handgrip strength to LMI, handgrip strength to ALMI, creatinine to LMI, and creatinine levels to ALMI were all lower in diabetic patients. There were significant correlations between creatinine levels and LMI, creatinine levels and handgrip strength, and handgrip strength and LMI in diabetic and non-diabetic patients. Regression slopes were significantly different for the relationship between handgrip strength and LMI for diabetics and non-diabetics, but the slopes of the relationship between creatinine and handgrip strength or creatinine and LMI were not significantly different. The slopes for creatine levels and LMI were significantly different in larger groups of similar patients, where the slope for diabetics was lower

than the non-diabetics. The authors concluded that muscle quality is well-reflected by creatinine levels in diabetic patients undergoing hemodialysis, and may be more representative of poor muscle quality than reduced muscle mass or malnutrition.

Overall, there are apparent differences among aging men and women's muscle mass and functional performance. There are several studies that have compared the accuracy of different measurements of muscle mass ranging from MRI, CT, DEXA, and BIA.[38,43,48,58,68] Additionally, using DEXA, there are multiple studies that have identified cut points for identification of sarcopenia in older adults, however, there is an inconsistency among these different studies' cut points.[2,17,57] Although each study has justification for the cut points identified, there needs to be a single factor that clinicians can use to correctly identify the incidence of sarcopenia in any population.

CHAPTER III

METHODOLOGY

Subjects and Research Design

Data from 167 men and women between the ages of 55 and 90 years were included in the present *ex post facto study* design. Table 1 contains the mean ± standard deviation of the sample demographics. Baseline data from two separate clinical trials were analyzed ex post facto. The two separate studies included: a two-phase clinical trial (phase one = A08; phase two = A09) sponsored by Abbott Nutrition conducted in 2008 and 2009 entitled "Evaluation of AN777 in Elderly Subjects," and a clinical trial (G10) sponsored by General Nutrition Corporation conducted in 2010 entitled "Effects of Whey Protein Supplementation on body Composition, Muscular Strength, and Mobility in Older Adults." For each clinical trial, participants were recruited from the University of Oklahoma-Norman Campus faculty and the surrounding Oklahoma City metropolitan area by flyers and verbal recruitment (Appendix F).

The inclusion and exclusion criteria for these clinical trials were similar. All participants were free from diabetes, active cancers, kidney or liver diseases, and they had not taken any protein, weight loss, calcium, or vitamin D nutritional supplements within at least 2 months of their screening visit. None of the subjects had undergone any type of in-patient surgery within 1 month of their screening visit. All participants for trials A08 and A09 had a BMI of 20-30 kg/m^2 and for trial G10 their BMI was 18.5-28.5 kg/m^2; therefore, the overall BMI for all subjects was between 18.5-30 kg/m^2. All subjects were untrained in resistance and aerobic exercise, and there was no

presence of uncontrolled blood pressure or arthritis that may have inhibited handgrip measurement. Blood analyses of BUN and creatinine, as confirmed by attending physicians, indicated no renal impairment.

Each subject completed testing for (a) body composition, (b) handgrip strength, (c) functionality, and (d) bench press and leg press strength. All procedures were explained to participants and they then signed an informed consent (Appendix C), HIPAA (Appendix D), and health and exercise status questionnaire (Appendix E). Any additional variables that were used in this study were calculated using one or any combination of these four aforementioned raw data. Of the 167 participants in all of the studies, 53 did not complete 5-RM bench press and leg press strength testing, but did complete all other assessments.

Procedures

Body Composition

All body composition was assessed using dual-energy x-ray absorptiometry (DEXA) (Lunar Prodigy Advance, PA+300532, Madison, WI). The device was calibrated daily with a quality assurance phantom with varying bone mineral density standards and percent fat standards and scans were not performed on subjects unless the quality assurance passed, where the measured density was within a predetermined range of the phantom's actual densities. Participants visited the laboratory after an 8-hour fast. Prior to each scan the participant's height, body mass, gender, birthdate, and race were entered in to the enCORE software (v.10.50.086, GE Healthcare, Madison, WI) by a certified technician. Each participant was asked to remove all removable metal objects from their body, and they were instructed to lay supine on the padded scan table with

the hands pronated (lying flat on the scanner bed) and positioned near their body (but not touching the hip). The legs were kept adducted by a Velcro strap wrapped around the distal leg, just above the ankles. A total body scan was selected with the appropriate body thickness (thin, standard, or thick, based on chest depth), and each scan lasted approximately 6 minutes. Scans were saved and analyzed after dividing the body into specific regions of interest (ROI). The right and left arms were separated from the torso by positioning the ROI through the neck of the humerus and the hands were separated from the hips. The lower-body was divided with a midline between the thighs and legs, and it was separated from the axial skeleton with a line through the necks of the femurs. Using results from the whole body scan, segmental lean mass (right arm, right leg, left arm, and left leg), total arm lean muscle mass, total leg lean muscle mass, total-body lean mass, and fat mass were used in further analyses. Additional variables used were also calculated from primary variables derived from the DEXA scan including ALST[32] (Eq. 1), non-skeletal muscle lean mass (Eq. 2), and TBSM (Eq. 3). ALST (Eq. 1) was calculated as the sum of the adipose-free muscle mass of the arms and legs. TBSM was predicted using an equation developed by Kim et al.[44] using ALST, age, and gender (Eq. 3).

$$\mathbf{ALST} = \sum(\mathbf{total\ arm\ lean\ muscle\ mass} + \mathbf{total\ leg\ lean\ muscle\ mass}) \quad \textbf{(Eq. 1)}$$

$$\textbf{Non-skeletal muscle lean mass} = \textbf{total lean mass} - \textbf{ALST} \quad \textbf{(Eq. 2)}$$

$$\textbf{TBSM} = (1.13 \times \textbf{ALST}) - (0.02 \times \textbf{age}) + (0.61 \times \textbf{gender*}) + 0.97 \quad \textbf{(Eq. 3)}$$

***Gender: male = 1 and female = 0**

Sarcopenia Classification

Four different methods of classifying sarcopenia were adopted from the literature. Table 2 displays each literature source, the population used to develop the classification system, the cut-off values for classification, and how those cut-off values were determined.

The first method used was proposed by Baumgartner et al.[2] using the DEXA scan, appendicular lean mass (ALM, which is also referred to as ASM and ALST in this and other studies) was determined as the sum of lean mass in the arms and legs as suggested by Heymsfield.[32] ASM was expressed relative to height squared (similar to how BMI is expressed), and consequently was considered the skeletal muscle index (SMI). The cut-off values used to classify sarcopenia were gender-specific and used 2 standard deviation units below the mean young adult sample used in the study.

Newman et al.[57] proposed two methods to classify sarcopenia. The first method used the same SMI value (ASM/ht^2) (Newman (a)). The second method used by Newman et al.[57] predicted gender-specific ALM (Newman (b)) with the following equations:

Men: predicted ALM (kg) = -22.48 + 24.14 x height (m) + 0.21 x fat mass (kg)

(Eq. 4)

Women: predicted ALM (kg) = -13.19 + 14.75 x height (m) + 0.23 x fat mass (kg)

(Eq. 5)

The difference between the predicted ALM and the actual ALM was considered the residual. The cut-off points for both methods proposed by Newman et al.[57] were determined as the lowest 20% of the sample studied in that paper. Finally, Delmonico et al.[17] used the ALM/ht^2 method suggested by Baumgartner et al.[2] and adapted it to

their population of older men and women. The cut-off values established by Delmonico and colleagues[18] were also set at the lowest 20% of the study's population.

Handgrip Strength

Isometric handgrip strength of the dominant hand was assessed using either a hand-held digital handgrip dynamometer (Detecto, DHS Series, Webb City, MO) or a hydraulic adjustable-handle handgrip dynamometer (Jamar, Sammons Preston Roylan, Boilingbrook, IL). A non-adjustable grip width (Detecto) was used for all subjects in A08 and A09, while subjects in G10 used the Jamar dynamometer with an adjusted grip width such that the 2nd phalanx of the middle finger was perpendicular to the device. Subjects performed the handgrip tests in a standing position with the arm near the torso, the elbow flexed at 90°, the forearm pronated to a neutral position, and the dynamometer head facing the tester directly in front of the participant. The scores from three trials were recorded, and the average of the two highest trials was used for subsequent analyses. Furthermore, handgrip muscle quality was calculated with the following equation:

Handgrip Muscle Quality = Handgrip strength (kg) / dominant-arm lean mass (kg) **(Eq. 6)**

Functionality

The timed get-up-and-go was performed on a measured and marked 3-meter course on solid laminate tile flooring using an armless wooden chair and a digital

stopwatch. Each participant began the test in a seated position with their feet behind a line marked on the floor. They were instructed to stand up, walk along the 3-meter line, turn around at the end of the line, and return to the start position as quickly as they could. The stopwatch began at the first sign of movement and stopped once the participant returned to the seated position. Time was recorded in seconds.

Bench Press and Leg Press Strength

Participants in A09 and G10 (n=114) completed bench press and leg press strength assessments, respectively. The 5-RM bench press exercise was performed on a standard free-weight bench (TuffStuff, Pomona, California) with an Olympic bar. The 5-RM leg press exercise was performed using a plate-loaded hip sled with a 45º incline (Paramount Fitness Corp., Los Angeles, California). For the bench press, participants were instructed to lay flat on the bench with their eyes directly under the bar, hands about shoulder width apart with a closed pronated grip. After receiving a lift-off from a spotter, subjects lowered the bar to their chest, paused briefly, and then pressed the bar to full extension of the elbows without locking them out. For the leg press, subjects were instructed to sit in the seat with their back flat against the backrest and were instructed to grasp the handles of the device tightly to avoid the buttocks losing contact with the seat during the exercise. Subjects placed their feet in the middle of the platform about shoulder-width apart, and this foot position remained constant for all the subsequent leg press tests. Subjects were instructed to lower the platform until the legs reached 90º of flexion at which point they were instructed to fully extend the legs (i.e., 0° of leg flexion). After a complete demonstration and explanation of the

exercises, each participant completed two warm-up sets with the first set of 10 repetitions at about 55-65% and the second set of 6-7 repetitions at about 75-80% of their estimated 1-RM. Three to 5 minutes of rest was allowed between sets. The load was increased to the subject's perceived 5-RM. Participants were instructed to complete 5 repetitions through their full range of motion. If they completed 5 repetitions and failed on the 6th attempt, testing was complete. If they performed more or fewer than 5 repetitions, they rested and the load was increased or decreased 5-10%, respectively, for their subsequent attempt. The 5-RM was determined within 5 attempts for the majority of participants. Once the 5-RM load was determined, the 1-RM was estimated with a prediction chart[20] using the following equation:

Epley[20] Predicted 1-RM = (1 + (0.0333 x reps)) x rep load **(Eq. 7)**

The estimated bench press and leg press 1-RM values were used in subsequent analyses. The bench press testing was always performed prior to the leg press. Muscle quality was calculated with the following equations:

Upper-body muscle quality = bench press 1-RM (kg) / ∑(right arm LM + left arm LM) **(Eq. 8)**

Lower-body muscle quality = leg press 1-RM (kg) / ∑(right leg LM + left leg LM) **(Eq. 9)**

Data Analyses

Eight separate independent t-tests were performed to examine age differences among individuals classified as sarcopenic or non-sarcopenic using each of the four methods. Fourteen separate two-way (2 x 4) full-factorial analyses of variance

(ANOVA) (gender [men vs. women] x age [50s vs. 60s vs. 70s vs. 80s]) were used to analyze LM, FM, ALM, TBSM, handgrip strength, TGUG, SMI, SMI residuals, non-skeletal muscle lean mass, bench press and leg press 1-RM, HGMQ, UMQ, and LMQ. Follow-up analyses included independent t-tests (men vs. women) collapsed across age and 1-way ANOVAs (50s vs. 60s vs. 70s vs. 80s) collapsed across gender with post-hoc analyses using pairwise comparisons.

Prevalence rates were calculated as ratios of the sample classified as sarcopenic to the entire sample (expressed as a percentage of the sample) for each of the four methods.[2,23,34,46,66] Use of prevalence rates within each study sample has been previously documented.[2,10,34,46,51]

Pearson's Product moment correlations were performed separately for each gender to determine the relationships among age, TGUG, bench press and leg press 1-RM, handgrip strength, LM, SMI, and HGMQ, UMQ, and LMQ.

To select the best model in determining sarcopenia status, Kendall's W coefficients of concordance were performed to demonstrate any differences among the different models' distributions of the categorical variable, SMI classification (sarcopenic vs. non-sarcopenic) for men and women. This test is used in comparing ranking by multiple judges where there may be ties in the rank, in this case 1 is better than 0 (no value) where 1 is non-sarcopenic.[64] Chi-squared ($_X^2$) tests of independence also compared any relationships among the different methods' distributions,[48,50,59] similar to those performed by Inaba et al.[35] and Newman et al.[57] where $_X^2$ were used to test the differences in categorical variable distribution.

As an exploratory analysis, binary logistic regression[12,22,52,65] analyses were used to predict one of two outcomes: sarcopenic or non-sarcopenic (as opposed to multiple logistic regression where there would be three or more potential outcomes).[22,65] Four separate logistic regression analyses[6] were performed with using the enter method as per previous recommendation,[27,65] each of the four methods for predicting sarcopenia classification selected as the dependent variables. Age, gender, LM, handgrip strength, and TGUG were used as theory-based predictors of sarcopenia classification, which were mathematically independent of the dependent variable.

All data were analyzed using computer software (PASW Statistics, version 18.0, Chicago, Illinois, United States). An alpha of $P \leq 0.05$ was used to determine statistical significance for all analyses.

CHAPTER IV

RESULTS

Age-related Differences Among Sarcopenia Classification

Figures 1-8 display differences across age for women and men for skeletal muscle and lean mass estimations, mobility and functionality performance, muscle quality, and SMI classifications.

Using the Baumgartner et al.[2] cut-off criteria, men and women classified as sarcopenic were significantly older than non-sarcopenic (mean differences 5.28 years, p=0.020; 5.76 years, p=0.002, respectively). Using the Newman et al.[57] (a) cut-off criteria, men and women classified as sarcopenic were significantly older than non-sarcopenic (mean differences 5.28 years, p=0.020; 6.12 years, p<0.001; respectively). Using the Newman et al.[57] (b) residuals cut-off criteria, men and women were not significantly different ages after being classified as sarcopenic or non-sarcopenic (mean differences 3.49 years, p=0.130; 3.38 years, p=0.054; respectively). Using the Delmonico et al.[17] cut-off criteria, men and women classified as sarcopenic were significantly older than non-sarcopenic (mean differences 5.28 years, p=0.020; 6.12 years, p<0.001; respectively).

Age- and Gender-related differences in Body Composition, Functionality, Strength, and Muscle Quality

There was no two-way interaction for ALM (p=0.105), however, there were main effects for gender (p<0.001) and age (p<0.001) (Figure 1a). ALM (collapsed across age) was significantly greater in men (p<0.001, mean difference 9.1 kg) than in

women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 50s, 60s, and 70s had significantly greater ALM (collapsed across gender) than those in 80s (p=0.001, mean difference 4.4 kg; p<0.001, mean difference 4.1 kg; p=0.002, mean difference 3.3 kg, respectively) (Figure 2a).

There was no two-way interaction for TBSM (p=0.102), however, there were main effects for gender (p<0.001) and age (p<0.001) (Figure 1b). TBSM (collapsed across age) was significantly greater in men (p<0.001, mean difference 10.8 kg) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 50s, 60s, and 70s had significantly greater TBSM (collapsed across gender) than those in 80s (p<0.001, mean difference 5.6 kg; p<0.001, mean difference 5.0 kg; p=0.002, mean difference 4.0 kg, respectively) (Figure 2b).

There was no two-way interaction for LM (p=0.209), however, there were main effects for gender (p< 0.001) and age (p<0.001) (Figure 1c). LM (collapsed across age) was significantly greater in men (p<0.001, mean difference 17.4 kg) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 50s, 60s, and 70s had significantly greater LM (collapsed across gender) than those in 80s (p=0.001, mean difference 8.4 kg; p<0.001, mean difference 7.4 kg; p=0.003, mean difference 6.1 kg, respectively) (Figure 2c).

There was no two-way interaction for non-skeletal muscle LM (p=0.588), however, there were main effects for gender (p<0.001) and age (p=0.036) (Figure 1d). Non-skeletal muscle LM (collapsed across age) was significantly greater in men (p<0.001, mean difference 6.6 kg) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 60s had significantly greater non-

skeletal muscle LM (collapsed across gender) than those in 80s (p=0.036, mean difference 2.3 kg) (Figure 2d).

There was no two-way interaction for FM (p=0.220) and no main effect for age (p=0.200). However, there was a main effect for gender (p=0.021) (Figure 1e). FM (collapsed across age) was significantly greater in women (p=0.021, mean difference 4.2 kg) than in men (Figure 2e).

For handgrip strength, there was a two-way interaction (p=0.023) (Figure 3a). A one-way ANOVA indicated a significant difference across age (p<0.001 and p=0.004) for both women and men, respectively. Follow-up post-hoc analyses with Bonferroni corrections indicated that handgrip strength was significantly greater for women in 50s than 70s and 80s (p=0.001 and p<0.001, respectively) and women in 60s had greater grip strength than 80s (p=0.013). Men in 50s, 60s, and 70s had significantly greater handgrip strength than men in 80s (p=0.003, p=0.003, and p=0.002, respectively). Additional independent t-tests indicated that men had greater handgrip strength than women in 50s, 60s, and 70s (p<0.001) (Figure 4a).

There was no two-way interaction for TGUG (p=0.879), and there were no main effects for age (p=0.364) or gender (p= 0.095) (Figures 3b and 4b).

There was no two-way interaction for bench press 1-RM (p=0.343), however, there were main effects for gender (p=0.006) and age (p<0.001) (Figure 3c). Bench press 1-RM (collapsed across age) was significantly greater in men (p<0.001, mean difference 27.0 kg) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 60s had significantly greater bench press 1-RM (collapsed across gender) than those in 80s (p=0.014, mean difference 13.4 kg) (Figure 4c).

There was no two-way interaction for leg press 1-RM (p=0.299), however, there were main effects for gender (p=0.003) and age (p<0.001) (Figure 4d). Leg press 1-RM (collapsed across age) was significantly greater in men (p<0.001, mean difference 79.3 kg) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 50s, 60s, and 70s had significantly greater leg press 1-RM (collapsed across gender) than those in 80s (p=0.001, mean difference 76.3 kg; p=0.016, mean difference 52.1 kg; p=0.026, mean difference 50.4 kg, respectively) (Figure 4d).

There was no two-way interaction for HGMQ (p=0.117) and there was no main effect for age (p=0.080). However, there was a main effect for gender (p=0.013) (Figure 5a). HGMQ (collapsed across age) was significantly greater in women (p=0.013, mean difference 1.5 $kg \cdot kg^{-1}$) than in men (Figure 6a).

There was no two-way interaction for UMQ (p=0.575) and there was no main effect for age (p=0.186). However, there was a main effect for gender (p<0.001) (Figure 5b). UMQ (collapsed across age) was significantly greater in men (p<0.001, mean difference 1.6 $kg \cdot kg^{-1}$) than in women (Figure 6b).

There was no two-way interaction for LMQ (p=0.578), however, there were main effects for gender (p=0.029) and age (p=0.001) (Figure 5c). LMQ (collapsed across age) was significantly greater in men (p=0.001, mean difference 2.0 $kg \cdot kg^{-1}$) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 50s had significantly greater LMQ (collapsed across gender) than those in 80s (p=0.021, mean difference 3.3 $kg \cdot kg^{-1}$) (Figure 6c).

There was no two-way interaction for SMI (p=0.566), however, there were main effects for gender (p=0.023) and age (p<0.001) (Figure 7a). SMI (collapsed across age) was significantly greater in men (p<0.001, mean difference 2.2 $kg \cdot m^{-2}$) than in women. Pairwise comparisons with Bonferroni corrections indicated that individuals in 60s had significantly greater SMI (collapsed across gender) than those in 80s (p=0.014, mean difference 0.8 $kg \cdot m^{-2}$) (Figure 8a).

There was no two-way interaction for SMI-residuals (p=0.892) and there was no main effect for age (p=0.250). However, there was a main effect for gender (p=0.008) (Figure 7b). SMI-residuals were significantly greater in women (p=0.008, mean difference 1.5 kg) than in men (Figure 8b).

Relationships Among Age, Body Composition, Functionality, Strength, and Muscle Quality

In women, age was negatively correlated with LMQ (r= -0.35, p<0.01), HGMQ (r= -0.34, p<0.01), handgrip strength (r= -0.54, p<0.01), UMQ (r= -0.28, p<0.05), and leg press 1-RM (r= -0.46, p<0.01). UMQ was negatively correlated with TGUG (r= -0.27, p<0.05) and positively correlated with LMQ (r= 0.39, p<0.01). LMQ was positively correlated with handgrip strength (r=0.32, p<0.05) and bench press 1-RM (r=0.49, p<0.01). Handgrip strength was positively correlated with leg press 1-RM (r=0.47, p<0.01). Additionally, SMI was positively correlated with bench press 1-RM (r=0.53, p<0.01), leg press 1-RM (r=0.61, p<0.01), LM (r=0.70, p<0.01), ALST (r=0.82, p<0.01), TBSM (r=0.82, p<0.01), LMQ (r=0.36, p<0.01), handgrip strength

(r=0.37, p<0.01), and FM (r=0.29, p<0.01) and negatively correlated with age (r= -0.37, p<0.01).

In men, age was negatively correlated with handgrip strength (r=-0.30, p<0.05) and bench press 1-RM (r=-0.37, p<0.05). UMQ was positively correlated with leg press 1-RM (r=0.48, p<0.01). LMQ was positively correlated with HGMQ (r=0.32, p<0.05), handgrip strength (r=0.43, p<0.01), and bench press 1-RM (r=0.58, p<0.01). HGMQ was positively related to bench press 1-RM (r=0.58, p<0.01). Handgrip strength was positively correlated with bench press 1-RM (r=0.67, p<0.01) and leg press 1-RM (r=0.57, p<0.01). Additionally, SMI was positively correlated with bench press 1-RM (r=0.53, p<0.01), leg press 1-RM (r=0.65, p<0.01), handgrip strength (r=0.52, p<0.01), LM (r=0.72, p<0.01), ALST (r=0.83, p<0.01), LMQ (r=0.43, p<0.01), and TBSM (r=0.83, p<0.01) and negatively correlated with TGUG (r=0.42, p<0.01).

Prevalence Rates

Three of 86 (3%) women and six of 68 (9%) men had impaired (>09.00s) TGUG using cut-off values determined by Bohannon[8] and 26 out of 91 (28.6%) women and eight out of 76 (10.5%) men were classified as having impaired handgrip strength as established by Laurentani et al.[47]. The percentage of men and women whom were classified as sarcopenic with each of the four SMI cut-off criteria are presented in Table 2.

Sarcopenia Cut-off Criteria Comparisons

The percentage of men and women whom were classified as sarcopenic with each of the four criteria are presented in Table 2. Three of the 4 different cut-off criteria use the same equation (ALM/ht^2) to identify SMI, but in attempt to compare all four's distributions of the sarcopenia classifications, Kendall's coefficient of concordance resulted in a significant difference among the distributions for women (p=0.025) and no differences among the distributions for men (p>0.05). Further analyses included chi-squared ($_X^2$) test of independence to make pairwise comparisons. In men, the chi-squared analysis resulted in a significant lack of independence across all methods (p<0.001), however, the strongest relationships emerged among the Baumgartner, Newman (a), and Delmonico methods (r=1.00, p<0.001). The Newman (b) method's $_X^2$ was significant, however, the relationships among Newman (b) and all other methods was not as strong (r=0.744, p<0.001) as other pairings. In women, the $_X^2$ analysis resulted in a significant lack of independence across all methods (p<0.05). Comparison of the Newman (a) and Delmonico methods resulted in a perfect relationship (r=1.00, p<0.001), Baumgartner with Newman (a) and Delmonico resulted in a strong relationship (r=0.760, p<0.001), and the weakest relationships resulted from Baumgartner and Newman (b) (r=0.374, p=0.001), and Newman (a) and Delmonico with Newman (b) (r=0.267, p=0.013).

Exploratory Logistic Regression

Four separate binary logistic regression analyses were conducted on SMI classification using each method [1-Baumgartner[2], 2-Newman[57] (a), 3-Newman[57] (b), and 4-Delmonico[17]] as the outcome of an individual being sarcopenic or non-

sarcopenic and five potential predictors of SMI: age, gender (female=0; male=1), LM, handgrip strength, and TGUG. Backwards likelihood ratio analyses were completed to determine the maximal likelihood estimate of an individual being sarcopenic or non-sarcopenic. The results of the logistic regression using cut-off criteria suggested by Baumgartner et al.[2] indicated a significant model ($p<0.001$) including handgrip strength ($B=0.069$, $p=0.095$), gender ($B=5.102$, $p<0.001$) and LM ($B=0.307$, $p<0.001$) as independent predictors and the intercept at $B= -16.816$. The model correctly predicted 82.5% of the cases. The models resulting from Newman et al.[57] (a) and Delmonico et al.[17] were identical with a significant final model ($p<0.001$) that included gender ($B=5.711$, $p<0.001$), LM ($B=0.452$, $p<0.001$) as independent predictors and the intercept at $B= -20.8$. The models correctly predicted 83.1% of the cases. Lastly, the model resulting from Newman et al. (b) residual method indicated a significant final model ($p<0.001$) which included handgrip strength ($B=0.122$, $p<0.001$) and TGUG ($B=-0.49$, $p<0.001$) as independent predictors and the intercept at $B=0.581$. This model correctly predicted 80.5% of the cases.

CHAPTER V

DISCUSSION

Age-Related Changes in Body Composition, Functionality, Strength, and Muscle Quality

Similar to previous studies, men were taller, weighed more, and had lower body fat percentages than women in all studies ($p<0.05$), in agreement with previous studies.[17,47,57] It has been well documented that muscle mass and strength decreases with advancing age.[4,5,28,33] Previous studies have reported that muscle mass remains stable through the fourth decade followed by a slow decrease in muscle mass from there on.[36] The present study's results indicated there was a decrease in LM, ALM, and TBSM following the 7th decade. Although there were decreases in LM, there were no age-related changes in fat mass. Fat mass has been previously reported to increase with age,[26] primarily due to genetics, inactivity, chronic illness, neural and hormonal changes, and concomitant diseases.[1,25] In addition, the results from the present study indicated that there were age-related decreases in muscular strength. Handgrip strength was lower in the 80s than at any other age in men, but in women handgrip strength was lower in the 70s and 80s than in the 50s and 60s. Bench press and leg press 1-RM was greater for men than women and collapsed across gender, bench press 1-RM was greater in the 60s than 80s and leg press 1-RM was lower in the 80s than at any other age. Interestingly, there were no age-related differences for HGMQ or UMQ, which indicated that strength in the upper body was maintained relative to the amount of muscle mass lost. This is similar to that reported by Newman et al.[56] in that the weight bearing muscles (lower-body) had a greater decline in muscle mass

than non-weight bearing muscles (upper-body). The present results indicated women had greater HGMQ than men, but lower UMQ and LMQ. LMQ was greater for individuals in their 50s than those in their 80s. Sayer et al.[62] recently reported that the relationship between muscle mass early in life (infancy) and early adulthood may indicate a predisposition to developing sarcopenia. Ultimately, maintenance or an increase in strength while maintaining or reducing the amount of muscle loss will improve muscle quality in older individuals. Tracy et al.[67] reported improvements in knee extensor strength, quadriceps muscle volume, and muscle quality with resistance exercises in older men and women. Thus, suggesting that resistance exercises may support muscle growth and subsequent increases in strength while combating a reduction in functionality and mobility with aging.

Recently, ALM has been assessed relative to height to normalize the data across gender and is referred to as SMI.[2,32]. Regardless of the height normalization, men had higher SMI than women, and although the cut-off values used to determine sarcopenia status reflect the gender differences, there are still inconsistencies among the cut-off values. In men, the cut-off values range from 7.23-7.26 kg/m^2,[2,17,57] however, in women there is a larger cut-off value range (5.45-5.67 kg/m^2).[2,17,57] Therefore, inconsistencies in cut-off values may potentially increase the errors in the classification of sarcopenia. In addition, mean SMI values with the residuals method calculated[57] resulted in greater values in women compared to men, which suggested that there was a higher amount of variability in the prediction of ALM when using height and FM from a whole-body DEXA scan.[57] Furthermore, the EWGSOP established the working definition of sarcopenia as the decrease in skeletal muscle

mass as the primary factor and functional deficits of mobility or handgrip strength as secondary factors.[15,23,54] In the present study, women had lower handgrip strength than men and a greater percentage of women had impaired handgrip strength according to established criteria by Lauretani et al.[47] of <20 kg (28.6%), while only 10.5% of the men had impaired (<30 kg) handgrip strength. If the same 28.6% of women and 10.5 % of men also have a SMI that falls below the cut-off value, then they would be considered sarcopenic according to the EWGSOP definition. However, 42.3%, 46.2%, 50.0%, and 50.0% of women with impaired handgrip strength were also classified as sarcopenic by Baumgartner et al.,[2] Newman et al.[57] (a and b), and Delmonico et al.,[17] respectively, not 100%. In addition, 75.0%, 75.0%, 62.5%, and 75.0% of men with impaired handgrip strength were also classified as sarcopenic by Baumgartner et al.,[2] Newman et al.[57] (a and b), and Delmonico et al.,[17] respectively.

Relationships Among Age, Body Composition, Functionality, Strength, and Muscle Quality

Similar to previous reports,[57] the results from the present study indicated significant negative correlations among age and handgrip strength, LM, upper- and lower-body 1-RM strength, LMQ, and HGMQ in women and handgrip strength, LM, and upper-body 1-RM in men. In addition, there were positive correlations among handgrip strength, HGMQ, upper- and lower body 1-RM, LMQ and UMQ for both genders, which is expected because the strength assessments are included in the calculation of muscle quality.

SMI is the most commonly used index of sarcopenia as established by Baumgartner et al.[2] SMI (ALM/ht^2) was strongly correlated with TBSM, ALST, and LM in men and women. SMI was also strongly positively correlated with upper- and lower-body 1-RM in men and women and handgrip strength in men. These relationships confirm the consensus definition of sarcopenia established by EWGSOP and their recommendations in classifying sarcopenia status based on muscle mass and function.[13,15,23,54] Of note, TGUG was not significantly correlated to SMI in women and had a weak correlation with SMI in men. Therefore the TGUG method may not be acceptable in an overall model to diagnose sarcopenia. The use of the SPPB is recommended by EWGSOP, primarily the 4-meter gait speed assessment of mobility,[15,23] however, the 4-meter gait speed test of the SPPB was not included in the present study.

Prevalence Rates of Sarcopenia

As the size of the aging population increases, there is a growing need for the determination of the extent of which sarcopenia is affecting older adults. Currently there are no databases forming DEXA-derived ALM normative values for young (18-39 years) middle-aged (40-60 years), or older adults (>60 years). In women, the prevalence rates of sarcopenia in the current sample differs, depending on which previous study's criteria is used: Baumgartner et al.,[2] Newman et al.,[57] or Delmonico et al.[17] The prevalence rates were higher than previously reported in women and lower than previously reported in men. The present study indicated that 30.8% of the women were considered sarcopenic according to the cut points established by

Baumgartner et al.,[2] whereas, the authors reported 33.9% prevalence in individuals ≥65 years. In addition, Baumgartner et al.[2] reported that 28.5% men were classified as sarcopenic, whereas, 13.2% of the sample were considered sarcopenic in the present study. Previously, Iannuzzi-Suchich et al.[34] reported prevalence rates of 22.6% and 26.8% for women and men, respectively, according to the Baumgartner et al.[2] cut-off values. Tankó et al.[66] reported 32.9% of women >70 years were considered sarcopenic using the Baumgartner et al.[2] criteria and 12.3% with their own reference group (18-39 years). Furthermore, Tanko et al.[66] used the same method as Baumgartner et al.[2] when determining a cut-off value (> -2 SD young adult mean) in women, which resulted in a lower value of 5.4 kg/m^2. The other criteria used in the present study, Newman et al. (a) and (b),[57] and Delmonico et al.[17] indicated a much higher prevalence in women of 41.8%, 40.7%, and 41.8%, respectively. In these studies the lowest 20% of the sample were considered sarcopenic and the cut-off values were set as the lowest quintile, despite the fact that in their population of 70-79 year old adults, 20% may be considered a conservative estimate. Men had a lower prevalence of sarcopenia with a calculated 11.8% for all models. Differences in the prevalence rates may be due to the study populations, with the present study having a lower age limit (≥55 years) and a larger age range (55-90 years, inclusive) than previous reports. Nonetheless, Tankó et al.[66] and Janssen et al.[40] reported a decline in muscle mass beginning in the fifth decade and suggests the present sample's age range (55-90) would capture accurate estimates of the prevalence of sarcopenia. In addition, previous studies used population-based samples from larger health surveys

where participants may not have visited a laboratory to undergo a DEXA to determine ALM, rather ALM was estimated from anthropometric measures.[2]

Alternative Classifications of Sarcopenia

The prevalence rates in the present study, Baumgartner et al.,[2] Iannuzzi-Suchich et al.,[34] and Tankó et al.[66] were greater than 20% (with the exception of men in the present study), which suggests that the arbitrary cut-off values at the lowest 20% of the samples of Newman et al.,[57] and Delmonico et al.,[17] may not be accurately representing the correct cut-off that should be used in the classification of sarcopenia. When using non-parametric analyses to determine the agreement among the different methods of classification, Kendall's W was used to compare the distributions of individuals considered sarcopenic. Kendall's W resulted in agreement of classification among all of the methods. Follow-up $_X^2$ analyses were performed to identify where (among the four methods) the strongest agreement occurred, and indicated that the strongest agreement was between Newman et al. (a)[57] and Delmonico et al.,[17] followed by Baumgartner et al.[2]

Since conception of the term 'sarcopenia', the interest and number of research studies conducted on sarcopenia has risen, and the number of different classification cut-off values has increased, subsequently resulting in an inconsistency in the classification of sarcopenia. In order to improve the classification, the present study explored an alternative use of binary logistic regression in the determination of sarcopenia with commonly used theory-based predictors encompassing the EWGSOP consensus definition of the age-related reduction in muscle mass and function. The

use of binary logistic regression with the independent predictors of age, gender, muscle mass, and a measure of functional performance may be useful in predicting sarcopenia status as "sarcopenic" or "normal" to incorporate uniformity in classification and may reduce the need to establish fixed cut-off values. The present study indicated that the most accurate model provided 83.1% correct predicted probability using Newman et al.[57] (a) or Delmonico et al.[17] and independent predictors of gender and LM. A model derived by Baumgartner et al.[2]criteria included gender, LM, and handgrip strength and provided 82.5% correct predicted probability. This method has been used in clinical settings to determine sepsis in the ICU.[27] Collectively, logistic regression can provide a binary determination of sarcopenia status where individuals with common characteristics (i.e., handgrip strength, LM, or age) would be classified into one of two outcomes. Future studies may warrant the use of this method in classification as opposed to identifying multiple cut-offs for function and muscle mass.

Conclusion

The classification of sarcopenia has most commonly been diagnosed by using the cut points established by Baumgartner et al.[2], however, using ALM/m^2 and cut points established by Newman et al.[57] (a) or Delmonico et al.[17] may more appropriately classify the current population as sarcopenic or non-sarcopenic. A larger epidemiological database needs to be established in order to generalize the proper cut-off values, perhaps those established by Newman et al.[57] (a) or Delmonico et al.[17] as

the dependent variable and the basis of binary logistic regression in actual classification of sarcopenia.

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APPENDIX A

TABLES

Table 1**.** Age, height, body mass, and percent body fat for all participants in A08, A09, G10, and together as a group.

Study		*n*	Age		Height (*cm*)		Body Mass (*kg*)		Body Fat (*%*)	
A08	*Men*	27	71.8	5.3	**177.4***	7	**83.3***	10.8	**29.6***	5.4
	Women	26	72.3	5.7	**162.4***	5.7	**64.5***	12.1	**39.0***	7.5
	All	53	72.0	5.5	170.0	9.9	74.1	14.8	34.2	8.0
A09	*Men*	27	72.6	6.0	**173.9***	5.3	**82.8***	11.4	**28.6***	5.6
	Women	27	72.3	6.9	**160.8***	6.1	**62.9***	10.4	**38.6***	6.1
	All	54	72.4	6.4	167.4	8.7	72.9	14.8	33.6	7.7
G10	*Men*	22	**67.1*#**	6.1	**177.2***	7.0	**81.0***	9.7	**27.4***	5.3
	Women	38	**63.1*#**	6.7	**162.9***	7.2	**66.0***	11.0	**38.4***	6.3
	All	60	64.6	6.8	168.2	9.9	71.9	13.0	34.4	7.9
TOTAL SAMPLE		167	69.5	7.2	168.5	9.5	72.9	14.1	34.1	7.9

Values represent mean ± SD; *denotes a difference between gender; #denotes a difference among studies

Table 2. Established cut points for classification of sarcopenia, the study sample, and reference population used to determine the cut points.

Author and year	Study	Location	Sample Size	Population	Reference Group	Reference Criteria	Men	Women
Baumgartner et al., 1998*	New Mexico Elder Health Survey/New Mexico Aging Process Study	New Mexico	808	Men and women ≥65 years old	229, 20-40 year old men and women from Rosetta Study and 301 men and women 65 and older	Greater than -2 SD below young adult average	<7.26 kg/m^2	<5.45 kg/m^2
Newman et al., 2003b*	Health, Aging, and Body Composition Study	Pennsylvania Tennessee	2984	70-79 year old men and women	Sample was used as reference	Lowest 20% of study distribution	>-2.29 kg	>-1.73 kg
Newman et al., 2003a*	Health, Aging, and Body Composition Study	Pennsylvania Tennessee	2984	70-79 year old men and women	Sample was used as reference	Lowest 20% of study distribution	<7.23 kg/m^2	<5.67 kg/m^2
Delmonico et al., 2007*	Health, Aging, and Body Composition Study	Pennsylvania Tennessee	2976	70-79 year old men and women	Sample was used as reference	Lowest 20% of study distribution	<7.25 kg/m^2	<5.67 kg/m^2
Tanko et al., 2002	Two cross-sectional studies from the Center of Clinical and Basic Research	Denmark	754	18-85 year old women	216, 18-39 year old women	Greater than -2 SD below young adult average	---	<5.4 kg/m^2
Iannuzzi-Sucich et al., 2002	Four studies from UConn Health Center and Claude Pepper Older Americans Independence Center	Connecticut	337	64-93 year old men and women	229, 20-40 year old men and women from Rosetta Study	Greater than -2 SD below young adult average	<7.26 kg/m^2	<5.45 kg/m^2

* Denotes the cut points that were compared in the present study.

Table 3. Sarcopenia cut points by gender and relative prevalence of sarcopenia in the current study.

Study	*units*	Women	# Sarcopenic (*%*)	Men	# Sarcopenic (%)
Baumgartner et al., 1998	*(kg/m^2)*	<5.45	28 (*30.8*)	<7.26	9 (*11.8*)
Newman et al., 2003a	*(kg/m^2)*	<5.67	38 (*41.8*)	<7.23	9 (*11.8*)
Newman et al., 2003b	*(kg)*	>-1.73	37 (40.7)	>-2.29	9 (*11.8*)
Delmonico et al., 2007	*(kg/m^2)*	<5.67	38 (*41.8*)	<7.25	9 (*11.8*)
Classified by all 4 as sarcopenic			19 (20.9)		7 (*9.2*)
Classified by all 4 as non-sarcopenic			37 (40.7)		55 (72.4)

APPENDIX B

FIGURES

Figure 1. Appendicular skeletal muscle mass (a), total body skeletal muscle (b), lean mass (c), non-skeletal muscle lean mass (d), and fat mass (e) for women (black) and men (grey) across age. Results are displayed with line of best fit and equation, R^2, standard error of the estimate, and SE_E expressed as a percentage of the mean.

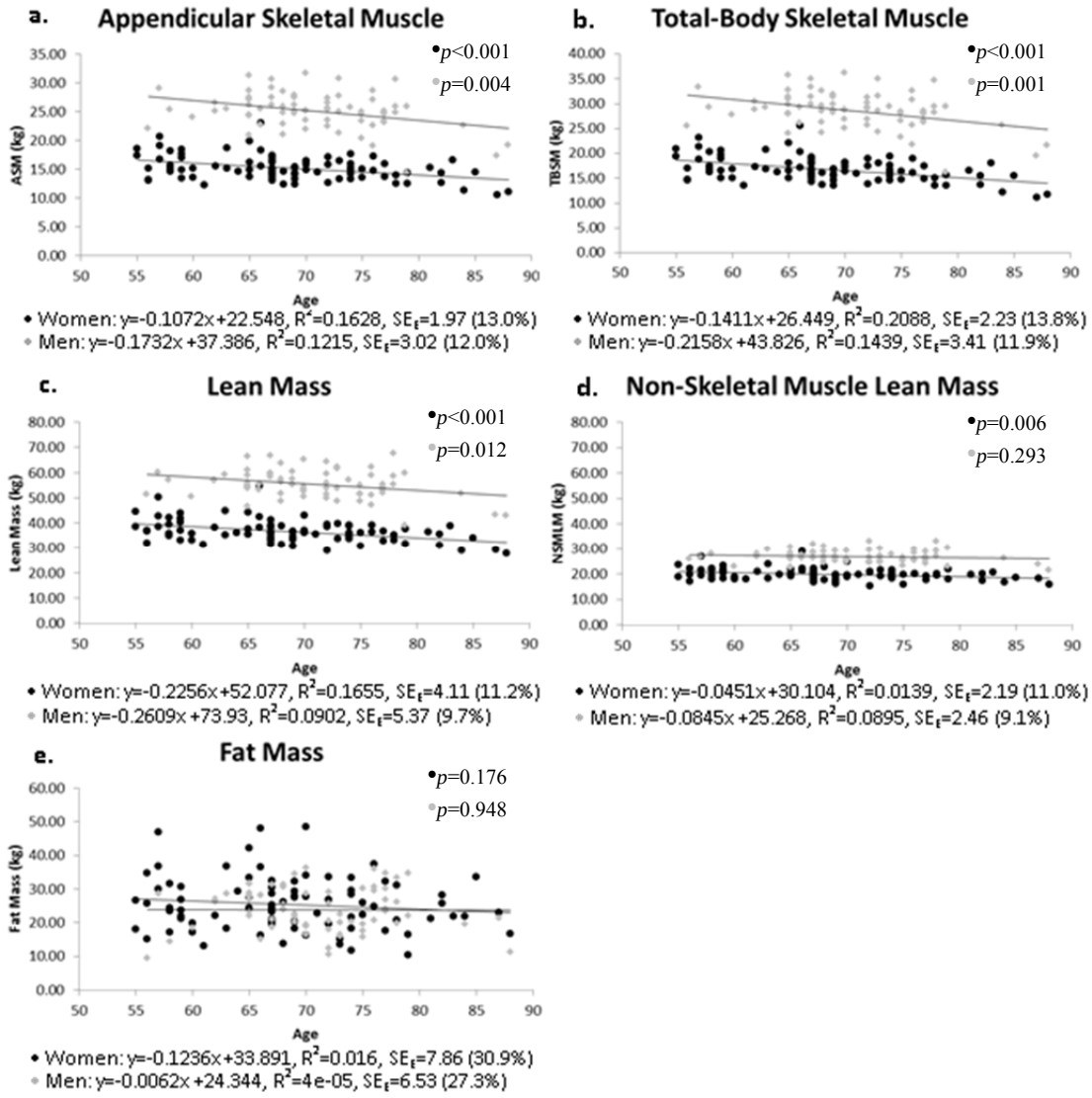


Figure 2. Age and gender differences for appendicular skeletal muscle mass (a), total body skeletal muscle (b), lean mass (c), non-skeletal muscle lean mass (d), and fat mass (e). Results are displayed as mean + SE values, women-black, men-grey.
* denotes a significant difference among age groups; # denotes a significant difference between genders.

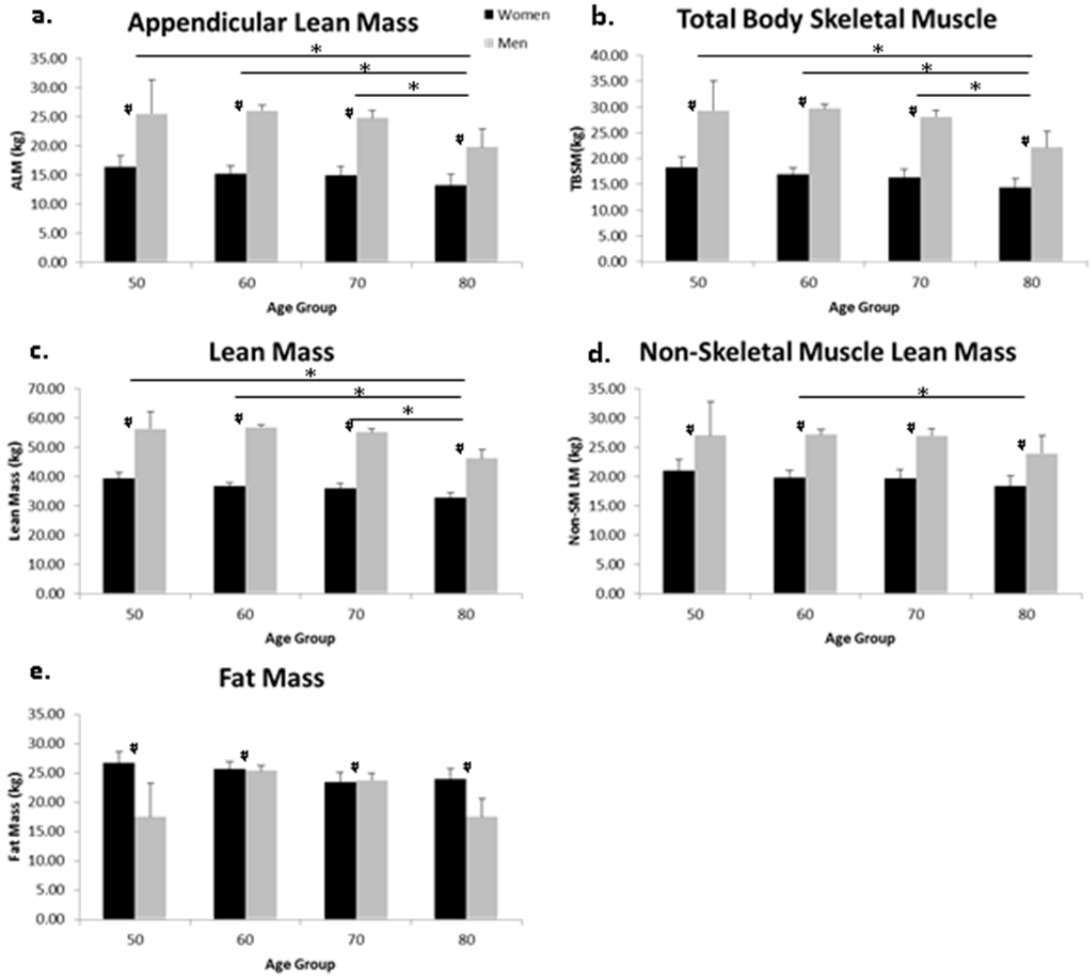


Figure 3. Handgrip strength (a), timed get-up-and-go (b), bench press 1-RM (c), and leg press 1-RM (d) for women (black) and men (grey) across age. Results are displayed with regression line and equation, R^2, standard error of the estimate, and SE_E expressed as a percentage of the mean.

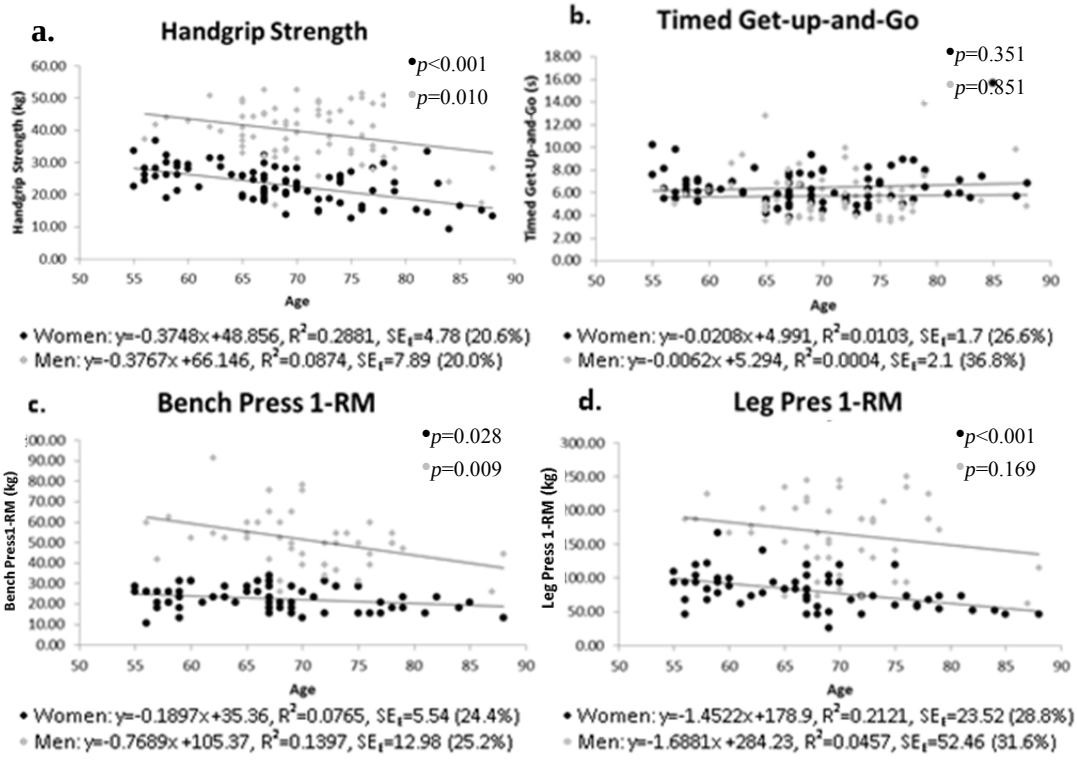


Figure 4. Age and gender differences for handgrip strength (a), timed get-up-and-go (b), bench press 1-RM(c), and leg press 1-RM (d). Results are displayed as mean + SE values, women-black, men-grey. * denotes a significant difference among age groups; # denotes a significant difference between genders.

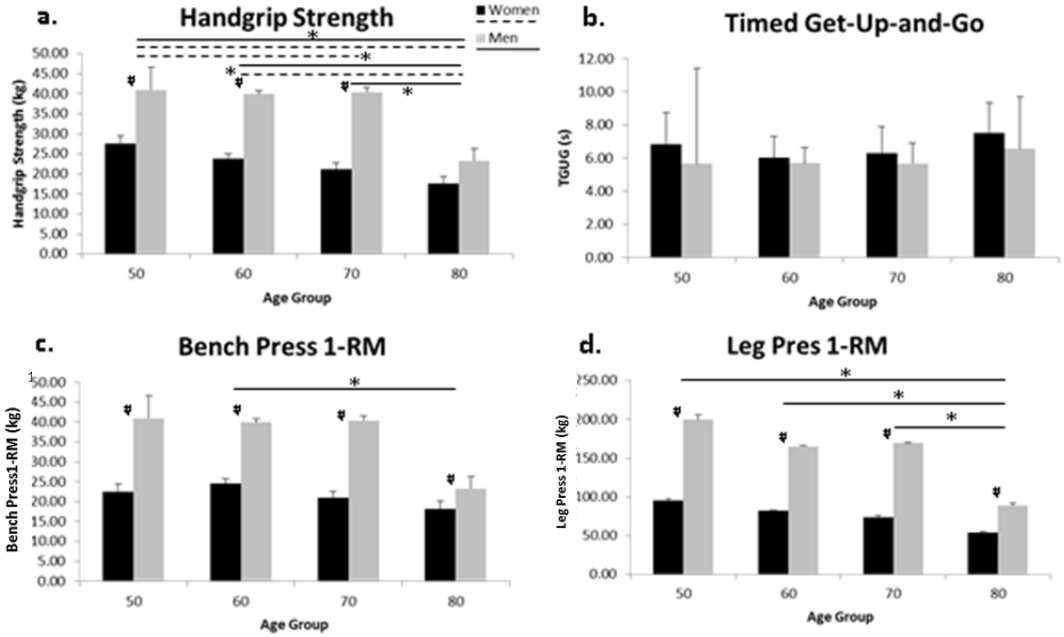


Figure 5. Hand grip muscle quality (a), upper- (b), and lower-body muscle quality (c) for women (black) and men (grey) across age. Results are displayed with regression line and equation, R^2, standard error of the estimate, and SE_E expressed as a percentage of the mean.

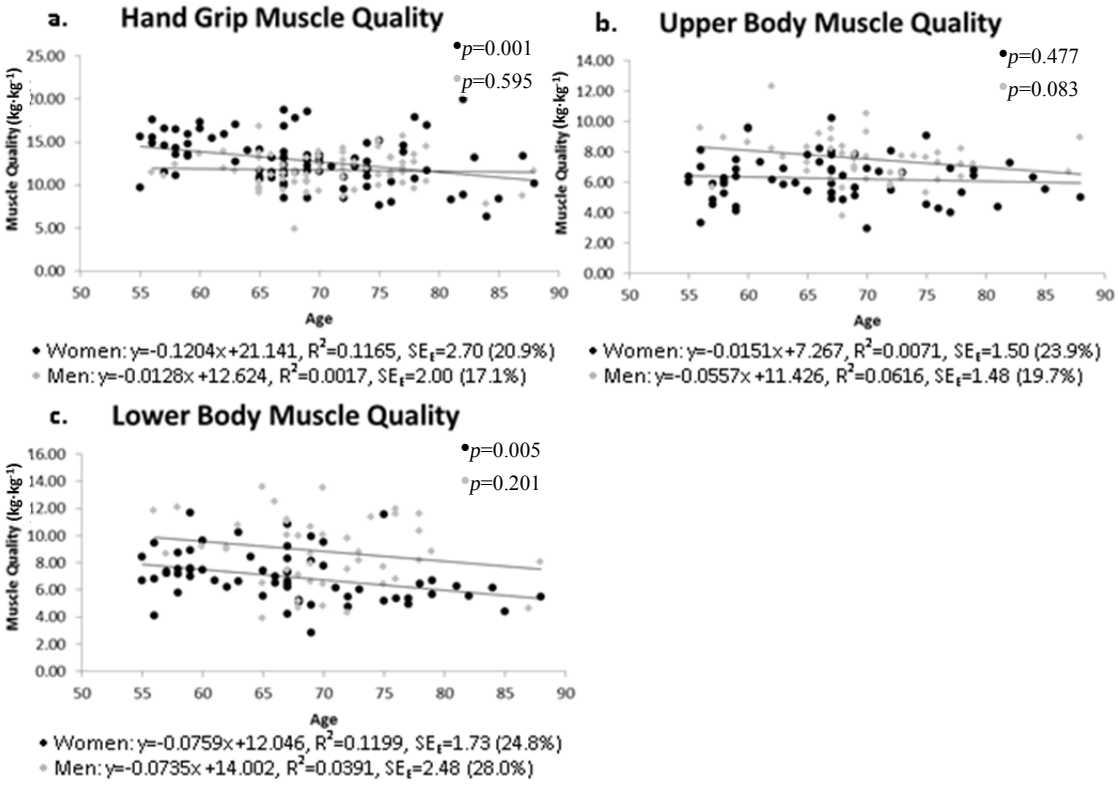


Figure 6. Age and gender differences for handgrip muscle quality (a), upper-body muscle quality (b), and lower-body muscle quality (c). Results are displayed as mean + SE values, women-black, men-grey. * denotes a significant difference among age groups; # denotes a significant difference between genders.

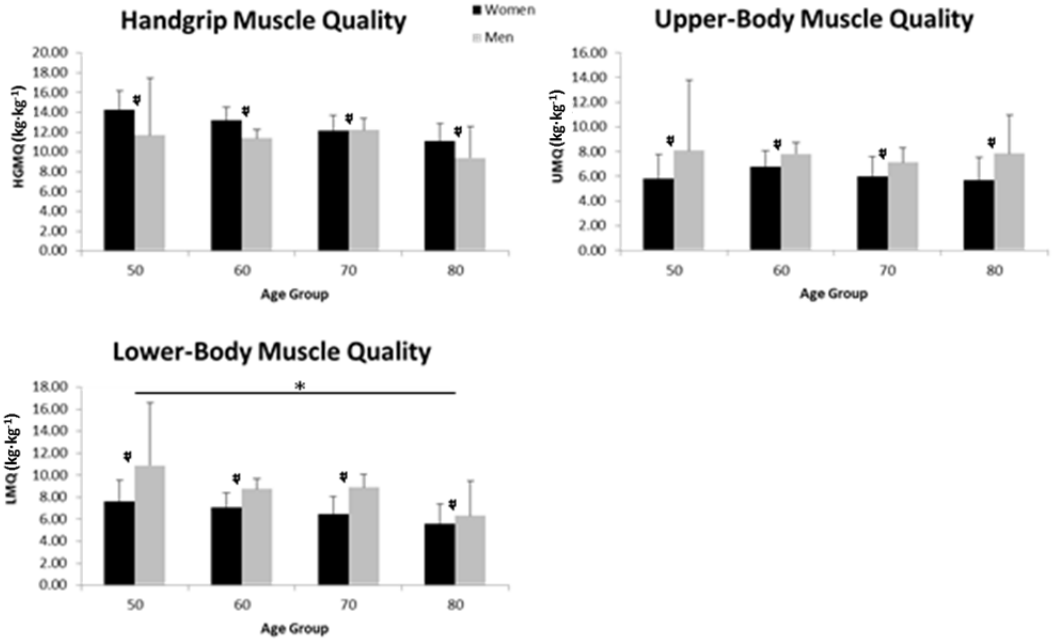


Figure 7. Skeletal muscle index expressed as ALM/ht^2 (a), and residuals (b) for women (black) and men (grey) across age and SMI cut-off criteria for women (c) and men (d). Results are displayed with regression line and equation, R^2, standard error of the estimate, and SE_E expressed as a percentage of the mean.

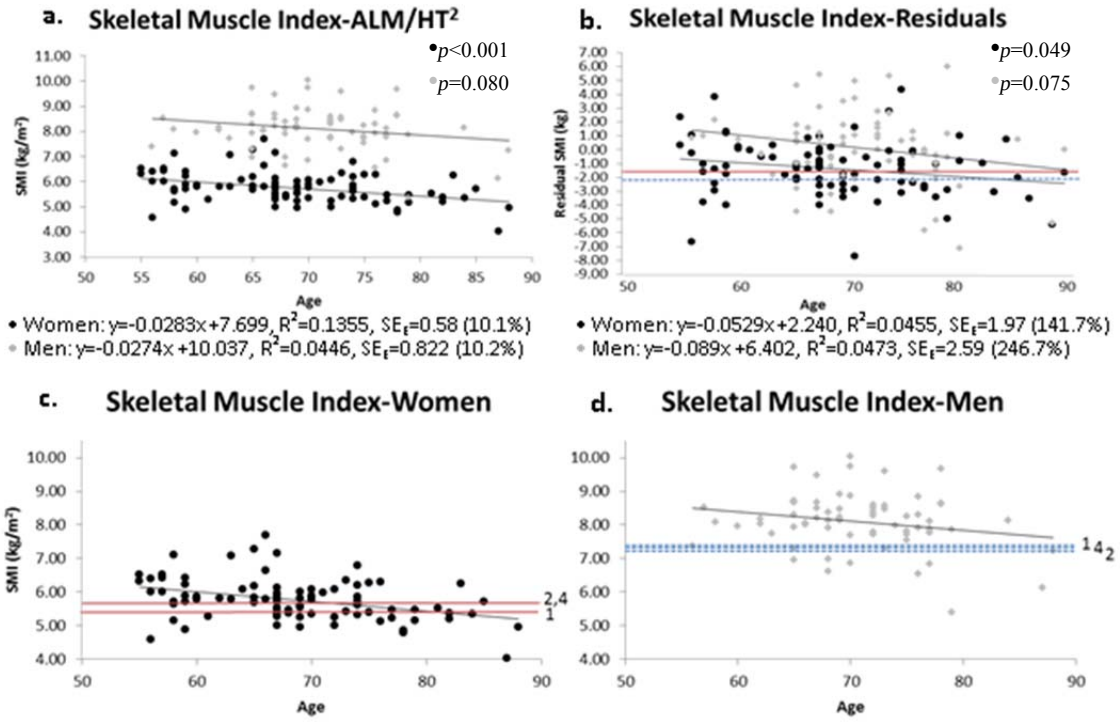
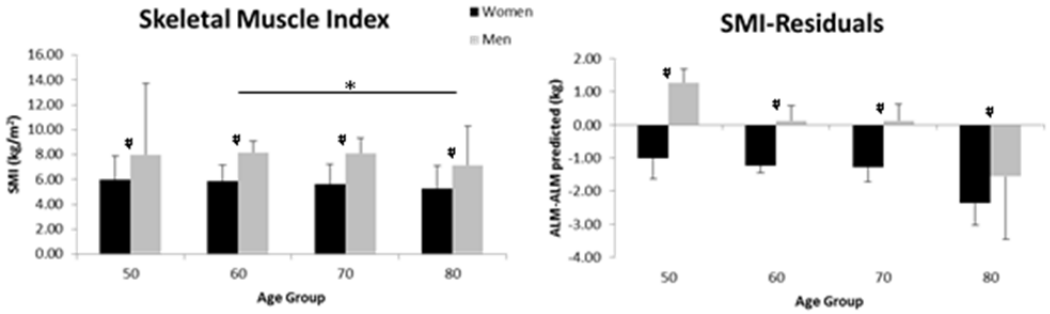


Figure 8. Age and gender differences for skeletal muscle index expressed as ALM/ht^2 (a) and residuals (b). Results are displayed as mean + SE values, women-black, men-grey. * denotes a significant difference among age groups; # denotes a significant difference between genders.



APPENDIX C

INFORMED CONSENT FORMS

701-A-1

University of Oklahoma
Institutional Review Board
Informed Consent to Participate in a Research Study

Project Title: Evaluation of AN777 in Elderly Subjects
Protocol Number: BK32
Sponsor: Abbott Nutrition, Abbott Laboratories
Columbus, Ohio
United States

Principal Investigator: Jeffrey R. Stout, PhD, FNSCA, FACSM, FISSN
Director Metabolic and Human Body Composition Laboratories
Department of Health and Exercise Science
University of Oklahoma
1401 Asp Avenue
Norman, OK 73019
Phone: 405-325-9023
Fax: 405-325-0594

You are being asked to volunteer for this research study. This study is being conducted at the University of Oklahoma Health and Exercise Science Department located in the Huston Huffman Center. You were selected as a possible participant because you are greater than or equal to 65 years of age, you agree and are able to comply with prescribed activity level, you are able to walk on your own, and you have certain nutritional risk factors.

This consent form may contain words that you do not understand. Please ask the study investigator or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

SUMMARY

- You are being asked to participate in a research study
- Your decision to be in this study is voluntary
- If you decide that you will be in this study and then you change your mind, you can leave the study at any time
- The care you receive in this study is not standard medical care. Your usual medical care from your doctor should not be replaced
- You will be randomly assigned to a placebo or the experiment/product group. You will then be placed into the exercise or non-exercise group.
- If you are in the non-exercise group, you will be in this study for about 6 months and have 4 study visits. You may receive reminder phone calls between visits. You may receive a follow-up phone call 7 to 10 days after you finish the study.
- If you are in the exercise group you will be in the study for about 6 months and have the same 4 study visits as stated above with the addition of 3 strength

testing sessions and 21 weeks of strength training sessions 3 days per week. The following is the order

- If you agree to be in this research study, your medical records will become part of this research. They may be looked at or copied by the sponsor of this study or government agencies (including the U.S. Food and Drug Administration) or other groups associated with the study.
- If you are injured in this study, your medical insurance may be billed for any treatment you may need, or for standard medical care that you receive that is not part of this study
- The study product is a metabolite of leucine, an essential amino acid found in protein foods. Benefits of this product MAY include the following; increased muscle tissue growth, and/or reduced muscle protein breakdown, increased immunity, decreased body fat, and lowering of blood cholesterol.
- You must agree to comply with prescribed activity level, i.e., either to maintain current activity or participate in resistance training
- Physician's clearance is necessary for participation in this study. Your physician will be contacted (via fax) with your approval. Typically this is free of charge, however, you will be responsible for paying any charges that a personal physician may implement to complete the release form or any charges for an office visit, if your physician requires that you make a personal visit in order to complete the release form. There is a study physician that may provide an exam for participation, free of charge.

More detailed information about this study is in this consent form. Please read this form carefully and ask any questions that you may have before agreeing to take part in this study.

Please read this form and ask any questions that you may have before agreeing to take part in this study.

Purpose of the Research Study

The purpose of this study is:

The purpose of this study is to evaluate the effect of a study product compared to a control product on muscle mass change in the non-exercising and exercising elderly population while on an adequate protein diet.

Number of Participants

About 108 people will take part in this study.

Procedures

If you agree to be in this study, you will be asked to do the following:

You will be asked to consume one packet of your assigned study product, with water, two times per day. The times of day in which you supplement are at your discretion. You cannot choose which study product you will get. This is decided by chance at Visit 1. You will have an equal chance of receiving the study product or the control product.

Neither you nor your study investigator will know which study product you receive. Your study investigator can find this out in an emergency.

You will make four study visits to the study site, each of which will last about 4 hours, with the exception of the screening visit. Study staff may phone you between visits to see how you are doing, remind you of study visit preparations and record keeping, and/or clarify information you have previously shared. You may also receive a follow-up phone call 7 to 10 days after study exit, if you have an ongoing medical event at exit.

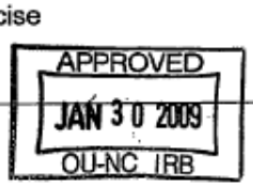
You will be required to visit the Human Performance Lab, located within the Huston Huffman Center, for all visits.

If you are participating in the non-exercising group, you will be asked to maintain your current activity level. If you are participating in the exercising group, you will schedule training times with a personal trainer and instructed on resistance training 3 days per week (i.e., Monday, Wednesday, and Friday) with at least 24 hours between sessions at the OU-Norman Health and Exercise Science training facilities (Huston Huffman Center) for a total of 21 weeks. Each training session will last up to 30-45 minutes.

For those in the exercise group, resistance training will consist of the following: Prior to starting resistance training, you will complete testing to have your one-repetition maximum determined using bilateral leg press, leg extension exercises, and chest press. These tests will be performed after both the screening visit following Visit 1, Visit 2 and the Final Visit/Exit on the same day or within 48 hours after completion of visits Visit 1, Visit 2 and the Final Visit/Exit. Prior to all testing attempts, you will first complete a standardized warm-up consisting of 5 minutes of stationary cycling at a light workload. You will then complete two warm-up sets of 10 repetitions at 55% and 65% of your perceived 1 repetition max. Between attempts, you will rest 3-5 minutes. You will complete three sets of 8-12 repetitions at approximately 80% of your pre-determined 1 repetition max first with the hack squat, then the bilateral leg press and finally with the leg extension for the lower body. Next, or prior to the leg exercises, you will perform a chest press and lateral pull down exercises utilizing the same percentage (approximately 80%) of your 1 repetition maximum. All resistance will be adjusted accordingly if you cannot complete the exercise so that each set of exercise stays within the desired range of 8-12 repetitions. Each set of exercise will be separated by a 2 to 5-minute recovery period allowing for full recovery. Weight will be increased as your trainer determines is appropriate. If you complete 12 repetitions for the last set of an exercise for two consecutive lifting sessions, weight will be increased by 2.5-10% depending on the exercise. Below is the exercise schedule based on week:

Week 1 - Testing
Weeks 2 and 3 – 1 Set per exercise
Weeks 3 and 4 – 2 Sets per exercise
Weeks 5 through 10 – 3 Sets per exercise
Week 11 (pre mid testing download week)
- Day 1 - 2 Sets per exercise
- Day 2 - 1 Set per exercise
- Day 3 - 2 Sets per exercise

Week 12 – Testing



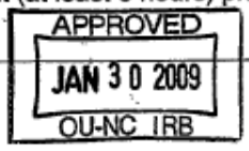
Weeks 13 through 22 – 3 Sets per exercise
Week 23 (pre mid testing download week)
Day 1 - 2 Sets per exercise
Day 2 - 1 Set per exercise
Day 3 - 2 Sets per exercise
Week 24 – Testing

During the screening, visit 1 and the final visit/exit you will be escorted to the Goddard Health Center, located on the OU-Norman Campus. The visits to Goddard will last approximately 0.5-1.0 hours, and are for blood work to assess total cholesterol (low density lipoproteins and high density lipoproteins), complete blood counts (CBC) and a CHEM-20, to assess albumin and HMB levels. Each blood draw requires 11.5 mL of blood. These tests help evaluate the function of your liver and kidneys; the visit-1 test will be used to assess your inclusion within the study, whereas visit-6 testing will be used to assess health-related response to the test intervention and supplementation compliance. If blood draw results are abnormal, you will be referred to your personal physician or Goddard Health Center for follow-up.

Screening Visit:

You will be asked to read and sign this consent form before any study-related procedures are performed. During the Screening Visit, the following will be done:

- Your age, race and gender will be collected
- Your self-reported medical history
- Your medication use
- Your self-reported physical activity
- Your body measurements (weight, height, knee height, and body mass index) will be measured
- A sample of your blood will be drawn and analyzed to assess your current nutritional status
- Your diet history will be collected
- You will receive a 3-day food diary to document three days of intake in the week prior to your next visit
- If you are participating in the exercising group, you will receive resistance training instructions.
- You will be reminded to fast prior to your next visit and that the first morning urine void will need to be collected at the facility during your next visit. In case of emergency, you will be provided with a sample collection container and instructed on how to collect a "clean catch" urine sample.
- You will be contacted by phone by the study staff prior to your next visit to be reminded of the following:
  - Fast overnight (at least 8 hours) prior to the visit



- Complete the 3-day diet diary in the week prior to the next visit
- First morning urine void on the day of Visit 1 needs to be collected at the site

Visit 1, Visit 2 and Final Visit/Exit

Visit 1 will take place about 7 days after your Screening Visit. Visit 2 will take place about 12 weeks after Visit 1. The Final Visit/Exit will take place about 24 weeks after Visit 1. The following will be done at each visit:

You will be asked about your medical history since the last visit, including the following:

- Changes in your health status
- Use of any medications, vitamins/mineral supplements, liquid or bar nutritional supplements, or other dietary supplements

- You will be randomly assigned, by chance (like the flipping of a coin) to one of the two study groups: (Visit 1 only)
  - Study product
  - Control product
- You will then be assigned to one of the following groups:
  - with exercise group
  - without exercise group
- Your weight will be measured
- A fasting blood draw will be performed (Visit 1 and Final Visit/Exit only)
- A first morning urine void will be collected
- Your body composition measurements include the following:
  - After a 12-hour fast, with water consumption allowed up to one hour prior to testing, you will participate in a series of data collection stations; each station will be made private by the use of room dividers and/or separate closed rooms. A female research assistant will accompany female subjects, who are being tested by a male researcher, during each testing station. The complete body composition measurements will last approximately 4-4.5 hours, per visit. You will be required to wear either a bathing suit or tight-fitting clothing, such as Spandex, during these visits.
  - **Bioelectrical Impedance (BIA/BIS)** (15-20min) – (Body tissue impedance is measured when a small, harmless electrical signal is passed through your body, carried by water and fluids) You will be asked to lie flat on your back and will have two electrodes attached to your right foot and hand; at the ankle and wrist, and the toe and finger, respectively. This widely used and FDA-approved commercial device conducts a harmless and painless electrical current through your body.
  - **Dual X-Ray Absorptiometry (DEXA) (10-15min) – *(The DEXA is essentially a padded table with a mechanical arm that uses low-dose radiation to measure bone mineral density)*** You will be asked to lie flat on your back, with your arms at your sides, legs extended and feet together. The "arm" of the DEXA will then slowly move over your body, without contact. This test will be used to assess total bone mineral density and upper and lower body muscle mass. You will be exposed to very

small levels of radiation – exposure that has been determined to be no greater than that which most Americans receive in several days from natural background radiation (~300 mrem/year) sources, such as radioactivity released from the soil. You will be required to wear either a bathing suit or tight-fitting clothing, such as Spandex, during this test.

- **BOD POD Measurements (10-15min) –** ***(The BOD POD is an egg-shaped device for someone to sit in, and is used for measuring and tracking body fat and lean mass using patented air displacement technology)*** You will be weighed, given a swimming cap to cover your hair, and then asked to sit in the Bod Pod for approximately three minutes. You will be asked to breathe normally and not to move while the machine calculates your body volume.
- **Total Body Water (TBW) and Extra cellular Water (4hrs) –** ***(The amount of water within your body; both the water within and outside your body cells, as measured by a urine test after drinking a chemical solution)*** You will be asked to drink a solution containing 10mL of deuterium oxide (D2O). The substance is non-radioactive, but may taste quite salty. Prior to drinking the solution, you will be asked to urinate into an 8 fl-oz cup, filling the cup about halfway. You will then be asked to ingest the D2O solution and then refrain from consuming any food or beverage for the next four (4) hours. All other body composition stations will be completed during this 4-hour period. After the four hours have expired, you will be asked to again urinate in an 8 fl-oz cup, as described above.
- **Ultrasound Thickness Measurements (10-15 min) –** ***(The thickness of your skin and muscle tissue)*** This technique involves applying a thick layer of gel on the surface of your skin and a metallic transducer gently applied over the surface of your skin. During the measurement, the ultrasound transducer is slid back and forth along the surface of your skin. Measurements will be taken at the following eight sites: biceps, abdomen, thigh, calf, hamstring, front hip (female only), triceps (female only), and chest (male only).

- You will be asked to complete a questionnaire to assess activities of daily living
- Your 3-day food diary will be collected and reviewed by your study investigator
- You will receive study product and instructions on how to prepare the product (Visit 1 and Visit 2 only)
- If in the exercising group, you will be reminded of your training schedule (Visit 1 and Visit 2 only)
- You will receive a new 3-day food diary to document three days of intake in the week prior to your next visit (Visit 1 and Visit 2 only) and product intake forms to record daily study product intake (Visit 1 and Visit 2 only)
- You will be tested for strength and functionality within 1-3 days after the body composition tests as follows;

- **Your upper body strength test** requires that you squeeze a handheld dynamometer handle as forcefully as possible for three to five seconds. You will be asked to do this three times using your dominant arm and the three tests will be averaged for your max strength score. **Your lower body strength** will be assessed using an isokinetic dynamometer and will require you to sit in an oversized chair with your knee joint aligned with the dynamometer axis of rotation, and straps will be positioned at the hips, shoulders, and over the right thigh to prevent extraneous movement. The fulcrum of the lever will be positioned on the shin, just above the ankle. You will be asked to perform three maximal effort knee extension and flexion movements at an angular speed of 60-degrees/second and 180-degrees/second. Three trials will be performed, with three minutes of rest in between trials, and the average of the three trials will be considered your max lower body strength. **The 'Get-up-and-Go' test** consists of timed measurements starting from a seated position of a chair, standing, walking forward 3 meters, turning around, walking back to the chair, and sitting down.

- You will be reminded to fast prior to your next visit and that the first morning urine void will need to be collected at the facility during your next visit. In case of emergency, you will be provided with a sample collection container and instructed on how to collect a "clean catch" urine sample.
- You will be contacted by phone by the study staff prior to your next visit to be reminded of the following:
  - Fast overnight (at least 8 hours) prior to the visit
  - Complete the 3-day diet diary in the week prior to the next visit
  - First morning urine void on the day of Visit 2 needs to be collected at the site
- You will return any remaining study product (Final Visit/Exit only)

7 Day Follow-up Phone Call:

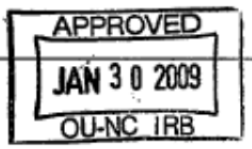
If you have an ongoing medical event (such as a cold) at the time of study exit, you will be contacted 7 to 10 days after the exit visit. You will be asked if the medical event has resolved.

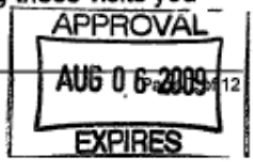
Length of Participation

You will be in this study for about 6 months and have 4 study visits. You may receive reminder phone calls between visits. You may receive a follow-up phone call 7 to 10 days after you finish the study.

Visit one (Screening visit) will last approximately 30-60 minutes, where you will complete the required forms, health history questionnaire and sign the informed consent. During this visit you will also be instructed on how to fill out the provided Nutrition Logs.

Visits 1, 2 and Final Visit/Exit will last approximately 4 hours. During these visits you will be assessed for body composition.





The visits for strength and functionality (within 1-3 days after the body composition tests) will last approximately 45-60 minutes.

This study has the following risks:

You will be asked health-related questions (i.e., allergies, current/recent medications, medical conditions, etc.) that will be recorded and used for screening purposes only. If you do not meet the inclusion criteria, as provided by the researchers, or are not cleared by your physician, you will not be allowed to participate.

Because the study product is considered investigational, there may be other risks to you that are unknown at this time. If you have any problems with the study product, you should contact the study investigator or study coordinator as soon as possible.

Very Likely To Occur:

- Pain, bruising, feeling faint, arm soreness or slight risk of infection from having your blood drawn. Blood draws will be performed by a phlebotomist at the Goddard Health Center and will require a single needle to puncture your skin. If any of the results are abnormal, you will be given a copy and advised to visit your personal physician or referred to Goddard Health Center for follow-up.
- Muscle fatigue, during and immediately following upper and lower-body maximal exercise tests and training and functionality tests
- Feelings of hunger from observing a 12-hour fast, on two test days
- Small amount of radiation from DEXA. Although the amount of radiation exposure received in the study is minimal, it is important that you are aware that the risk from radiation exposure is cumulative over a lifetime. If you participate in the study you will receive three DEXA scans (a type of x-ray), and thus be exposed to additional amounts of radiation that you would not have received otherwise. Women should always inform their physician or X-ray technologist if there is any possibility that they are pregnant. No complications are expected with the DEXA procedure.

May Possibly Occur:

- Emotional/psychological discomfort, distress and/or anxiety due to the type of clothing required and/or human-human contact and interaction required for accurate and successful completion of the body composition assessment. Tests, however, take only a few minutes to complete.
- Muscle soreness may begin within 24 hrs following maximal upper- and lower-body maximal testing and training, and may last for up to several days.
- Anxiety resulting from closed-in spaces (BOD POD)

Some research designs require that the full intent of the study not be explained prior to participation. Although we have described the general nature of the tasks that you will be asked to perform, the full intent of the study may not be explained to you until after the completion of the study. At that time, we may provide you with a full debriefing which

will include an explanation of the hypothesis that was tested and other relevant background information pertaining to the study. You will also be given an opportunity to ask any questions you have about the hypothesis and the procedures used in the study.

New Findings

Any new information that is discovered during the study, which may change your decision to continue participation in the study, will be made available to you in a timely manner.

Benefits of being in the study are

This is not a treatment study. You are not expected to receive any direct medical benefits from being in this study. The information from this research study may benefit others in the future.

The benefits of knowing your body composition, regional distribution of muscle mass, upper and lower-body strength, functionality, and general blood parameters (cholesterol, triglycerides, glucose, white blood cells and red blood cells, etc) are wide and numerous and include potentially identifying underlying problems that you may not currently be aware of.

The benefit of knowing your recommended protein intake may help you to understand healthier eating habits.

The study product is a metabolite of leucine, an essential amino acid found in protein foods. Benefits of this product MAY include the following; increased muscle tissue growth, and/or reduced muscle protein breakdown, increased immunity, decreased body fat, and lowering of blood cholesterol.

Additionally, participants in the exercise groups will have personalized resistance training sessions with a trainer.

Alternate Procedures

This is not a treatment study. Your other option is to not participate in this study.

Costs

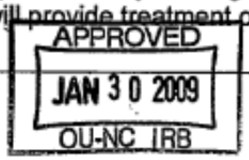
The sponsor will provide study product. There are no charges for the study visits. You will be billed for all medical treatment that is not part of this study. These charges may include charges relating to your medical care (hospital and physician fees), which are not a part of this study.

SOURCE OF FUNDING FOR THE STUDY

The study investigator is being paid by Abbott Nutrition, Abbott Laboratories to conduct this research.

Injury

If you are injured as a direct result of participation in this study, contact the study investigator immediately. The study investigator will review the situation. If necessary, the study investigator will provide treatment or refer you for treatment.



If the study investigator determines that any complication, injury, or illness requiring emergency medical treatment is a result of participation in this study, appropriate acute medical care will be provided at no cost to you. Abbott agrees to pay all reasonable medical expenses necessary to treat such injury:

(1) to the extent you are not otherwise reimbursed by medical insurance, and
(2) provided you have followed the directions of the study investigator and/or study staff.

This agreement to provide medical treatment does not include complications, injuries, or illnesses you might get while in the study if these complications are not a result of the study product. There are no plans for additional payment for lost wages, pain and suffering, or for other losses.

The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

By signing this consent form, you will not give up any legal rights for yourself.

In case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company may be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

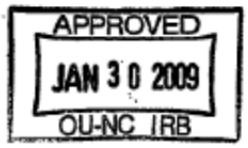
Confidentiality

In published reports, there will be no information included that will make it possible to identify you without your permission. Research records will be stored securely and only approved researchers will have access to the records.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the Abbott Nutrition, Abbott Laboratories and the OU Institutional Review Board.

Compensation

You will be reimbursed for you time and participation in this study. Participants in the non-exercise groups will receive a stipend in the amount of $200 upon completion of the study, otherwise, a prorated amount will be awarded. Stipends will be prorated based on weekly participation. The total duration of the study is 6 months (24 weeks). Weekly participation will be compensated $8.33. Participants in the exercise groups will receive a stipend in the amount of $300 upon completion of the study, otherwise, a prorated amount will be awarded. Stipends will be prorated based on weekly participation. The total duration of the study is 6 months (24 weeks). Weekly participation will be compensated $12.50. If you withdraw from the study you will be paid according to the last completed week.



Voluntary Nature of the Study

Your participation in this study is voluntary. You may decide not to participate or you may leave the study at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your participation in this study may be stopped at any time by the study investigator or the sponsor without your consent if it is determined that it is in your best interest or in the best interest of this study.

If you are removed from the study prior to your final study visit, then you may be asked by the study investigator to return to the study site one last time to return study product, forms and answer questions about any changes in your health status.

Participation in this study should not replace routine medical care by your primary care physician or specialist.

Contacts and Questions

If you have concerns or complaints about the research, the researcher(s) conducting this study can be contacted at

Jeffrey R. Stout, PhD at **405-325-9023** or jrstout@ou.edu for any of the following reasons:

- If you have questions concerning your participation in this study,
- If at any time you feel you have experienced a research-related injury or reaction to the study product, or
- If you have questions, concerns, or complaints about the research.

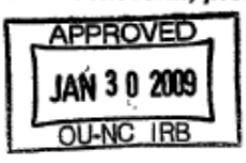
You may also contact: Abbie E. Smith, MS at 405-325-5211 or abbiesmith@ou.edu
Jordan Moon, MS at 405-325-1368 or JordanMoon@ou.edu

Contact the researcher(s) if you have questions or if you have experienced a research-related injury.

If you have any questions about your rights as a research participant, concerns, or complaints about the research and wish to talk to someone other than individuals on the research team or if you cannot reach the research team, you may contact the University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

You will be given a copy of this information to keep for your records. If you are not given a copy of this consent form, please request one.



Statement of Consent

I have read the above information. I have asked questions and have received satisfactory answers. I consent to participate in the study.

I authorize the use and disclosure of my health information to the parties listed in the "authorization to use or disclose protected health information for research" form and the authorization section of this consent for the purposes described above.

By signing this consent form, I have not given up any of my legal rights.

_____

Printed Name of Subject

_____ _____

CONSENT SIGNATURE: **Date**

_____

Printed Name of Legally Authorized Representative

_____ _____

Signature Legally Authorized Representative Date

_____

Authority of Subject's Legally Authorized Representative or Relationship to Subject (when applicable)

_____

Printed Name of Person Conducting Informed Consent Discussion

_____ _____

Signature of Person Conducting Informed Consent Discussion Date

Consent Version 3, September 2010 *IRB No: 15263*

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
Norman Campus
"Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults"
Sponsored by General Nutrition Corporation
Dr. Joel T. Cramer

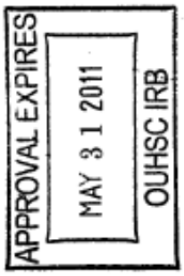
This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to take part in this trial/study because you meet the criteria of being a healthy individual between 55 and 80 years of age, with a BMI between 18.5 and 28.5.

Why Is This Study Being Done?
The purpose of this study is to determine the effects of 12 weeks of whey protein supplementation on body composition (lean mass), muscle strength, mobility and physical function, bone health (bone density), and quality of life.

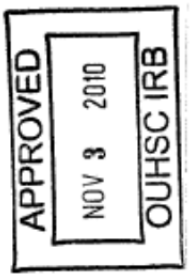
What is the Status of the Drugs (Devices or Procedures) involved in this study?
Advanced Whey Protein is an investigational product which is not approved by the US Food and Drug Administration.

How Many People Will Take Part In The Study?
About 150 people will take part in this study worldwide/nationwide. About 75 of these individuals will participate at this location.



What Is Involved In The Study?

For Randomized Trials:
You will be randomized to receive either study supplement *Advanced Whey Protein* or placebo (inactive substance, which will look like the study drug). Randomization means that you are put in a group by chance. You have a 50% chance of receiving the study product. A computer program of the study sponsor will make this random assignment. Neither you nor the investigators will choose which group you will be in. Neither you nor the investigators will know which group you have been assigned to.



If you take part in this study, you will have the following tests and procedures:

1. Preliminary screening of inclusion and exclusion criteria
2. Height, weight, resting heart rate and blood pressure

3. Demographics (age, gender, activity level)
4. Fasting blood sample for routine laboratory analysis
5. Intercurrent illnesses (any illness that occurs while you are involved with the study) and concomitant medications (medications, prescription or over-the-counter, that you are taking to treat the illness) will be evaluated upon enrollment in this study
6. Weight, resting heart rate and blood pressure
7. Body composition and bone density assessed by a dual-energy x-ray absorptiometry (DEXA) whole body scan
8. Muscle strength (1-repetition maximum bench press and leg press assessment determined from 5-RM model and hand grip)
9. Mobility and physical function
10. Quality of life questionnaire

The timeline of participation will go as follows:

- *Visit 1 – Screening (day -3±2)*

You will visit the study site and sign a dated Informed Consent Form (this form) and complete a pre-participation health history questionnaire. Vital signs, weight, height, concomitant medications, intercurrent illnesses and demographics will also be assessed and recorded. A fasting blood sample will be collected for routine laboratory analysis.

- *Visit 2 – Baseline (day 0)*

Subjects who meet the study criteria will return to the site and be familiarized with the experimental procedures. Weight, vital signs, intercurrent illnesses and concomitant medications will be recorded. You will undergo testing for body composition and bone density (DEXA). Maximal muscle strength for the upper and lower body (1-RM bench press and leg press estimated using the 5-RM model) and grip strength will be determined. Mobility (the heel to toe stand and the heel to toe walk and distance walked in 30 minutes), physical function (get up and go test) will be assessed. Quality of life (SF-36 questionnaire) will be determined. You will be randomized into one of two groups:

- Group 1: Advanced whey protein formula + training, (50/ group; 25 males and 25 females)
- Group 2: Placebo + training (50/ group; 25 males and 25 females)

Test product: 15 g maltodextrin + 20 g whey protein isolate + 6.2 g leucine + 1000 mg calcium + 1000 IU vitamin D

A 12 week supply of test product will be dispensed. Dosing instructions, a dosing diary, a 3-day food log and guidance on a healthy, nutritionally adequate diet will be provided to you. You will be instructed to bring unused study product and completed dosing diaries and food logs back to the study site at the end of the study.

- *12-week Training - (day 0- day 84)*

You will participate in a 12-week resistance training and aerobic exercise (walking) program 3 days per week at the Huston-Huffman Center. On training days, study site staff will ensure that you consume 1 serving of the study product immediately after

exercise at the study site. The second serving will be consumed in the evening before food. On non-training days participants in the treatment and placebo groups will be instructed to ingest the supplement once in the morning before food and once in the evening before food. Training and supplement compliance, adverse events, concomitant medications and intercurrent illnesses will be assessed at training visits.

- *Visit 3-Post Training I (day 85±2)-Final Visit*

You will visit the study site. You will return your completed 3 day food log, dosing diaries and any unused study product. Supplement and training compliance, adverse events and use of concomitant medications will be assessed. You will undergo measurement of weight and vital signs. A fasting blood sample will be collected for routine laboratory analyses. Body composition and bone density will be measured using DEXA. Maximal muscle strength for the upper body (1-RM bench press and leg press estimated using the 5-RM model) and grip strength will be determined. Mobility (the heel to toe stand and the heel to toe walk and distance walked in 30 minutes), physical function (get up and go test) will be assessed. Quality of life (SF-36 questionnaire) will be determined.

How Long Will I Be In The Study?

We think that you will be in the study for approximately 12 weeks (85 days).

There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent.

Examples:

- The Investigator feels that it is in your medical best interest.
- New information becomes available.
- You fail to follow study requirements.
- The study is stopped by the sponsor.

You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher. There will be no penalty for not completing the study and you will be compensated for your time to that point.

What Are The Risks of The Study?

While on the study, you are at risk for these side effects. You should discuss these with the researcher and/or your regular doctor.

Blood Draw:

Common risks involved with a blood draw include pain at site of draw, bruising, feeling faint and slight risk of infection. In the event of an abnormal blood draw result, the investigator will be notified by the blood analysis laboratory (DLO). The principle investigator (or research team) will provide you the results, inform you of the abnormality, and encourage you to consult your physician. At the time this information is given to you, the principle investigator and you will decide whether or not it is safe to continue enrollment in this study.

Exercise Testing Risk:
There is a possible risk of muscle strain or injury during the 5-repetition maximum (5-RM) testing protocol. Your physical risks will be minimized by having each testing session conducted by qualified investigators. Testing measurements will be observed and monitored during each session by a CPR-certified trainer. All testing procedures will be done in a controlled manner. All additional research staff members directly involved with testing of the subjects are familiar with the National Strength and Conditioning Association's standards and protocols for exercise testing and emergency management. Rise in heart rate and blood pressure associated with exercise may also occur.

Radiation Exposure:
In addition to any radiographic procedures that are being done as part of this research, you may also be exposed to radiation from procedures that are part of your normal care. The number and frequency of these procedures are based on standard clinical practices for a person with good health; however, your doctor may order an additional radiographic test if he/she thinks it is necessary for your care.

The risk from radiation exposure increases over your lifetime as you receive additional exposure to radiation. Radiation exposure from the whole body DEXA scan is minimal (0.0004 mSv or 0.04 mrems). For comparison, in the United States we receive about 3.0 mSv (300 mrem) of exposure from natural background radiation every year.

Consumption of Advanced Whey Protein:
The issue of a safe upper limit of total protein intake has been considered due to a concern that excessive intakes of protein may be associated with various health risks. An official upper tolerable limit has not been determined due to inconsistent data. The Recommended Daily Allowance is 0.8g/kg/day or slightly lower for older adults and studies have shown that protein is well tolerated at such levels over 1.2g/kg/day. If randomly selected to consume the protein supplement, you will receive 20g of whey protein per serving and will consume 2 servings per day (an additional 40g per day). If in the placebo group, you will not receive any additional protein.

According to US guideline, the acceptable macronutrient distribution range (AMDR) is 5-35% calories from protein. The introduction of an additional 40 grams of protein per day would not result in deviating from the AMDR. In the US, the average protein intake for adults ages 51-70 years is higher than the RDA. The average daily intake in males is 1.2g/kg body weight (based upon weight of approximately 73.6kg; accounts for 16.2 % of total calories) and in females is 1.1g/kg body weight (based upon weight of approximately 60.6 kg; accounts for 15.7% of total calories)

Leucine, a branched-chain amino acid, is widely available in foods such as brown rice, beans, meats, nuts, soy and whole wheat products. Leucine has been shown to stimulate protein synthesis when taken regularly while on a strength training regimen without any significant side effects.
Whey protein, a dairy based amino acid source, is a popular protein source for supplements. Whey has been shown to have higher biological value and solubility than

whole proteins. It is widely available in foods such as milk, ice cream, bread, canned soup, and other processed foods. In studies demonstrating that whey protein has positive effects on muscle size and strength, no significant side effects were observed.
Calcium and **vitamin D** help enhance and maintain bone health. The recommended doses for older adults is 1200mg/day of calcium and 400-600 IU/day of vitamin D for maintenance. The addition of protein to these levels can further decrease the risk of bone deterioration (osteoporosis).

Are There Benefits to Taking Part in The Study?

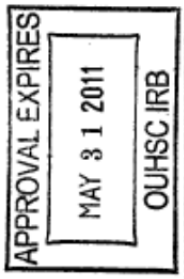
If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope that the information learned from this study will benefit other patients with this disease in the future.
Direct benefits from assessments may include:

- Identification of bone health and body composition
- Participation in a structured exercise program for 12 weeks may enhance mobility and physical function
- Feedback given regarding muscular strength
- Efficacy of treatment on quality of life and health status in older adults.

What Other Options Are There?
You may choose not to participate in the study.

What About Confidentiality?
Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

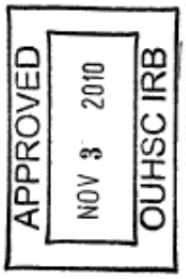
There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the US Food & Drug Administration, General Nutrition Corporation (study sponsor), the investigators (faculty members and graduate students appointed to this protocol from the Health and Exercise Science department at the University of Oklahoma), governmental agencies and authorities, and the OUHSC Institutional Review Board.



What Are the Costs?
There is no cost involved with participation in this study:

Will I Be Paid For Participating in This Study?

You will be reimbursed for your time and participation in this study. If you complete the study, you will receive a stipend in the amount of $200; otherwise a prorated amount will be awarded. If by any chance you do not complete the study, you will receive a prorated stipend based on the stage of completed participation. The following is the payment per stage and the proration schedule:



Payment per stage:
$50 for completing the first blood draw and the pre-testing measurements.
$100 for completing the training sessions*.
$50 for completing the second blood draw and the post-training measurements.

Proration schedule:
$50.00—first blood draw and all pre-testing completed.
$150.00—all pre-testing (including blood draw) and training sessions completed*.
$200.00—all testing (pre- and post-) including both blood draws and training sessions* completed.

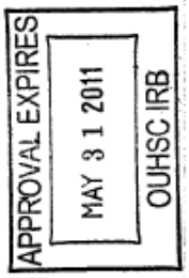
* You must complete at least 85% of the training sessions (31 out of 36 sessions) to be considered compliant. If you complete fewer than 20 sessions, you will be paid $2.78 per session completed ($2.78 x 36 = $100).

What if I am Injured or Become Ill While Participating in this Study?
In the case of injury or illness resulting from this study, emergency medical treatment is available. No funds have been set aside by The University of Oklahoma Health Sciences Center, General Nutrition Corporation, or The University of Oklahoma Health and Exercise Science Department, to compensate you in the event of injury.

What Are My Rights As a Participant?

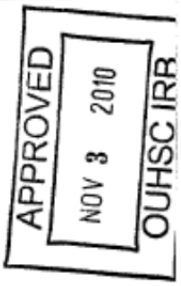
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, at certain times during the treatment, it may be dangerous for you to withdraw, so please be sure to discuss leaving the study with the principal investigator or your regular physician. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.



Whom Do I Call If I have Questions or Problems?
If you have questions, concerns, or complaints about the study or have a research-related injury, contact the Principle Investigator, Dr. Joel T. Cramer, 24 hours a day and 7 days per week at (405) 501-0651, or the Study Coordinator, Diane McBride at (405) 325-5211, during normal business hours.

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.



For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

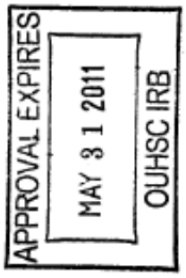
Signature:

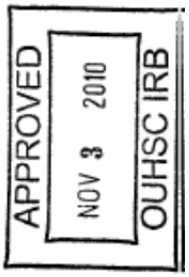
By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18) *(Or Legally Authorized Representative)*	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

IRB Office Version Date: 07/07/2009





APPENDIX D

HIPAA FORMS

UNIVERSITY OF OKLAHOMA – NORMAN CAMPUS
INSTITUTIONAL REVIEW BOARD

AUTHORIZATION TO USE or DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.

Title or Research Project: Evaluation of a leucine metaboliteon muscle mass in elderly subjects

Principal Investigator: Jeffrey R. Stout, PhD.

IRB Number:

Address: Health and Exercise Science, 1401 Asp Ave., Huston-Huffman Center, Norman, OK 73019

Phone Number: 405-325-9023

If you decide to join this research project, University of Oklahoma (OU) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private information To be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to evaluate the study supplement and analyze the study results.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop procedures or

commercial products. They may share your private information with the research sponsor, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services (HHS). The researchers may also share your private information with agents working on behalf of the Sponsor to help collect and analyze the results of the study .

Confidentiality. Although the research may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

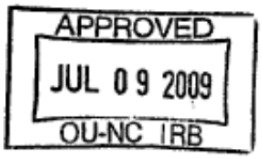
Voluntary Choice. The choice to give OU researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OU.

Revoking Permission. If you give OU researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OU researchers to use or share your private information for their research will be granted. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma
1000 Stanton L. Young Blvd., STE 221,
Oklahoma City, OK 73117
If you have questions, call: (405) 271-2511



Giving Permission. By signing this form, you give OU and OU's researchers led by Jeffrey R. Stout, permission to share your private information for the research project called Evaluation of a leucine metabolite on muscle mass in elderly subjects.

Subject Name:

_____ _____

Signature of Subject Date
Or parent if Subject is a Child

Or

_____ _____

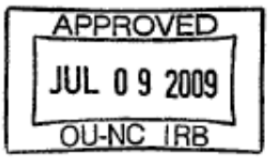
Signature of Legal Representative** Date

**If signed by a legal Representative of the Subject, provide a description of the relationship to the Subject and the Authority to Act as Legal Representative:

_____

OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.



AUTHORIZATION TO USE or DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.
Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults**

Leader of Research Team: **Joel T. Cramer, Ph.D.**

Address: **1401 Asp Avenue, HHC 104 Norman, Oklahoma 73019**

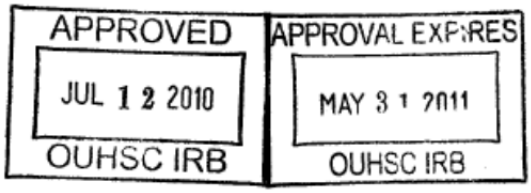
Phone Number: **(405) 325-1371**

If you decide to join this research project, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private Information To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to analyze and interpret all findings from this investigation for use in presentation and publication of its findings. Nowhere during this process, will you or your information be identified. Your results will be combined with others from the study to effectively analyze and interpret the findings.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research sponsor, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS). The researchers may also share your private information with General Nutrition Corporation (study sponsor), the investigators (faculty members and graduate students appointed to this protocol from the Health and Exercise Science department at the University of Oklahoma), governmental agencies and authorities, and the OUHSC Institutional Review Board.



Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

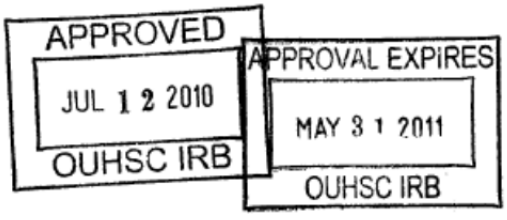
Voluntary Choice. The choice to give OUHSC researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OUHSC.

Revoking Permission. If you give the OUHSC researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OUHSC researchers to use or share your private information for their research will never end. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma Health Sciences Center
PO Box 26901, Oklahoma City, OK 73190
If you have questions call: (405) 271-2511



Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by Joel T. Cramer, permission to share your private information for the research project called "Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults".

Patient/Subject Name: _____

_____ _____
Signature of Patient-Subject or Parent if subject is a child — Date

Or

_____ _____
Signature of Legal Representative** — Date

**If signed by a Legal Representative of the Patient-Subject, provide a description of the relationship to the Patient-Subject and the Authority to Act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

IRB No.: [~~Provide IRB number~~ 15263]

APPROVED
JUL 12 2010
OUHSC IRB

APPROVAL EXPIRES
MAY 31 2011
OUHSC IRB

APPENDIX E

HEALTH & EXERCISE STATUS QUESTIONNAIRES

University of Oklahoma

Evaluation of a leucine metabolite on muscle mass in elderly individuals

Health History & Exercise Status Questionnaire

Side A

Demographics:

Name: _____ Subject number: _____

Date: _____ Age: _____ Date of Birth: _____

Daytime phone: _____ Evening contact number: _____

Family History:

Has anyone in your immediate family had any of the following: Please circle **Yes** or **No.**

Heart disease	Yes	No	Diabetes	Yes	No
High blood pressure	Yes	No	Cancer	Yes	No
Stroke	Yes	No	Tuberculosis	Yes	No
Sudden Death (before 50)	Yes	No	Asthma	Yes	No
Epilepsy	Yes	No	Gout	Yes	No
Migraine Headaches	Yes	No	Marfan's Syndrome	Yes	No
Eating Disorder	Yes	No	Sickle Cell	Yes	No

Please explain all Yes responses; denoting relationship and age of onset/occurrence of the family member in question (if known):

_____

_____

_____

Personal History:

1. Have you ever been hospitalized? Yes No
   Have you ever had surgery? Yes No
   Are you presently under a doctor's care? Yes No
   Have you ever been diagnosed with a sleeping disorder or clinical depression? Yes No
   Please explain and give dates for all Yes responses: _____
   _____
   _____
2. Please list any medications you are currently taking and for what conditions: _____
   _____
   _____
3. Please list any known allergies: _____
   _____
4. Have you ever had a head injury / concussion? Yes No
   Have you ever been "knocked-out" or unconscious? Yes No
   Have you ever had a seizure, "fit" or epilepsy? Yes No
   Have you ever had a "stinger," "burner" or pinched nerve? Yes No
   Do you have recurring headaches or migraines? Yes No
   Please explain and give dates for all Yes responses:
   _____
   _____
   _____
5. Have you ever had the chicken pox? Yes No
   If Yes, at what age? _____
6. Have you ever had the mumps or measles? Yes No
7. Do you have a history of asthma? Yes No

8. Are you missing an eye, kidney, lung or testicle? Yes No

9. Do you have any problems with your eyes or vision? Yes No

University of Oklahoma

Evaluation of a leucine metabolite on muscle mass in elderly individuals

Health History & Exercise Status Questionnaire

Side B

10. Have you ever had any other serious medical problems (mononucleosis, diabetes, anemia, etc)? Yes No

11. Have you ever taken any supplements for improved performance? Yes No

12. Are you presently taking any supplements for diet or performance (creatine, protein, etc.)? Yes No

13. What is the lowest bodyweight you have been at, within the last **3 months**? _____
Highest? _____ What is your ideal weight? _____

14. Do you have trouble breathing, or do you cough during or after exercise? Yes No

15. Have you ever had heat cramps, heat illness or muscle cramps? Yes No

16. Do you have any skin conditions (ex: itching, rashes, acne, rosacea, etc)? Yes No

Please explain all Yes responses for question 5 -16: _____

17. Have you ever fainted during or after exercise? Yes No
Have you ever been dizzy during or after exercise? Yes No
Have you ever had chest pain during or after exercise? Yes No
Have you ever had high blood pressure? Yes No
Have you ever been told you have a heart murmur? Yes No
Have you ever had racing of your heart or a skipped heartbeat? Yes No
Have you ever had an EKG or echocardiogram? Yes No

Please explain all Yes responses for question 17: _____

18. Have you ever sprained / strained, dislocated, fractured, or had repeated swelling or other injury of any bones or joints? Please explain all Yes responses.

Head / Neck	Yes	No	_____
Shoulder	Yes	No	_____
Elbow & Arm	Yes	No	_____
Wrist, Hand & Fingers	Yes	No	_____
Back	Yes	No	_____
Hip / Thigh	Yes	No	_____
Knee	Yes	No	_____
Shin / Calf	Yes	No	_____
Ankle, Foot & Toes	Yes	No	_____

19. Have you participated in a study involving a DEXA scan in the past year? YES NO

20. Have you had a CT scan in the last year? YES NO, How Many? _____

CT = 2,500 mrem
X-ray = 8 mrem
< 5000mrem per year

21. Have you had any x-ray scan in the last year? YES NO, How Many? _____

Please Sign:
I hereby state that, to the best of my knowledge, my answers to the above questions are correct.

Subject's Signature: _____ Date: _____

PRE-EXERCISE TESTING HEALTH & EXERCISE STATUS QUESTIONNAIRE

Name _____ Date_____

Birthday (mm/dd/yy)____/_____/_____ Home Phone _____(Cell)_____

Home Address_____

Gender_____ Age _____(yrs) Height _____(ft)_____(in) Weight_____(lbs)

Does the above weight indicate: a gain____ a loss____ no change____ in the past year?
If a change, how many pounds?_____(lbs)

Person to contact in case of emergency_____ Phone_____

Personal Physician _____ Physician's Phone_____

A. JOINT-MUSCLE STATUS (✓Check areas where you currently have problems)

Joint Areas
( ) Wrists
( ) Elbows
( ) Shoulders
( ) Upper Spine & Neck
( ) Lower Spine
( ) Hips
( ) Knees
( ) Ankles
( ) Feet
( ) Other_____

Muscle Areas
( ) Arms
( ) Shoulders
( ) Chest
( ) Upper Back & Neck
( ) Abdominal Regions
( ) Lower Back
( ) Buttocks
( ) Thighs
( ) Lower Leg
( ) Feet
( ) Other_____

B. HEALTH STATUS (✓Check if you currently have any of the following conditions)

( ) High Blood Pressure
( ) Heart Disease or Dysfunction
( ) Peripheral Circulatory Disorder
( ) Lung Disease or Dysfunction
( ) Arthritis or Gout
( ) Edema
( ) Epilepsy
( ) Multiply Sclerosis
( ) High Blood Cholesterol or Triglyceride Levels
( ) Allergic reactions to rubbing alcohol
( ) Acute Infection
( ) Diabetes or Blood Sugar Level Abnormality
( ) Anemia
( ) Hernias
( ) Thyroid Dysfunction
( ) Pancreas Dysfunction
( ) Liver Dysfunction
( ) Kidney Dysfunction
( ) Phenylketonuria (PKU)
( ) Loss of Consciousness

** NOTE: If any of these conditions are checked, then a physician's health clearance will required.*

C. PHYSICAL EXAMINATION HISTORY

Approximate date of your last physical examination_____

Physical problems noted at that time_____

Has a physician ever made any recommendations relative to limiting your level of physical exertion? _____YES _____NO
If YES, what limitations were recommended?_____
_____

D. CURRENT MEDICATION USAGE (List the drug name and the condition being managed)

MEDICATION	CONDITION
_____	_____
_____	_____
_____	_____

E. PHYSICAL PERCEPTIONS (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after *physical activity* (PA); or during *sedentary periods* (SED))

PA	SED		PA	SED	
( )	( )	Chest Pain	( )	( )	Nausea
( )	( )	Heart Palpitations	( )	( )	Light Headedness
( )	( )	Unusually Rapid Breathing	( )	( )	Loss of Consciousness
( )	( )	Overheating	( )	( )	Loss of Balance
( )	( )	Muscle Cramping	( )	( )	Loss of Coordination
( )	( )	Muscle Pain	( )	( )	Extreme Weakness
( )	( )	Joint Pain	( )	( )	Numbness
( )	( )	Other_____	( )	( )	Mental Confusion

F. FAMILY HISTORY (✓Check if any of your blood relatives . . . parents, brothers, sisters, aunts, uncles, and/or grandparents . . . have or had any of the following)

( ) Heart Disease
( ) Heart Attacks or Strokes (prior to age 50)
( ) Elevated Blood Cholesterol or Triglyceride Levels
( ) High Blood Pressure
( ) Diabetes
( ) Sudden Death (other than accidental)

G. EXERCISE STATUS

Do you regularly engage in aerobic forms of exercise (i.e., jogging, cycling, walking, etc.)? **YES** **NO**

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you regularly lift weights? **YES** **NO**

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

Do you regularly play recreational sports (i.e., basketball, racquetball, volleyball, etc.)? **YES** **NO**

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

H. **DIET** (✓Check the nutritional supplements you are currently taking or have taken within the past 9 weeks.) If checked, please list product and dose/frequency

( ) Protein:_____

( ) Protein Drinks:_____

( ) Creatine Monohydrate:_____

( ) Vitamins (multi-vitamins, etc):_____

( ) Calcium:_____

( ) Other: _____

ENROLLMENT/SCREENING FORM

Name:_____ Phone number 1:_____

Date:_____ Phone number 2:_____

E-mail address:_____

1.) How old are you? _____

2.) How many hours of exercise do you participate in per week regularly? _____

3.) Are you participating in another clinical trial or have you received an investigational product within the past 30 days?
YES / NO (circle one)

4.) Have you *lost* or *gained* more than 10 pounds in the past 3 months?
YES / NO (circle one)

If so, please describe:_____

If so, when did this take place:_____

5.) Do you eat meals at regular intervals? YES / NO (circle one)

6.) Do you have a history of alcohol or drug abuse in the past year? YES / NO (circle one)

7.) Do you use tobacco products regularly (i.e. cigarettes & chewing tobacco)?
YES / NO (circle one)

8.) Do you currently have a history of high blood pressure (i.e. systolic BP> 140 and/or diastolic BP>90)?
YES / NO (circle one)

9.) Do you have any of the following disorders that are treated or untreated?

i.	bleeding disorder	YES / NO (circle one)
ii.	diabetes mellitus (Type 1 diabetes)	YES / NO (circle one)
iii.	thyroid disease	YES / NO (circle one)
iv.	tachyarrhythmia (Fast Heart Rate)	YES / NO (circle one)
v.	heart disease	YES / NO (circle one)
vi.	kidney disease	YES / NO (circle one)
vii.	liver disease	YES / NO (circle one)
viii.	sleep disorders	YES / NO (circle one)
ix.	clinical depression	YES / NO (circle one)
x.	eating disorders	YES / NO (circle one)
xi.	psychiatric conditions	YES / NO (circle one)

10.) Have you ever had an abnormal electrocardiogram (ECG)? YES / NO (circle one)

11.) Do you currently suffer from a sleep disorder and/or do you have a known history of (or are being treated for) clinical depression, eating disorder(s), or and other psychiatric condition(s)? YES / NO (circle one)

12.) Do you have any allergies or sensitivity to the following ingredients: whey protein or milk products?
YES / NO (circle one)

13.) Are you taking medicines for high blood pressure? YES / NO (circle one)

14.) Are you currently taking or have you taken any nutritional supplements (such as ribose, protein drinks, creatine, or multivitamins) within the past 9 weeks? YES / NO (circle one)

If so, please describe:_____

15.) Are you currently taking or have you taken any medications in the past 30 days?
YES / NO (circle one)

If so, please describe:_____

If so, what are you taking them for:_____

Medication/Supplement Name	Dose /units	Route	Freq	Start Date	End Date	On-going	Indication (Diagnosis preferred)
						☐	
						☐	
						☐	

16.) Do you understand and agree to the time commitment and physical demands that will be necessary for you to complete this study?
YES / NO (circle one)

17.) Have you worked out a schedule for testing and training with the investigators that you understand and agree to?
YES / NO (circle one)

18.) Do you have any history or existing medical conditions involving any of the following system

HEENT (Head, Eyes, Ear, Nose, Throat)	YES / NO (circle one)
Cardiovascular	YES / NO (circle one)
Respiratory	YES / NO (circle one)
Gastrointestinal	YES / NO (circle one)
Renal	YES / NO (circle one)
Hepatic	YES / NO (circle one)
Genitourinary	YES / NO (circle one)
Reproductive	YES / NO (circle one)
Endocrine	YES / NO (circle one)
Hematologic	YES / NO (circle one)
Lymphatic	YES / NO (circle one)
Neurologic	YES / NO (circle one)
Pscyhiatric	YES / NO (circle one)
Immunologic	YES / NO (circle one)
Dermatologic	YES / NO (circle one)
Musculoskeletal	YES / NO (circle one)

If so, please describe:_____

If so, when did you have this medical condition:_____

19.) You will receive 0.0008 μSv of radiation during the pre- and post-training measurements combined by participating in this study. Please list all previous (within the last year) radiological procedures (and dates) you have been exposed to, such as CAT scans, CT scans, DEXA scans, dental X-rays, or other X-rays. _____
_____
_____

20.) Do you currently take any prescribed or over-the-counter medications or supplements? YES / NO (circle one)

If so, please describe below:

Medication	Dose /units	Route	Freq	Start Date	End Date	On-going	Indication (Diagnosis preferred)
						☐	
						☐	
						☐	
						☐	
						☐	
						☐	
						☐	
						☐	
						☐	
						☐	

By signing this form, you agree that the information that you provided above is accurate and that you fully understand and agree to the time commitment and demands of this study.

Signature:_____ Date:_____

APPENDIX F

VERBAL RECRUITMENT SCRIPTS

RECRUITMENT FLYERS/ADVERTISEMENTS

Evaluation of a leucine metabolite on muscle mass in elderly subjects

Verbal Recruitment Script

Hello, my name is _____. I'm a faculty member/student in the Department of Health and Exercise Science at the University of Oklahoma. I hope that you will consider participating in a research study I am conducting. The title of the study is "Evaluation of a leucine metabolite on muscle mass in elderly subjects." I'm trying to determine the effects of consuming a leucine metabolite, for 24 weeks on muscle mass, body composition, upper- and lower-body strength, functionality, quality of life, and lipid profile in healthy elderly (≥ 65 year old) adults.

Testing will consist of four visits, during the 24-week study, to the Human Performance Lab within the Department of Health and Exercise Science (Room HHC 14). Your body composition will be assessed by use of several methods. One of the tests involves exposure to minimal radiation from two DEXA scans, which is a type of x-ray procedure used to predict body composition and bone density. Also, some of the body composition tests require that you wear either a bathing suit or tight fitting clothing such as Spandex, and each body composition visit will last approximately 2-4 hours.

Maximal upper- and lower-body strength will be assessed by grip-strength and leg extension and flexion ability, respectively. During these visits you will also be assessed for functionality, by measuring the amount of time it takes for you to stand, from a seated position, walk 3 meters forward and back and return to the seated position. Quality of life will also be evaluated using a survey with a series of daily living questions.

Testing will also include 3 visits to the Goddard Health Center for blood work assessing cholesterol (low density lipoproteins and high density lipoproteins), complete blood count, and a CHEM 20. These are tests that evaluate the function of your liver and kidneys.

Each participant will be randomly assigned to one of two supplement groups. Of these two groups, depending on availability, you will chose to be in the exercise or non-exercise groups. Exercise groups require resistance training, [redacted] for 21 weeks 3 days per week and additional strength testing during weeks 1, 12, and 24. All groups will continue their normal diet and will be asked to log their food intake (two weekdays and one weekend day) for a baseline, 12 week and 24 week food intake assessment. Participants in both groups will be asked to consume one packet of their assigned supplement, two times per day, with water for 24 weeks. The experimental group will consume packets containing 1.5 grams of the study product, 12 calories, 4 gram carbohydrates and 200 milligrams calcium. The placebo group will consume packets containing 12 calories, 4 grams carbohydrates and 200 milligrams calcium. The study product contains no novel ingredients.

You will be required to sign an informed consent document before participating in this study, showing that you understand all of the procedures as well as your rights as a research subject. If you complete the entire study, you will be compensated for your participation.

At any time during your participation, you may stop and choose not to complete the testing.

Thank you.

Evaluation of a leucine metabolite on muscle mass in elderly subjects

Participants 65 + years old Needed!

We are looking for individuals 65 ≥ years old to participate in a study in the Department of Health and Exercise Science, OU-Norman campus, entitled: "Evaluation of a leucine metabolite on muscle mass in elderly subjects." Our main purpose is to evaluate the effects of consuming the study product for 24 weeks with and without exercise, on muscle mass, body composition, upper- and lower-body strength, functionality, quality of life, and lipid profiles in healthy older (≥ 65 year old) adults.

Testing will consist of four visits, during the 24-week study, to the Human Performance Lab within the Department of Health and Exercise Science (Room HHC 14) and 21 weeks of resistance training for those participating in the exercise group. Your body composition will be assessed by use of several methods. One of the tests involves exposure to minimal radiation from two DEXA scans, which is a type of x-ray procedure used to predict body composition and bone density. Also, some of the body composition tests require that you wear either a bathing suit or tight fitting clothing such as Spandex, and each body composition visit will last approximately 2-4 hours.

Maximal upper- and lower-body strength will be assessed by grip-strength and leg extension and flexion ability, respectively. During these visits you will also be assessed for functionality, by measuring the amount of time it takes for you to stand, from a seated position, walk 3 meters forward and back and return to the seated position. Quality of life will also be evaluated using a survey with a series of daily living questions.

Testing will also include 3 visits to the Goddard Health Center for blood work assessing cholesterol (low density lipoproteins and high density lipoproteins), complete blood count, and a CHEM 20. These are tests that evaluate the function of your liver and kidneys.

Each participant will be randomly assigned to one of two supplement groups.

- Study product with exercise group
- Control product without exercise group
- [redacted]
- exercise group
- without exercise group

All groups will continue their normal diet and will be asked to log their food intake (two weekdays and one weekend day) for a baseline, 12 week and 24 week food intake assessment. Participants in both groups will be asked to consume one packet of their assigned supplement, two times per day, with water for 24 weeks. The experimental group will consume packets containing 1.5 grams of the study product, 12 calories, 4 gram carbohydrates and 200 milligrams calcium. The placebo group will consume packets containing 12 calories, 4 grams carbohydrates and 200 milligrams calcium. The study product contains no novel ingredients.

If you are assigned to the non exercising group, you will be asked to maintain your current activity level. If you are assigned to the exercising group, you will be assigned training times with a personal trainer and instructed on resistance training 3 days per week (i.e., Monday, Wednesday, and Friday) with at least 48 hours between sessions at the OU-Norman Health and Exercise Science training facilities for a total of 21 weeks. [redacted]

For those in the exercise group, resistance training will consist of the following: Prior to starting resistance training, you will complete testing to have your one-repetition maximum determined using bilateral leg press, leg extension exercises, and chest press. These tests will be performed after both the screening visit following [redacted]. Prior to all testing attempts, you will first complete a standardized warm-up consisting of 5 minutes of stationary cycling at a work

rate of 300 kg*m/min. You will then complete two warm-up sets of 10 repetitions at 55% and 65% of your perceived 1 repetition max. Between attempts, you will rest 3-5 minutes. You will complete three sets of 8-12 repetitions at approximately 80% of your pre-determined 1 repetition max first with the hack squat, then the bilateral leg press and finally with the leg extension for the lower body. Next, or prior to the leg exercises, you will perform a chest press and lateral pull down exercises utilizing the same percentage (approximately 80%) of your 1 repetition maximum. All resistance will be adjusted accordingly if you cannot complete the exercise so that each set of exercise stays within the desired range of 8-12 repetitions. Each set of exercise will be separated by a 2 to 5-minute recovery period allowing for full recovery. Weight will be increased as your trainer determines is appropriate. If you complete 12 repetitions for the last set of an exercise for two consecutive lifting sessions, weight will be increased by 2.5-10% depending on the exercise.

You will be required to sign an informed consent document before participating in this study, showing that you understand all of the procedures as well as your rights as a research subject. If you complete the entire study, you will be compensated for your participation.

If you are interested and have questions, or know of any family/friends that may be interested please contact me by phone or email.

Abbie Smith

405-325-5211

abbiesmith@ou.edu

*The OU IRB has approved the content of this message, but not the method of distribution. The OU IRB has no authority to approve distribution by mass email.

*This institution in compliance with all applicable Federal and State laws and regulations does not discriminate on the basis of race, color, national origin, sex, age, religion, disability, political beliefs, or status as a veteran in any of its policies, practices, or procedures.

BK32 IRB #12188
Version 4, 11-21-08
This institution in compliance with all applicable Federal and State laws and regulations does not discriminate on the basis of race, color, national origin, sex, age, religion, disability, political beliefs, or status as a veteran in any of its policies, practices, or procedures.

PARTICIPANTS WANTED FOR RESEARCH STUDY:

Evaluation of a leucine metabolite on muscle mass in elderly subjects

To Participate:

Must be age 65 or older.
Must be in good physical condition.
Must not have: history of diabetes or currently taking dietary supplements.
Must agree to comply with prescribed activity level, i.e., either to maintain current activity or participate in resistance training for up to 21 weeks.

Details:

*Determine your body composition and bone density
*Assess daily functionality and quality of life!
*Possibly 21 weeks of personal training
*Compensation for participation

*Upper and lower-body Strength
*Lipid/Blood Profile

(minimal radiation from DEXA)

If you are eligible and interested, please contact:

[redacted]
1401 Asp Ave. Huston-Huffman Center
Norman, OK 73019
405-325-9023 or jordanmoo@ou.edu

[redacted]

Leucine RESEARCH
Jordan Moon
405-325-9023
rdanmoon@ou.edu

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rdanmoon@ou.edu

Verbal Recruitment Script

Hello, my name is Joel Cramer, and I am an assistant professor in the Department of Health and Exercise Science at the University of Oklahoma. I hope that you will consider participating in a research study we are conducting. The title of the study is "The Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults."

We are trying to determine if supplementation with whey protein with added branched chain amino acid leucine, calcuim, and viatmin D in conjunction with resistance training and a walking program will change bone health, body composition, muscular strength, mobility, or functionality. We are looking for healthy older adults aged 55 to 80 who are not taking nutiritional supplements.

Testing will consist of 2 testing visits to the Biophysics Lab within the Department of Health and Exercise Science (Room HHC 12). An additional 36 training visits will also be required. The first and last visit (about 3 hours each) will include body composition assessment and bone mineral density (from DEXA), determination muscular strength and mobility assessments. These visits will also require a fasted blood draw that will be conducted on site. The training visits will last approximately 45 minutes each.

After the initial testing visit you will set up a training schedule. You will also be given your supplements and supplement information. All testing exercises include bench press and leg press, while training sessions will include dumbbell bent over row, step up, and chest press. The exercises performed may cause some mild muscle soreness after the testing that may last up to 48 – 72 hours. All subjects will be required to sign an informed consent document showing they understand all of the procedures and their rights as a research subject.

As a participant, you will receive information about your body composition and muscular strength. Additionally, subjects who complete the entire study will receive compensation. Those who do not complete study will receive a prorated stipend based on percentage of completed participation. I would be happy to answer any additional questions that you may have about the study. Do you think you might be interested?

Thank you.

Hello, my name is Joel Cramer, and I am an assistant professor in the Department of Health and Exercise Science at the University of Oklahoma. I will be conducting a research study very soon and I hope that you will consider participating. The title of the study is "The Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults."

We are trying to determine if supplementation with whey protein with added branched chain amino acid leucine, calcuim, and viatmin D in conjunction with resistance training and a walking program will change bone health, body composition, muscular strength, mobility, or functionality. We are looking for healthy older adults aged 55 to 80 who are not taking nutiritional supplements.

Testing will consist of 2 testing visits to the Biophysics Lab within the Department of Health and Exercise Science (Room HHC 12). An additional 36 training visits (3 days per week for 12 weeks) will also be required. The first and last visit (about 2-3 hours each) will include body composition assessment and bone mineral density (from DEXA), determination muscular strength and mobility assessments. The screening and final visit will also require a fasted blood draw that will be conducted on site. The training visits will last approximately 45-60 minutes each.

After the initial testing visit you will set up a training schedule. You will also be given your supplements and supplement information. All testing exercises include bench press and leg press, while training sessions will include dumbbell bent over row, step up, and chest press. The exercises performed may cause some mild muscle soreness after the testing that may last up to 48 – 72 hours. Prior to conducting any tests, all subjects will be required to sign an informed consent document which will indicate your full understanding of all the procedures and your rights as a research subject.

As a participant, you will receive information about your body composition and muscular strength. Additionally, subjects who complete the entire study will receive compensation. Those who do not complete the study will receive a prorated stipend based on percentage of completed participation. I would be happy to answer any additional questions that you may have about the study.

Do you think you might be interested? If so, I would like to set up a time in the near future where you can come in and begin the enrollment process, which includes filling out some paperwork, testing of vital signs, and obtaining a fasted blood sample. This initial visit should take no longer than 20-30 minutes.

Thank you for your time. Please feel free to contact us if you have any further questions.

IRB Approved #15263

VOLUNTEERS WANTED

NUTRITIONAL SUPPLEMENT/EXERCISE STUDY

Requirements:

- Healthy men and women who are >55 years old
- Able to perform upper and lower body exercises
- 2 blood draws will be taken
- **Must not** be taking nutritional supplements or concomitant medications
  If you have taken any in the past 3 months you are ineligible

Study Scope:

- 12 weeks resistance training plus 30 minutes walking
- Body composition and bone density assessment (via DEXA*)
  *Low-dose radiation will be used
- Muscular strength, physical performance, and mobility

►If eligible, you will be randomly placed in to placebo or enhanced whey protein supplement group◄

Subjects who complete the entire study will receive a stipend

Those who do not complete study will receive a prorated stipend based on percentage of completed participation

If you are eligible and interested, please contact:

Ashley Walter 1401 Asp Ave. Huston-Huffman Center
Biophysics Lab: ashannwalter@ou.edu; 325-1368

SUPPLEMENT STUDY
~ASHLEY WALTER~
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APPENDIX G

PHYSICIAN CLEARANCE FORMS

Department of Health and Exercise Science - University of Oklahoma-Norman Campus
Study Investigators: Jeffrey R. Stout, PhD, Abbie E. Smith, MS, & [redacted]
Phone: 405-325-9023 Fax: 405-325-0594

Study Name: *Evaluation of a leucine metabolite on muscle mass in elderly subjects*

To the Attending Physician of: _____

Your patient is interested in participating in a research study in the health and exercise department at the University of Oklahoma. In short, this test involves 4 visits to the lab and 21 weeks of resistance training:

Screening Visit

- **Determining if the participant is eligible for participation**: Complete an enrollment form and a pre-exercise testing health and exercise status questionnaire to be reviewed for current/recent medications, past surgeries, and general health condition.

Visits 1, 2 and Final Visit/Exit

- Random assignment into one of the [redacted] study groups:

[redacted]

- Body Composition (Visits 1,2 and Final Visit/Exit) - after a 12-hour fast, with water consumption allowed up to one hour prior to testing, participants will undergo a series of body composition tests, including: bioelectrical impedance (BIA/BIS), dual x-ray absorptiometry (DEXA), air displacement plethysmography (BOD POD), total body water using deuterium oxide/sodium bromide technique, and ultrasound to measure muscle and fat tissue thickness.
- Fasting blood collection (Visit 1 and Final Visit/Exit) – Complete metabolic panel – samples will be sent to Diagnostic Laboratory of Oklahoma to determine albumin, alkaline phosphatase, ALT, AST, Bilirubin (total), calcium, carbon dioxide, chloride, creatinine, glucose, potassium, protein (total), sodium, urea nitrogen (BUN) *free of charge to the participant.* Complete blood count – will determine WBC, RBC, differential WBC, and platelet count.
- Leg strength test (Visits 1, 2 and Final Visit/Exit) – your patient will undergo lower-body strength testing using an isokinetic dynamometer. He/she will perform a battery of warm-up exercises prior to all maximal testing sessions. Your patient will complete three leg extension/flexion trials, with three minutes of rest in between trials. The average of the three trials will be considered maximal lower body strength.
- Upper body strength test- your patient will be asked to maximally squeeze a handheld dynamometer with his or her dominant arm, three different times, with the average of the three representing maximal upper body strength.
- Muscle function test – your patient will undergo "get-up-and-go" test consisting of timed measurements of the subject starting from a seated position of a chair, standing, walking forward 3 meters, turning around, walking back to the chair, and sitting down to determine muscle coordination and functionality.
- Quality of life - using The Lawton Instrumental Activities of Daily Living (IADL). The IADL is an appropriate instrument to assess independent living skills. These skills are considered more complex than the basic activities of daily living. The instrument is most useful for indentifying how a person is functioning at the present time and to identify improvement or deterioration over time. There are eight domains of function measured with the Lawton IADL scale: ability to use phone, shopping, food preparation, housekeeping, laundry, mode of transportation, responsibility for own medications, and ability to handle finances. Summary scores range from 0 (low function, dependent) to 8 (high function, independent) for women and 0 to 5 for men.
- Resistance training - Subjects assigned to the exercising group, you will be assigned training times with a personal trainer and instructed on resistance training 3 days per week with at least 48 hours between sessions at the OU-Norman Health and Exercise Science training facilities for a total of 21 weeks. Resistance training will consist of the following: Prior to starting resistance training, subjects will complete testing to have their one-repetition maximum determined using bilateral leg press, leg extension exercises, and chest press. These tests

will be performed after both the screening visit following [redacted]. Prior to all testing attempts, subjects will first complete a standardized warm-up consisting of 5 minutes of stationary cycling at a work rate of 300 kg*m/min. Subjects will then complete two warm-up sets of 10 repetitions at 55% and 65% of your perceived 1 repetition max. Between attempts, subjects will rest 3-5 minutes. Subjects will complete three sets of 8-12 repetitions at approximately 80% of your pre-determined 1 repetition max first with the hack squat, then the bilateral leg press and finally with the leg extension for the lower body. Next, or prior to the leg exercises, subjects will perform a chest press and lateral pull down exercises utilizing the same percentage (approximately 80%) of their 1 repetition maximum. All resistance will be adjusted accordingly if subjects cannot complete the exercise so that each set of exercise stays within the desired range of 8-12 repetitions. Each set of exercise will be separated by a 2 to 5-minute recovery period allowing for full recovery. Weight will be increased as their trainer determines is appropriate. If subject complete 12 repetitions for the last set of an exercise for two consecutive lifting sessions, weight will be increased by 2.5-10% depending on the exercise.

Note: you may request that the patient receives copies of the tests above to bring to your office during their next visit.

Pertaining to the above mentioned patient, I advise the following:

☐ To my knowledge, there is no reason why this patient should not be allowed to participate in this study.

☐ I recommend that this patient be allowed to participate in the study with the following restrictions:_____

☐ I recommend that this patient should **not** be allowed to participate in the study.

☐ Please ask the participant to bring the results of the tests to my office during their next check-up.

Physician's Signature_____ Date: _____

Physician's Name _____

The University of Oklahoma
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

Dear Dr. _____,

My name is Joel Cramer, I am an assistant professor in the Department of Health and Exercise Science at the University of Oklahoma. I am writing to you regarding a potential research participant for our study titled "The Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults".

The potential subject is your patient _____, age ___, phone number (___) ___-____. I have included with this sheet a brief medical clearance form the University of Oklahoma requires for individuals age 55 to 80 to participate in our study. At your convenience, can you please fill out this form and return via fax or email to ensure _(pt. name)_____ is healthy enough to participate.

We thank you in advance for your help and support of research here in the Department of Health and Exercise Science.

Kind regards,

Joel T. Cramer, Ph.D.

Lab phone: 405-325-1371
Department phone: 405-325-5211
Fax: 405-325-0594
Email: jcramer@ou.edu

Department of Health and Exercise Science - University of Oklahoma-Norman Campus

The Effects of whey protein supplementation on body composition, muscular strength, and mobility in older adults.

To the Attending Physician of: _____

This individual has indicated that he wishes to participate in a research study investigating the effects of an advanced whey protein supplement and light resistance training with a walking program on body composition, muscular strength, and mobility in older men and women. This project has been approved by the Institutional Review Board at the University of Oklahoma.

Description of the Study

We are trying to determine if supplementation with whey protein with added branched chain amino acid leucine, calcuim, and viatmin D in conjunction with resistance training and a walking program will change bone health, body composition, muscular strength, mobility, or functionality. We are looking for healthy older adults aged 55 to 80 who are not taking nutiritional supplements.

This study will consist of two testing visits to the Biophysics Laboratory, separated by twelve weeks of light resistance training and 30 minutes of walking 3 days per week. During this first visit, all participants will have a DEXA scan for bone density and muscle mass determination and simple physical function and mobility teats such as the 3 meter get-up-and-go, heel-to-toe stand and walk, and a hang grip strength test. They will also complete a 5-repetition maximum (RM) bench press and leg press to estimate 1-RM upper and lower body strength. These testing visits will also require a fasted blood draw that will be conducted on site.

After the initial testing visit participants will set up a training schedule. That will also be given their supplements and supplement information. They will be randomly assigned to one of two groups, advanced whey protein, or placebo. The advanced whey protein (AWP) contains 15g maltodextrin for flavoring, 20g whey protein isolate, 6.2g leucine (a branched-chain amino acid), 1000mg calcium (as calcium citrate), and 1000IU vitamin D (as Cholecalciferol). The placebo will contain maltodextrin and artificial sweeteners to match the color and flavor of the AWP, however, with fewer calories. Two servings of either AWP or the placebo will be taken each day.

All testing exercises include bench press and leg press, while training sessions will include dumbbell bent over row, step up, and chest press. Training will include 3 sets of 8-12 repetitions at a comfortable weight, followed by 30 minutes of walking on an indoor track at a comfortable pace. The exercises and walking are to be performed under the supervision of a trained and certified personal trainer. The participants will be monitored to ensure they do not over-exert themselves.

Please advise the investigators regarding any physical limitations and/or contraindications that this patient might have from engaging in this exercise study.

Participants will not be allowed to participate (exclusion criteria) in this study if they:

1.) have any orthopedic problems (e.g., previous surgery, joint replacements, etc.) or previously diagnosed neuromuscular disease that will prevent them from participating in the strength or mobility assessment or;
2.) have any absolute or relative contraindication for exercise testing as outlined by the American College of Sports Medicine (provided below):

Absolute Contraindications to Exercise Testing (check all that applies)

- ❑ A recent significant change in the resting ECG suggesting significant ischemia, recent MI or other acute cardiac event
- ❑ Unstable angina
- ❑ Uncontrolled cardiac dysrhythmias causing symptoms or hemodynamic compromise

- ❑ Symptomatic severe aortic stenosis
- ❑ Uncontrolled symptomatic heart failure
- ❑ Acute pulmonary embolus or pulmonary infarction
- ❑ Acute myocarditis or pericarditis
- ❑ Suspected or known dissecting aneurysm
- ❑ Acute systemic infection, accompanied by fever, body aches, or swollen lymph glands

Relative Contraindications to Exercise Testing (check all that applies)

- ❑ Left main coronary stenosis
- ❑ Moderate stenotic valvular heart disease
- ❑ Electrolyte abnormalities (e.g. hypokalemia, hypomagnesemia)
- ❑ Severe arterial hypertension (i.e. SBP>200 and/or DBP >110) at rest
- ❑ Tachyarrhythmia or bradyarrhythmia
- ❑ Hypertrophic cardiomyopathy and other forms of outflow tract obstruction
- ❑ Neuromuscular, musculoskeletal, or rheumatoid disorders that are exacerbated by exercise
- ❑ High-degree atrioventricular block
- ❑ Ventricular aneurysm
- ❑ Uncontrolled metabolic disease (e.g., diabetes, thyrotoxicosis, or myxedema)
- ❑ Chronic infectious disease (e.g., mononucleosis, hepatitis, or AIDS)
- ❑ Mental or physical impairment leading to inability to exercise adequately

Pertaining to the above mentioned patient, I advise the following:

☐ To my knowledge, there is no reason why this patient should not be allowed to participate in this study.

☐ I recommend that this patient be allowed to participate in the study with the following restrictions:_____

_____

_____

☐ I recommend that this patient should **not** be allowed to participate in the study for the following reasons:_____

_____

_____

Physician's Name (Printed) _____ Date_____

Physician's Signature _____

If you have any questions about this form, please contact: Joel T. Cramer, Ph.D., Assistant Professor, Director of the Biophysics Laboratory at **Phone: 405-325-5211, Fax: 405-325-0594, Email: jcramer@ou.edu**