EXERCISE AND NUTRITION TO COUNTER AGE-RELATED DECREMENTS IN MUSCLE HEALTH AND FUNCTION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Muscle atrophy and strength decrements can occur following disease (e.g., cachexia), with increasing age (e.g., sarcopenia), or from disuse related to injury or occupational environment (e.g., microgravity). High-load resistance training and adequate protein and amino acid intake are effective countermeasures. However, high-load training may not be feasible in all populations. Lower intensity resistance training with blood flow restriction (BFR) is an effective alternative and its benefits may be increased when combined with supplementation of protein and leucine. Two studies explored the relationships between nutrient intake and physical activity and the effects of short-term combination on muscle health in middle age. **Methods:** To compare muscle strength and size, 98 participants were divided by age and physical activity (young active adults = 23.0 ± 3.1 years, sedentary young adults = 26.3 ± 4.7 years, middle-aged active adults = 57.3 ± 4.0 years, middle-aged sedentary adults = 57.9 ± 4.4 years). Relationships between muscular health, nutrient intake, and physical activity were also examined. Next, 16 participants (age = 36.0 ± 10.1 yrs., BMI = 27.2 ± 5.0 kg/m^2) completed BFR training with supplementation of 28g of leucine-rich protein or an isocaloric placebo to evaluate the effects on health and performance. **Results:** There were significant group effects for muscle strength ($p = .003$-$.010$) and size ($p=.002$). Physical activity, protein intake, and leucine were significantly and positively associated with knee flexor size and strength ($R^2 = 0.28$-0.71, $p<.05$). Physical activity and protein intake were negatively associated with dorsiflexor strength ($r^2 = 0.48$-0.58, $p<0.05$). Total leucine intake (g/day) increased by ~38% with supplementation. Training volume in all exercises, sit-to-stand repetitions, gait speed increased significantly with BFR exercise while resting heart rate significantly decreased ($p<0.05$). **Conclusion:** These results indicate that muscle size and strength are lower in middle age and increased physical activity, protein intake,

and leucine intake may be able to preserve muscle size and strength in larger muscle groups of the lower body. Additionally, they show that BFR exercise can improve muscular and cardiovascular health in middle-aged adults, however, there was no short-term benefit to increasing protein and leucine intake above the recommended dietary allowance.

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DEDICATION

This dissertation is dedicated to: my parents, Susan and Greg Hobbs, who graciously made sacrifices to support me throughout this journey and continuously encouraged me to persevere; my late grandparents, Anna and Ted Huddleston, who instilled in me the ambition, determination, and compassion necessary for my success; and my best friend, Zeus, who loved me unconditionally, made me laugh, and got me moving when I thought I couldn't.

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LIST OF ABBREVIATIONS

HRT ..... High-load Resistance Training

BFR ..... Blood Flow Restriction

CSAq ..... Quadriceps Cross Sectional Area

KEPT ..... Knee Extensors Peak Torque

KFPT ..... Knee Flexors Peak Torque

PFPT ..... Plantar Flexors Peak Torque

DFPT ..... Dorsi Flexors Peak Torque

aLM ..... Appendicular Lean Mass

LBM ..... Lean Body Mass

SMI ..... Skeletal Muscle Index

MVPA ..... Moderate to Vigorous Physical Activity

YA ..... Young Adults

MA ..... Middle-aged Adults

RDA ..... Recommended Dietary Allowance

DRI ..... Dietary Reference Intake

IGF-1 ..... Insulin-like Growth Factor 1

mTORC1 ..... Mammalian Target of Rapamycin Pathway

NMJ ..... Neuromuscular Junction

MUNIX ..... Motor Unit Number Index

MUSIX ..... Motor Unit Size Index

SPPB ..... Short Physical Performance Battery

EMG ..... Electromyography

ALS ..... Amyotrophic Lateral Sclerosis

MVC..... Maximal Voluntary Contraction

GH..... Growth Hormone

DHEA..... Dehydroepiandrosterone

DHEAS..... Dehydroepiandrosterone Sulfate

ATP ..... Adenosine Triphosphate

S6K1..... Ribosomal Protein S6 Kinase 1

4E-BP1 ..... Eukaryotic Initiation Factor 4E-Binding Protein 1

MuRF-1 ..... Muscle RING Finger 1

MafBx..... Muscle Atrophy F-box

PKB..... Protein Kinase B

IMAT..... Intramuscular Adipose Tissue

DXA ..... Dual Energy X-ray Absorptiometry

CSA..... Cross Sectional Area

ACSA ..... Anatomical Cross Sectional Area

PCSA..... Physiological Cross Sectional Area

ET-1..... Endothelin-1

NO..... Nitric Oxide

TUG..... Timed Get Up and Go Test

MPS..... Muscle Protein Synthesis

MPB ..... Muscle Protein Breakdown

ACSM..... American College of Sports Medicine

PA..... Physical Activity

RT..... Resistance Training

AT ..... Aerobic Training

CCT ..... Concurrent Training

GS..... Gait Speed

WBV ..... Whole Body Vibration

SARMs ..... Selective Androgen Receptor Molecules

THC..... Delta-9-tetrahydrocannabinol

PAR-Q ..... Physical Activity Readiness Questionnaire

NDSU ..... North Dakota State University

MRI ..... Magnetic Resonance Imaging

ANOVA..... Analysis of Variance

IPAQ ..... International Physical Activity Questionnaire

DVT..... Deep Vein Thrombosis

STS..... Sit-to-stand Test

AYA ..... Active Young Adults

SYA..... Sedentary Young Adults

AMA ..... Active Middle-aged Adults

SMA ..... Sedentary Middle-aged Adults

1. INTRODUCTION

1.1. Overview of the Problem

Age-related declines in skeletal muscle mass and strength affect many men and women as early as 20 years of age (Bae & Kim, 2017). Similar observations in muscle health, as well as cardiovascular health, have been reported in individuals following disuse and spaceflight (Jones et al., 2019). Inadequate nutrition and physical activity contribute to the development of sarcopenia (Beaudart et al., 2017). Exercise is the gold standard for mitigating muscular and cardiovascular health decrements; and increased dietary protein may provide additional benefits (Morley et al., 2014). Therefore, it is imperative that researchers develop novel interventions to preserve lean mass and strength for a variety of middle-aged populations. The current recommended dietary allowance for protein is 0.8 g/kg/day but a higher amount of 1.2-1.4g/kg/day has been suggested as optimal (Baum et al., 2016; Landi et al., 2016; Volpi et al., 2013). Dietary supplements containing whey, casein, or a combination of amino acids have been most effective when combined with high-load resistance training (HRT) (Rondanelli et al., 2016; van Dijk et al., 2016). However, HRT (70-80% of a one repetition maximum) is often difficult for certain populations because of other health conditions or the environment in which they are exercising (Wojtek J. Chodzko-Zajko et al., 2009; Garber et al., 2011). To overcome these challenges, various “alternative” training methods have been examined, including blood flow restriction (BFR) training (Cook et al., 2017; Hackney et al., 2016; Vechin et al., 2015). BFR exercise has been effective in mimicking the strength and hypertrophy increases typically observed with traditional HRT at a much lower intensity (20-30% of a one repetition maximum) (Freitas et al., 2020; Neto et al., 2017). These findings suggest feasibility and efficacy and should

be examined as countermeasures for muscle atrophy associated with certain conditions and occupational environments, like aging and long duration space travel.

1.2. Statement of Purpose

The overall purpose of this project was to examine the role of dietary protein and physical activity on muscle health in middle age through two studies. The first study was a cross-sectional approach examining dietary protein intake and habitual physical activity on muscle size and strength in healthy middle-aged adults. The current literature on this topic focuses mostly on those in their sixth decade of life and later. The second study examined the effects of a leucine-rich protein supplementation combined with short-term (< six weeks) low-intensity resistance training with BFR on body composition, muscular health, and cardiovascular function in middle-age. These investigations will examine a sample representing a neglected, but important, population of adults who could be at risk for muscle atrophy and reduced quality of life from normal processes of aging or environments.

1.3. Research Questions

1) *Investigation One*: Are there differences in muscle strength between sedentary and active young and middle-aged adults and how does dietary intake and habitual physical activity relate to muscular health?
2) *Investigation Two:* Does a leucine-rich protein supplement combined with BFR exercise result in favorable changes to body composition, muscular health, and cardiovascular health in only four weeks?

1.4. Dependent Variables

For the first study, the dependent variables were muscle size as measured by quadriceps cross sectional area (CSAq) and muscle strength as measured by peak torque (Nm) for the knee

extensors (KEPT), knee flexors (KFPT), plantar flexors (PFPT), and dorsi flexors (DFPT). For the second study, dependent variables for dietary intake included: energy intake (kcals·kg·day^{-1}), protein intake (g·kg·day^{-1}), leucine intake (g·day^{-1}), carbohydrate intake (g·day^{-1}), and fat intake (g·day^{-1}). Body composition was examined using appendicular lean mass (aLM) and lean body mass (LBM) in kg, skeletal muscle index (SMI) in kg/m^2, and percent body fat (%). Muscle strength was examined using the same dependent variables as the first study with the addition of maximal hand grip strength (kg). Muscle function was examined using 30 second sit-to-stand (repetitions), gait speed (m/s), balance composite score, and timed up and go. Cardiovascular health was examined using resting blood pressure (mmHg) and heart rate (bpm).

1.5. Independent Variables

For study one, the independent variables for dietary intake were total energy (kcals·kg·day^{-1}), protein (g·kg·day^{-1}), and leucine (g·day^{-1}). Physical activity was examined as minutes per day spent doing sedentary, light, moderate, vigorous, and moderate to vigorous activities (MVPA). Age was also examined as an independent variable. For study two, the independent variables were ingestion of the protein supplement or isocaloric placebo.

1.6. Limitations

The first study was limited by self-reported amounts and types of concurrent physical activity. In addition, though the wrist accelerometery accounts for rhythmic aerobic activity, it may not include stationary activities and resistive exercises. Nutrient intakes were also self-reported as participants were asked to estimate their food amounts using supplemental handouts equating portion sizes to the hands and common household items. The second study was additionally limited by a small volunteer sample. Additionally, though participants were asked to avoid any structured exercise and limit recreational participation in sports to once per week or

less, it was impossible to completely control for lifestyle physical activity from household duties and commuting. This sample was limited to healthy, non-sarcopenic individuals, normobaric/normogravity environments, and predominantly white individuals (~94%).

1.7. Delimitations

The first study included males and females who were aged 20-35 and 50-65 years, excluding those aged 36-49. This was done to compare an underrepresented middle-aged group to a group of healthy young adults. Additionally, an equal number of sedentary (e.g., not participating in regular, structured exercise two or more times per week) and active (e.g., participating in moderate to vigorous resistance and aerobic exercises 3-5 times per week) adults were recruited in each age group to examine the differences in muscle health between young and middle-aged sedentary and active adults. The second study used a ten repetition maximum (RM) test to determine training load as a percentage of 1-RM. Additionally, generally healthy, recreationally active adults who were not participating in structured exercise were recruited for this study to ensure that participants could safely participate and avoid deconditioning from a reduced intensity of resistance training. Participants aged 18-60 were included to replicate the age at risk for onset of muscle atrophy due to the aging process or environments such as microgravity.

1.8. Assumptions

Assumptions for the first study included participant honesty in completion of all pre-screening forms, Arizona Food Frequency Questionnaires, and food diaries. It was also assumed that participants wore their assigned accelerometers and gave their best effort on all muscle function testing. For the second study, it was assumed that participants refrained from training outside of the study, gave their best effort at each exercise and testing session, were truthful on

all screening forms and food diaries, and were consuming all shakes as prescribed or reporting missed shakes.

1.9. Significance of Study

The information from these studies will provide information about the role of dietary intake and physical activity on the muscular and cardiovascular health and body composition in middle-aged adults. Middle-aged adults are prone to muscle atrophy from natural processes of aging, occupational hazards, and disuse. They may also experience conditions which make traditional resistance exercise challenging. These investigations will also evaluate the effects of a low-intensity training regimen with BFR combined with leucine-rich protein supplementation on this under researched group. Potential benefits of exercise and/or supplementation may contribute to more feasible and efficient countermeasures for muscle loss in middle-aged adults who are aging, injured, or working and living in microgravity.

1.10. Definitions

Young Adults (YA) includes individuals aged 18-40 years of age (English et al. 2016).

Middle-aged Adults (MA) includes individuals aged 40-60 years of age (Carrasco-Rios et al. 2019).

Blood Flow Restriction (BFR) is applied using special cuffs worn on the limbs that are inflated to a pressure high enough to occlude venous return but low enough to allow arterial inflow (Loenneke et al. 2014).

Recommended Dietary Allowance (RDA) is a guideline of the average daily level of intake sufficient to meet the nutrient requirements of 97-98% of healthy people, divided by age and gender *(Institute of Medicine (IOM), 2006)*.

Sedentary Activity refers to the amount of time (min/day), as determined by hip accelerometry, spent in <100 counts/min (Freedson, Melanson, & Sirard, 1998).

Light Physical Activity refers to the amount of time (min/day), as determined by hip accelerometry, spent in 100-1951 counts/min (Freedson, Melanson, & Sirard, 1998).

Moderate to Vigorous Physical Activity (MVPA) is the sum of moderate and vigorous physical activities. It refers to the amount of time (min/day), as determined by hip accelerometry, spent in >1952 counts/min (Freedson, Melanson, & Sirard, 1998).

Lean Body Mass (LBM) refers to the total mass of the body that is not fat.

Body Fat Percentage is the percentage of body mass that is from fat.

Appendicular Lean Body Mass (aLM) is the sum of LBM for all limbs (kg).

Skeletal Muscle Mass Index (SMI) is the quotient of aLM and squared height (kg/m^2) (Cruz-Jentoft et al. 2019).

2. LITERATURE REVIEW

2.1. Introduction

Aging is associated with numerous physiological changes that lead to reduced physical function, reduced quality of life, and increased economic burden (Chodzko-Zajko et al., 2009). The population of individuals over the age of 60 years is estimated to be greater than 420 million worldwide and is expected to increase to approximately 1.4 billion and 2.1 billion by the years 2030 and 2050, respectively (Ilgili et al., 2014; World Population Ageing [WPA], 2015). In the United States, the number of individuals aged 65 and older equal greater than 47.8 million with an estimated two-fold increase by the year 2060 (Profile America Facts for Features, 2017). With these increasing numbers, there is a potential risk of increased economic burden if countries do not alter policies regarding pension systems, employment, and healthcare to enable the lifelong health and wellness of aging people (Ilgili et al., 2014; United Nations [UN], 2015). Furthermore, the increasing population suggests a growing importance of encouraging healthy aging through preventive care such as adequate nutrition, physical activity, and the cessation of harmful substances and activities (UN, 2015). To attenuate changes that undermine quality of life, it is imperative that researchers gain a greater understanding of the mechanisms behind these changes. Age-related declines in skeletal muscle mass and strength, known as sarcopenia and dynapenia, affect many individuals over 60 years of age and have been reported to occur as early as 27 years of age (Doherty, 2003; Mitchell et al., 2012). The European Working Group on Sarcopenia in Older People suggested specific causes of sarcopenia (e.g., inadequate nutrition and low physical activity), along with age-related or primary sarcopenia, might contribute to a secondary class of sarcopenia (Alfonso J. Cruz-Jentoft et al., 2019; von Haehling, Morley, & Anker, 2010). The effects are devastating to the aging adults' lives and have been shown to

reduce their ability to complete normal activities of daily living because of decreases in muscular strength, power, and quality (Cruz-Jentoft et al., 2010; Morley, 2012; Tieland, van de Rest et al., 2012). There is sufficient evidence that exercise, increased physical activity, hormonal, and nutritional interventions can mitigate functional incapacities in older people (Borst, 2004; Chodzko-Zajko et al., 2009; Hunter et al., 2004; Latham et al., 2004; Mitchell et al., 2012; Morley, 2012). However, because aging and the way individuals respond to these interventions are multi-faceted, further exploration of these mechanisms are warranted.

The development of sarcopenia, its associated increase in frailty, and loss of physical function has been tied to: 1) cardiovascular diseases and decreased capillary blood flow, 2) decreased motor unit function and ciliary nerve trophic factor, 3) changes in nutritional and activity needs, and 4) the reduction of growth hormone, insulin-like growth factor I (IGF-1), and testosterone (Chodzko-Zajko et al., 2009; Morley, 2012; Xue, 2011). It is well documented that resistance training can help to mitigate sarcopenia and dynapenia (Burd et al., 2012; Hunter et al., 2004; American College of Sports Medicine [ACSM], 2009; Reidy & Rasmussen, 2016; Vechin et al., 2015). It is also well documented that protein supplementation can improve muscle health through activation of mammalian target of rapamycin pathway (mTORC1) in older adults (Francaux et al., 2016; van Dijk et al., 2016). Though research examining protein supplementation alone has shown promise in reducing sarcopenia, some researchers suggest that a combination of supplementation and exercise will yield the greatest benefits (Borst, 2004; Latham et al., 2004; Mitchell et al., 2012; Morley, 2012; Rondanelli et al., 2016). However, the most appropriate mode of exercise and amino acid combination for aging individuals is not well understood. The high loads typically used in resistance training may not be appropriate for older individuals with limited abilities (Martín-Hernández et al., 2013; Nakajima et al., 2006; Scott et

al., 2014). Thus, it is important for researchers to explore low-intensity alternatives to find the safest and most effective intervention in the maintenance of functional independence in later decades.

2.2. Sarcopenia and Dynapenia

2.2.1. Introduction

Sarcopenia and dynapenia affect 8-58% of men and women over the age of 60 years (Doherty, 2003). Individual responses to aging are highly variant making the mechanisms of these processes difficult to identify (Chodzko-Zajko et al., 2009; Rygiel et al., 2016). With an expected twofold increase in the population of individuals over 60 years of age by 2050, a similar increase in healthcare costs is also anticipated (Danielson, 2013; Janssen et al., 2004; HHS, 2011; Rathge et al., 2012). Sarcopenia and dynapenia occur naturally with age and these conditions can be debilitating and expensive (Profile America Facts for Features, 2017; Scott et al., 2014; Vechin et al., 2015; UN, 2015). Thus, to reduce these effects, it is imperative that researchers gain a greater understanding of the mechanisms behind the conditions. Researchers have examined many potential mechanisms and suggested that one or more of the following systems: neuromuscular, endocrine, muscular, and cardiovascular factors collaboratively contribute to the loss of muscle mass and strength with age (Curcio et al., 2016; Morley, 2016; Morley & Malmstrom, 2013; Rygiel et al., 2016).

2.2.2. Mechanisms of Sarcopenia and Dynapenia

2.2.2.1. Neuromuscular

It is well understood that as humans age, they naturally experience changes in skeletal muscle size, strength, muscle fiber type distribution and size, muscular power and endurance, and sensory and motor systems (Chodzko-Zajko et al., 2009). However, the cause of these

changes is poorly understood. It has been suggested that changes in the neuromuscular junction (NMJ) can lead to changes associated with sarcopenia and dynapenia (Bütikofer et al., 2011; Curcio et al., 2016; Drey et al., 2013; Tintignac et al., 2015). It has further been suggested that agrin plays a major role in the development, innervation, and stability of the NMJ (McMahan, 1990; Wu et al., 2010). Furthermore, researchers have suggested that agrin cleavage caused by neurotrypsin leads to detectable C-terminal agrin fragments and NMJ degeneration (Bolliger et al., 2010; Bütikofer et al., 2011; Drey et al., 2013; Reif et al., 2007).

To gain a greater understanding of how these changes might affect skeletal muscle, Bütikofer et al. (2011) examined changes in grip strength, muscle cross-sectional area, muscle fiber cross-sectional area, fiber type distribution, footprint analysis, NMJ fragmentation, and motor neuron quantity in mice with varying degrees of neurotrypsin and agrin. Increased agrin cleavage led to 30% loss of muscle fibers in the soleus, a reduction in muscle CSA, and an increased number of type I fibers compared to type II similar to losses associated with sarcopenia (Bütikofer et al., 2011). Additionally, increased agrin cleavage led to an 11% decrease in grip strength and changes in gait patterns indicative of dynapenia (Bütikofer et al., 2011). Other rodent studies are suggestive of large, dysfunctional mitochondria and reduced peroxisome proliferator-activated receptor ɣ coactivator (PGC) 1α leading to an altered NMJ and denervation (Gouspillou et al., 2013; Rygiel et al., 2016). Lack of thorough research on the NMJs of humans combined with few comparisons of the NMJs between sarcopenic and healthy aging individuals makes understanding the mechanisms of sarcopenia and dynapenia difficult. Thus, several hypotheses have been developed and continue to emerge.

In human muscle, another well-established hypothesis is that the number of motor units decreases with age causing muscle fibers to denervate while remaining motor units become

larger and “sprout” to reinnervate those fibers (Clark & Fielding, 2012; Drey et al., 2013; Gordon et al., 2004). In 2013, researchers evaluated this concept using the motor unit number index (MUNIX) and motor unit size index (MUSIX) in 27 individuals deemed sarcopenic by skeletal muscle mass index (SMI) and short physical performance battery (SPPB) (Drey et al., 2013). Using surface electromyography (EMG), they found a mean compound muscle action potential of 9.7 ± 2.4mV. They also found an inverse relationship (r = -0.65) between MUNIX and MUSIX and that the distribution of MUNIX in sarcopenic individuals was significantly lower than healthy individuals and similar, though slightly higher, than what is typically observed in amyotrophic lateral sclerosis (ALS) patients. Lastly, they determined 25% of the sarcopenia cases to be due to lost motor neurons and 75% to have other unspecified causes (Drey et al., 2013). In 2014, another study was completed using EMG to examine the functioning motor units of 12 men ages 22-85 years (Power et al., 2014). Examination of compound muscle action potential showed no significant difference between the young (25 ± 3 years), old (68 ± 5 years), and older (79 ± 3 years) in reference to the negative-peak amplitude, however, a -19% difference was observed between older (79 ± 3 years) and young men (Power et al., 2014). Additionally, voluntary activation was similar and greater than 95% between the three groups though muscle strength and size was lower in the older groups (Power et al., 2014). The authors suggested that the weakness may be due to an imbalance, or threshold, between denervation and reinnervation (Power et al., 2014). This etiology appears to be well supported but not completely conclusive as a standalone cause for sarcopenia. Additionally, the magnitude of how these mechanisms contribute to dynapenia and the loss of physical function during aging remains unclear.

To examine differences in neuromuscular function between pre-sarcopenic, sarcopenic, and severe sarcopenic individuals, a study was developed to evaluate gait speed, handgrip strength , lean mass, strength, and voluntary action among 24 elderly individuals (Morat et al., 2016). Among the groups, only significant differences in the gait speed of severe sarcopenic and pre-sarcopenic individuals differed (Morat et al., 2016). Additionally, a negative relationship was observed between age and strength measured by maximal voluntary contraction (MVC) and a positive relationship was observed between handgrip strength and MVC (Morat et al., 2016). However, when comparing normalized data of contractile properties like voluntary action, MVC, and rate of torque development, no differences were seen amongst the groups (Morat et al., 2016). Though these variables indicate a decline in neuromuscular function with age, how they contribute to increasing severity of sarcopenia and dynapenia remains unclear. It has been suggested that gait speed correlates with inflammatory markers (e.g., interleukin 6 and tumor necrosis factor receptor 2) but does not account for other systems (e.g., neuromuscular and endocrine) that may contribute to these conditions (Curcio et al., 2016). Furthermore, it is unclear whether alterations to the NMJ are causal to sarcopenia or if they occur in response to dysfunction of the motor neuron or muscle fiber (Tintignac et al., 2015). To answer this, researchers must determine if variables are involved before the synapse, such is the case of muscle atrophy, or after the synapse, as observed in mitochondrial function (Tintignac et al., 2015). By examining the conflict in these example variables, it is clear that the etiology of sarcopenia and dynapenia is multifaceted.

2.2.2.2. Endocrine

Because humans experience multiple fluctuations in hormones throughout their lives, understanding the precise role of the endocrine system in the development of sarcopenia and

dynapenia has proven difficult for many researchers. It has been suggested that decreases in growth hormone (GH), insulin-like growth factor 1 (IGF-1), mechanogrowth factor, testosterone and increases in cortisol and insulin resistance contribute to sarcopenia (Abbatecola et al., 2005; Borer, 2013; Chodzko-Zajko et al., 2009; Mitchell et al., 2012). Additional research suggests that alterations in myostatin, follistatin, and dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) are also contributors (Curcio et al., 2016; Maggio et al., 2013). Some researchers have suggested hypoestrogenism and hypoandrogenism as lead contributors for sarcopenia and dynapenia in women and men, respectively (Sipilä et al., 2013).

Because glycolysis provides energy for efficient muscular contractions and insulin regulates glucose by altering amino acid availability, the increasing resistance of this hormone with age has been suggested as a potential mechanism for sarcopenia and dynapenia (Abbatecola et al., 2005; Fukagawa et al., 1988). However, additional age-related alterations in metabolism that occur concurrently in the muscular and cardiovascular systems make the mechanisms behind insulin resistance unclear (Abbatecola et al., 2011; Marzetti et al., 2013). Additionally, other hormones such as, testosterone, stimulate myogenesis through various pathways (Kovacheva et al., 2010; Morley & Malmstrom, 2013) and alteration of sex hormones, like testosterone and estrogen , have been shown to affect fuel utilization and muscle maintenance (Gheller et al., 2016; Haren et al., 2011) making deciphering the etiology of diseases associated with aging even more difficult. Sex hormones are known contributors to sarcopenia, dynapenia , and the syndrome frailty which includes sarcopenia, dynapenia, cognitive decline, and decrements to other bodily systems (Hyde et al., 2010). In a study examining the contribution of sex hormones to sarcopenia and dynapenia in the sixth and seventh decades of life, researchers saw a positive association between bioavailable testosterone and mass (r^2=0.11) but not between testosterone

and grip strength (Baumgartner et al., 1999). No associations were observed between sex hormones and mass or strength in women (Baumgartner et al., 1999). Another study examined relationships between testosterone and strength and functional tasks in African American males aged over seventy years of age (Perry et al., 2000). Associations were found between serum testosterone and upper body strength (r=.386-.391), lower body strength (r=.329-.456), opening and closing doors (r=-.306), and sit-to-stand (r=-.341) (Perry et al., 2000). Similar associations were found between bioavailable testosterone and shoulder strength, hip strength, and the functional tasks of opening and closing doors, completing the timed-get-up and go test, and completing the gait speed test (Perry et al., 2000). Additionally, researchers noticed a high correlation between bioavailable testosterone levels (r=0.744) and subnormal levels of serum testosterone in the older men(Perry et al., 2000). Later research by Maggio et al. (2011) showed that men with lower testosterone levels had lower grip strength values and SPPB scores when adjusted for age ($p = <0.04$) and BMI ($p = <0.001$). Similarly, data from Third National Health and Nutrition Evaluation Survey showed increased odds of frailty with low bioavailable testosterone and high sex hormone-binding globulin (Eichholzer et al., 2012). No associations were observed between testosterone or bioavailable estrogen and frailty (Eichholzer et al., 2012). These data are suggestive of a mechanistic role of androgens in the development of sarcopenia and dynapenia in men. Additionally, these conditions have potentially different mechanisms in women. However, women are underrepresented in current research making it difficult to understand the specific role of hypoestrogenism in declining muscle health with age.

Other potential contributors to sarcopenia include age-associated reductions in DHEA and DHEAS and increases in cortisol. DHEA and DHEAS can be converted into sex hormones by skeletal muscle (Vitale et al., 2016) making their reduction a potential secondary mechanism

to sarcopenia and dynapenia. Other research is suggestive of a more direct role as decreased DHEAS and increased cortisol are related to frailty through catabolism at a cellular level (Baylis et al., 2013). The Hertfordshire Aging Study assessed the odds of frailty after a 10 year follow up in 717 men and women and found associations between low testosterone, high cortisol, decreased DHEAS, and a high cortisol:DHEAS ratio (Baylis et al., 2013). Though these hormones may not be entirely responsible for frailty syndrome, they are associated with the five criteria defining frailty (e.g., weight loss, weakness, exhaustion, slowness, and low activity) in the above-mentioned studies. Furthermore, these criteria cannot be separated from sarcopenia and dynapenia as they are indirect measurements of muscle size and strength.

Decreases in GH and IGF-1 have been suggested as potential contributors to sarcopenia and dynapenia but appear to play a greater role in the maintenance of muscle mass (Morley & Malmstrom, 2013). Muscle mass can be affected directly by pulsatile GH or indirectly through IGF-1 produced by the liver or muscle derived mechanogrowth factor in response to secreted GH (Borer, 2013; Morley & Malmstrom, 2013; Rudman et al., 1990). However, when 18 men aged 65-82 years were treated with GH injections or placebo injections while completing 24 weeks of full body resistance training, no hypertrophic results were observed (Taaffe et al., 1996). Because pulses in GH occur nocturnally with the onset of sleep, serum IGF-1 or hormone replacement therapy using GH are more feasible variables for research (Herman-Bonert & Melmad, 2011). However, research findings regarding the role the GH-IGF-axis are unclear and often debated among scientists (Stewart & Pell, 2010). Animal research has shown differences in body mass standardized to tibia length (+5.9-20.7%, $p<0.05$) and muscle fiber CSA for types I, IIa, and IIb (+10-+21%, $p<0.05$) between transgenic and wild type mice, indicating IGF-1 stimulated hypertrophy (Shavlakadze et al., 2010). However, after further comparison between

the transgenic mice and dystrophic mice the effects appeared to be limited to developmental and regenerative hypertrophy (Shavlakadze et al., 2010). While the hypertrophic effects of IGF-1 appear straightforward in mice, the role of this hormone and related hormones appears to be more complex when examining human muscle atrophy. For instance, results from the Health in Men Study showed no significant change in odds ratio of frailty with low levels of IGF-1 (Yeap et al., 2013). Additionally, Yeap et al. (2013) observed increased likeliness of developing frailty when low levels of IGF-1 were combined with high levels of IGF-1 binding protein 1 or low BT (OR=2.08 and 2.13, respectively). It was further observed that the likelihood of frailty was greater in individuals with low levels of IGF-1 and testosterone than in those with only low testosterone (Yeap et al., 2013). These studies suggest that the GH-IGF axis and testosterone are contributors of sarcopenia but that they are not stand-alone mechanisms. Furthermore, these studies suggest that the milieu of sarcopenia and dynapenia is not only hormonal but may include multiple systems and alterations in stimuli.

2.2.2.3. Muscular

The terms sarcopenia and dynapenia translate to “poverty of flesh” and “poverty of power”, respectively (Clark & Manini, 2008). Therefore, it is obvious that the conditions are related to the muscular system. However, understanding the direct mechanistic role of the muscular system apart from the overlapping mechanisms of other systems is much more difficult. Nevertheless, researchers have established numerous potential mechanisms for sarcopenia and dynapenia that occur directly within muscle tissue. Though the characteristics of these mechanisms appear different based on the level of examination, at the cellular level they stem from unequal rates of decreased muscle protein synthesis (MPS) and increased muscle protein breakdown (MPB) (Curcio et al., 2016; Haran et al., 2012; Tieland et al., 2018). This

imbalance in muscle protein turnover is attributed to numerous abnormal responses to genetic, environmental, nutritional, chemical, and morphological anabolic stimuli.

In 2005, researchers published an example of how chemical and nutritional stimuli effect MPS through a comparison of components of the mammalian target of rapamycin (mTOR) pathway, and MPS rates in young and older men following essential amino acid supplementation (Cuthbertson et al., 2005). Age is related to a reduced concentration of mitochondrial DNA (Welle et al., 2003), increased free radical production of existing subsarcolemmal mitochondria (Adhihetty et al., 2005; Seo et al., 2008), and increased oxidation stimulated release of the cell death mediators, cytochrome c and apoptosis-inducing factor (Adhihetty et al., 2005). This is an important concept in understanding the role of age-related reductions in mitochondrial oxidative capacity as mitochondrial proteins are largely responsible for the production of adenosine triphosphate (ATP) needed for muscular contractions (Johnson et al., 2013). Lower sarcoplasmic and myofibrillar maximal fractional synthesis rates combined with 50-70% decreases in downstream targets of mTOR, ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), were observed in elderly muscle when compared to young muscle (Cuthbertson et al., 2005). Similar observations in the muscle protein synthesis of aging muscle have been observed following supplementation with 20g of casein protein (Wall et al., 2015). In addition to decreased MPS, rodent research is suggestive that aging adults may experience increased MPB through the ubiquitin proteasome pathway, or more specifically through the upregulation of Muscle RING Finger 1 (MuRF-1) and Muscle Atrophy F-box (MafBx) (Altun et al., 2010; Bodine et al., 2001). However, conflicting findings emerged when the response of these markers and markers of protein kinase B/mTOR complex 1 (PKB/mTORC1) pathway to combined nutritional and environmental stimuli were compared in

young and old men and women (Francaux et al., 2016). Significant impairment of PKB phosphorylation (-30-40%), trends toward impairment of S6K1($p=0.066$), and doubling in the expression of Regulated DNA Damage and Development 1 were observed with no significant changes in MuRF-1 or MafBx between age groups (Francaux et al., 2016). These findings suggest that the blunted response of aging muscle to chemical, environmental, genetic, and nutritional anabolic stimuli may contribute to sarcopenia through decreased MPS over increased MPB. Though this might explain the reduction of mitochondrial mass and function with age (Guillet et al., 2004; Miller et al., 2012; Rooyackers et al,, 1996), inconsistencies in research methodologies, models, and the multifaceted pathogenesis of sarcopenia and dynapenia (particularly regarding the endocrine and cardiovascular systems) make understanding the primary mechanisms of these diseases extremely difficult (Johnson et al., 2013).

Muscle protein synthesis may also be affected, to some extent, by changes in muscle morphology. Changes in muscle morphology that are directly related to sarcopenia include an age related reduction in muscle fiber number and size, primarily in type II fibers (Lexell, 1995). In general, type II fibers are up to eight times stronger than type I fibers and are significantly weaker in older men vs. young men (Frontera et al., 2000). Additional research showed that elderly individuals have a higher percentage of type I fibers than type II (53 ± 3 vs. 47 ± 3) which was significantly different from their young peers (Verdijk et al., 2007). Significant differences in the satellite cells of type II fibers vs. type I fibers and in the number of satellite cells in type II fibers between young and elderly participants ($p<0.001$) were also observed (Verdijk et al., 2007). Similar observations were made when examining type II fiber percentage and satellite cell content from birth to the eight decade of life (Verdijk et al., 2014). Upon further examination, researchers determined relationships between type II fiber size and age and type II

fiber satellite cell content and age ($r = -0.56$, $p<0.001$) (Verdijk et al., 2014). These findings indicate a role of morphological changes of the muscle itself in the development of sarcopenia and dynapenia. Findings from the Hertfordshire Sarcopenia Study have attempted to support this concept by examining satellite cell density, satellite cell/fiber ratio, and fiber size in sarcopenic men (Patel et al., 2015). Though this study was likely underpowered, trending differences ($p=0.06$) in satellite cell density and satellite cell/fiber ratio between sarcopenic and non-sarcopenic men (1.7 cells/mm^2 vs. 3.8 cells/mm^2 and 0.02 vs. 0.06, respectively) were reported (Patel et al., 2015). However, the majority of rodent studies have shown that deletion of satellite cells post-maturity does not elicit sarcopenia (Fry et al., 2015; Keefe et al., 2015; Lee et al., 2016). These studies and others have led researchers to question the true role of satellite cells in human muscle maintenance and aging (Gundersen & Bruusgaard, 2008; Murach et al., 2018).

Aging is also associated with increased adipose tissue infiltration of the muscle, another morphological change (Nakagawa et al., 2007). Research examining intramuscular adipose tissue (IMAT) in individuals with conditions associated with muscle loss showed a positive relationship ($r = 0.47$, $p = <0.05$) between IMAT of the thigh and age (Marcus et al., 2010). Additionally, significant relationships ($p<0.01$) between increased IMAT and "mobility function" , as measured by walk testing ($r = -0.33$), stair climbing ($r = 0.36$-0.39), and timed up and go ($r = 0.30$) have been previously recorded (Marcus et al., 2012). Years later, associations were observed between IMAT and interleukin- 6 (IL-6) ($r = 0.43$-0.50) and IL-6 and walk testing ($r = -0.63$), gait speed ($r = -.60$) and isometric strength ($r = -0.54$) suggesting that increased IMAT may lead to increased inflammation and decreased functional capacity in older adults (Addison et al., 2014; Zoico et al., 2010). Furthermore, greater NFkB activity, an inflammatory pathway associated with IL-6 and tumor necrosis factor alpha (TNF-α), in elderly muscle when

compared to young muscle might be responsible for downregulating mTOR and MPS (Cuthbertson et al., 2005). However, associations between IMAT and TNF-α were not observed with the abovementioned IL-6 data (Addison et al., 2014). Though the evidence of how morphology affects MPS is growing, it is all together inconclusive. Additional research is required to further support this as a mechanism for sarcopenia and dynapenia.

Though dynapenia is often under the umbrella-term "sarcopenia" it has been suggested that they remain separate terms to differentiate between the mechanisms which cause them (Clark & Manini, 2008). Because losses in muscle size are generally accompanied by losses in muscle strength (Dey et al., 2009; Frontera et al., 1991; Hayashida et al., 2014), some mechanisms for dynapenia may be overlooked. This could be detrimental to the progress in mitigating age-related losses in function and quality of life as recent literature has documented greater reductions of strength than of size (Delmonico et al., 2009; Francis et al., 2016; Goodpaster et al., 2006). For example, data from a large study cohort has suggested that changes in muscle size explain only about 5% of the variation in strength across different genders and races (Goodpaster et al., 2006). These findings have led researchers to examine age-related changes in muscle quality, strength per unit of muscle mass, in addition to examining changes in muscle size and strength when studying these age-related diseases. For example, Francis et al. showed differences in leg muscle size as determined by dual energy x-ray absorptiometry (DXA), isokinetic strength, and muscle quality between young and older participants (Francis et al., 2016). Additionally, Goodpaster et al observed declines of approximately 4% in DXA derived appendicular mass, 3% in computerized tomography (CT) derived cross sectional area (CSA), and 7-11% in isokinetic strength of elderly muscle after 3 years (Goodpaster et al., 2006). Delmonico and colleagues observed similar decreases in CT CSA and strength with the

additional observation of increased IMAT during a 5 year follow up (Delmonico et al., 2009). Further research has determined that declines in CSA and increases in IMAT are associated with decreased physical functioning and may have a mechanistic role in the development of dynapenia (Visser et al., 2002). However, it is unlikely that these mechanisms are independent. Sarcopenia has also been associated with architectural changes like decreased fascicle length (Narici & Maganaris, 2006; Narici et al., 2003; Thom et al., 2007), reduced pennation angle (Narici & Maganaris, 2006; Narici et al., 2003), and changes to the surrounding tendons (Narici & Maganaris, 2006). Narici et al. (2003, 2006) have suggested that these changes are not accounted for in the previous studies as the methods report an anatomical CSA (ACSA) and not a physiological CSA (PCSA). They have reported 19.1% lower CT derived ACSA and 15.2% lower ultrasound derived PCSA in older males when compared to young males with no differences in the ratios of ACSA to PCSA (Narici et al., 2003). Furthermore, they observed a significant but moderate correlation between ACSA and PCSA ($R^2 = 0.576$) with shorter fascicles and lower pennation angles in the elderly males indicating that these units of mass are not interchangeable (Narici & Maganaris, 2006). These findings and several others suggest the loss of sarcomeres contracting in series and in parallel with aging as a primary, but not independent, mechanism of dynapenia (Clark & Manini, 2008; Mitchell et al., 2012; Narici & Maganaris, 2006; Narici et al., 2003).

At the single fiber level, dynapenia, sarcopenia, and frailty have also been associated with decreases in maximum shortening velocity and specific tension, force per unit CSA, when compared to young healthy muscle (D'Antona et al., 2003; Larsson et al., 1997; Yu et al., 2007). These age-related changes in muscle quality might be triggered by changes in calcium release and reuptake of ryanodine receptors and the voltage-gated calcium channel alpha subunit 1 in the

sarcoplasmic reticulum leading to excitation-contraction uncoupling (Delbono, 2000; Hunter et al., 1999; Moreno et al., 2006; Renganathan & Delbono, 1998; Renganathan et al., 1997). However, significant differences in maximum shortening velocity ($p<0.05$) and differences of 20-28% in the specific tension between young and old adults have been observed in skinned muscle fibers with dysfunctional sarcoplasmic reticula (Yu et al., 2007). Nevertheless, this process should not be disregarded as a potential mechanism for the decreased muscle quality in vivo (Hunter et al., 1999; Moreno et al., 2006; Renganathan & Delbono, 1998; Renganathan et al., 1997; Yu et al., 2007). It is also possible that the above mentioned losses in specific tension and contraction velocity are related to reductions in myosin content with increasing age (D'Antona et al., 2003; Marx et al., 2002; Pette & Staron, 2000). Additionally, fibers tend to shift from type I myosin heavy chain (MHC) to type IIa MHC fibers with age which may contribute to changes in muscle fiber quality (D'Antona et al., 2003; Höök et al., 2001; Pette & Staron, 2000). These mechanisms are reversible making them attractive evidence for nutritional and exercise interventions (Hunter et al., 1999; Renganathan & Delbono, 1998).

2.2.2.4. Cardiovascular

There is a high prevalence of age-related diseases in older individuals with cardiovascular disease (Addison et al., 2018; Chainani et al., 2017). Furthermore, associations between cardiovascular disease risk factors, increased mortality, and frailty have been reported indicating a relationship between these diseases and the cardiovascular system (Chainani et al., 2017). Though overlapping mechanisms like those previously discussed make it difficult for researchers to understand the direction of this relationship, several studies point towards microvascular (Prior et al., 2016; Snijders et al., 2017), macrovascular (Abbatecola et al., 2012; Ochi et al., 2010; Timmerman & Volpi, 2013), and cardiac contributors (Chowdhary et al., 2002;

Chowdhary et al., 2000). Impaired macrovascular function (i.e. arterial stiffness) measured by pulse wave velocity has been previously associated with low SMI in males and white females ($p=0.01$) (Abbatecola et al., 2012). Similar associations in men, but not in women, have been observed between thigh muscle CSA/body weight and brachial-to-ankle pulse wave velocity ($r=-0.34$, $p<0.0001$) indicating a predominately hormonal mechanism being responsible for the differences in muscle mass (Ochi et al., 2010). However, the role of macrovascular dysfunction in the development of sarcopenia cannot be completely disregarded. In the previously mentioned 6-year longitudinal study, the highest tertile of pulse wave velocity was associated with the lowest SMI, regardless of participant body mass index (Abbatecola et al., 2012). Additional research showed that male and female participants with lower SMI were 2 times more likely to higher pulse wave velocity determined cardio-ankle vascular index (Sampaio et al., 2014).

In addition to macrovascular dysfunction, muscle fiber perfusion is limited by age related changes in microvascular function (Prior et al., 2016; Wilkes et al., 2009). Impaired microvascular function, measured as fewer capillary contacts per fiber and decreased muscle capillary density, have been previously observed in sarcopenic individuals when compared to young (Groen et al., 2014). Additionally, ACSA was significantly lower in older people (-16%) when compared to the young, and even lower in age-matched subjects with insulin resistance (-20%) suggesting that microvascular dysfunction, in addition to anabolic resistance, is a contributor to the development of sarcopenia (Groen et al., 2014). Similar findings suggest that low ACSA ($r =.55$, $p \leq .001$), degree of sarcopenia ($r =.51$, $p \leq .001$), and exercise capacity ($r =.37$, $p \leq .01$) are associated with low capillary-to-fiber ratio suggesting a mechanistic role of decreased capillarization in declining physical function associated with sarcopenia and dynapenia (Prior et al., 2016).

As previously discussed, efficient insulin sensitivity and nutrient delivery is essential for maintaining skeletal muscle function with aging. Insulin is a key hormone involved in the stimulation of nitric oxide, an endothelial vasodilator, and thus is a mediator of muscle perfusion through the regulation of blood flow (Timmerman et al., 2010; Timmerman & Volpi, 2013). Age related alterations in endothelin-1 (ET-1), nitric oxide (NO), inflammation, and oxidative stress may contribute to the development of age-related diseases through dysfunctional endothelial dilation (Timmerman & Volpi, 2013). Elevations in ET-1 expression in older vs. young males, as well as the relationship between ET-1 and decreased endothelium dependent dilation, have been previously observed (Donato et al., 2009). Furthermore, inhibition of ET-1 receptors has been related to greater increases in the blood flow of older men when compared to young men (Thijssen et al., 2007; Van Guilder et al., 2007). Primarily, elevations in this potent vasoconstrictor act on voltage-gated calcium channels (Yanagisawa et al., & Masaki, 1988) and insulin receptors (Jiang et al., 1999) in the endothelium of vascular smooth muscle. Thus, the associated secondary changes (decreased blood flow) in the vasculature may contribute to sarcopenia and dynapenia by reducing glucose uptake and availability in skeletal muscle (Shemyakin et al., 2010). However, changes in ET-1 are not solely responsible for the cardiovascular changes associated with aging. NO and its precursor, L-Arginine, are known regulators of cardiac vagal control (Chowdhary et al., 2002; Chowdhary et al., 2000). More specifically, the L-arginine/nitric oxide pathway promotes vasodilation through the conversion of L-Arginine to nitric oxide and L-citrulline via endothelial nitric oxide synthase (eNOS) and/or the phosphorylated isoform (PeNOS) (Arnal et al., 1999; Bai et al., 2009; Donato et al., 2009; Fleming & Busse, 1999). Because of this, decreased NO availability has been touted as a primary mechanism for endothelial dysfunction in older people (Taddei et al., 2001). However,

expression of PeNOS and eNOS did not appear to be reduced, and was observed to be greater, in older men when compared to young indicating the role of alternative biological contributors to dilatory responses with aging (Donato et al., 2009). Bioactivity of ET-1, oxidative stress, and inflammation may be greater contributors to the development of sarcopenia than synthesis of NO itself due to their mechanistic roles in the reduction of NO availability and decreased blood flow (Donato et al., 2009; Landmesser et al., 2003; Verma, Li, et al., 2002; Verma et al., 2002). In addition to these potential mechanisms, a more direct relationship between L-arginine and muscular hypertrophy has been established as L-arginine has been shown to activate MPS through the mTORC1 pathway by binding to an under researched protein, CASTOR1 (Chantranupong et al., 2016). Additionally, NO has shown to inhibit MPB through the ubiquitin proteasome pathway (Kapadia et al., 2009).

Evidence of sarcopenia in both skeletal and smooth muscle has led researchers to explore its incidence in cardiac muscle using senescent mice (Lin et al., 2008). Much like the previously discussed sarcopenic and dynapenic alterations to skeletal muscle, decreased number of myocytes, increased size and intracellular lipids of existing myocytes, decreased left ventricular wall thickness and ejection fraction were observed in senescent mice when compared to adult mice (Lin et al., 2008). Additionally, Lin et al. (2008) were the first to demonstrate a relationship between strain driven remodeling, resulting from vasodilation and increased pressure, and cardiac sarcopenia using linear-elastic cylindrical left ventricular modeling. Furthermore, this study is potentially indicative of a new syndrome, termed cardiac sarcopenia, which may have similar potential mechanisms to the condition in skeletal muscle. Though this, and previous (Kawaguchi et al. 2003), research indicate the potential of this model in studying the structural

and functional changes of the human heart during aging, this has yet to be completed making it difficult to fully comprehend.

2.2.2.5. Summary

As previously discussed, the etiology of the sarcopenia, dynapenia, and frailty is multifaceted and consists of numerous overlapping mechanisms involving different biological systems (Figure1). Thus, it is imperative that researchers examine the mechanisms with an overarching goal of improving individuals' quality of life and reducing economic burden. In doing so, researchers can better develop safe and effective countermeasures to mitigate losses associated with these syndromes. Though there are several appropriate countermeasures that could be discussed, this paper will focus primarily on the risks and benefits associated with exercise and nutritional interventions.

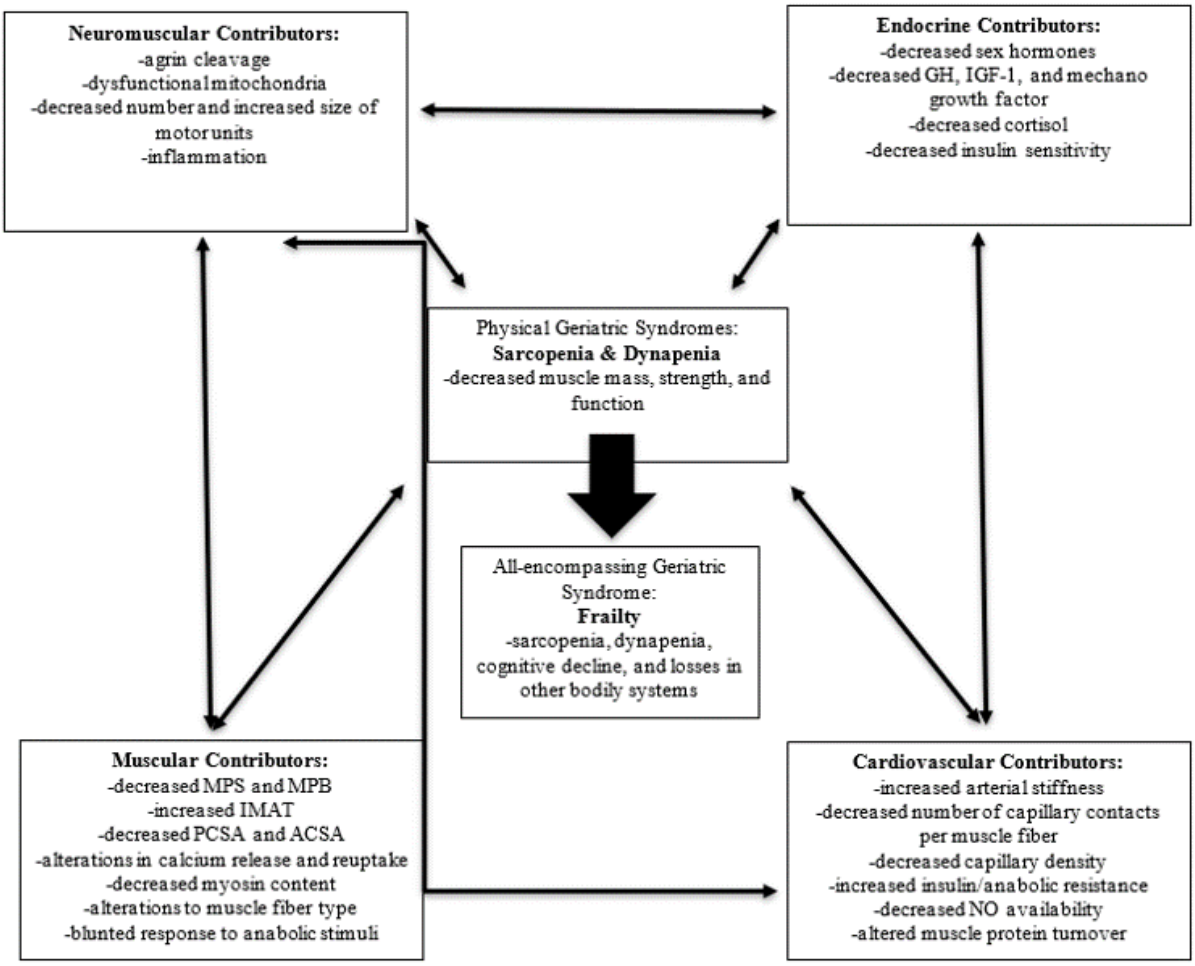

*Figure 1***.** The Multifactorial Etiology of Age-relate Diseases. This figure summarizes the differences and relationships between sarcopenia, dynapenia, and frailty (represented by thick arrow) and the overlapping mechanisms (represented by thin arrows) of the biological systems which contribute to them.

2.3. Interventions

2.3.1. Introduction

Sarcopenia is recognized as a disease under the code ICD-10-CM (Anker et al., 2016). The disease is defined as a combination of low muscle mass and strength (i.e. dynapenia) and/or physical performance (Cruz-Jentoft et al., 2014). As previously discussed, this disease leads to an even more debilitating disease, frailty, through numerous overlapping mechanisms. Additionally, sarcopenia and frailty are associated with rising functional and economic

consequences (Cruz-Jentoft et al., 2010; Danielson, 2013; Doherty, 2003; Janssen et al., 2004; HHS, 2011; Rathge et al., 2012; van Dijk et al., 2016). Because the prevalence of sarcopenia in community dwelling adults aged 30-80 years is relatively high (up to 30%), it is important for researchers to examine interventions that favorably alter the key contributors of the neuromuscular, muscular, endocrine, and cardiovascular systems (Cruz-Jentoft et al., 2014). Resistance training, aerobic training, and other exercise modalities have been extensively reviewed as potentially effective treatments to slow the progression of sarcopenia (Bauer et al., 2013; Bowen et al., 2015; Chodzko-Zajko et al., 2009; Cruz-Jentoft et al., 2014; Morley et al., 2010; Naseeb & Volpe, 2017; Strasser et al., 2018). Additionally, nutritional supplementation, pharmaceutical interventions, and combined therapies have been reviewed and appear to be advantageous in treating and attenuating sarcopenia and attenuating frailty (Bauer et al., 2013; Borst, 2004; Bowen et al., 2015; Cruz-Jentoft et al., 2014; Morley et al., 2010; Naseeb & Volpe, 2017; Strasser et al., 2018). However, because of inconsistencies in the methodology and reported variables of previous research, the most effective intervention for sarcopenia is currently unknown. There is a definite need to further examine the positive relationship between exercise and sarcopenia, particularly regarding the effects on physical performance as assessed by short physical performance battery (SPPB), stair-climbing, and the timed get up and go test (TUG) (Chodzko-Zajko et al., 2009; Naseeb & Volpe, 2017).

2.3.2. Exercise Interventions

2.3.2.1. Resistance Training

Exercise favorably alters MPS and MPB through several mechanisms that have been previously discussed in this paper (Bowen et al., 2015; Hasten et al., 2000). Additionally, exercise has the potential to exert powerful anti-inflammatory and anti-oxidant mechanisms that

help to reduce MPB (Bowen et al., 2015). Furthermore, exercise promotes and regulates healthy functioning of skeletal muscle mitochondria (Drake et al., 2016; Porter et al., 2015) and the development of myocytes through the activation of satellite cells (Hawke & Garry, 2001). The American College of Sports Medicine (ACSM) recommends that older adults perform moderate to vigorous progressive resistance training at least twice per week (Chodzko-Zajko et al., 2009). These guidelines have been applied to older adults in order to better understand the effects of resistance training on lean mass (Binder et al., 2005; Churchward-Venne et al., 2015; Fiatarone et al., 1990; Fragala et al., 2014; Leenders et al., 2013; Raue et al., 2009; Slivka et al., 2008), muscle strength (Binder et al., 2005; Churchward-Venne et al., 2015; Fiatarone et al., 1990; Fragala et al., 2014; Morganti et al., 1995; Raue et al., 2009; Serra-Rexach et al., 2011; Slivka et al., 2008; Strasser et al., 2009), and physical performance (Churchward-Venne et al., 2015; Fiatarone et al., 1990; Fragala et al., 2014; Leenders et al., 2013). Though there are differences in the variables examined, muscle groups trained, duration of training, and the age of participants, progressive resistance training (RT) has shown to be beneficial in participants over 60 years of age (Tables 1 & 2).

Several studies have examined the response of muscle strength to RT using one-repetition maximum (1-RM) (Binder et al., 2005; Churchward-Venne et al., 2015; Fiatarone et al., 1990; Leenders et al., 2013; Morganti et al., 1995; Raue et al., 2009; Slivka et al., 2008; Strasser et al., 2009) or isometric strength testing (Capodaglio et al., 2007; Capodaglio et al., 2005). Alternative methods like isokinetic dynamometry (Binder et al., 2005) and hand grip strength (Fragala et al., 2014; Leenders et al., 2013) have also been used. When examining the response of muscle size to training, methods including using lean body mass (LBM) measured by doubly labeled water (Fiatarone et al., 1990), DXA (Binder et al., 2005; Churchward-Venne et al., 2015; Fragala et al.,

2014; Leenders et al., 2013), and CT derived ACSA (Fiatarone et al., 1990; Leenders et al., 2013; Raue et al., 2009; Slivka et al., 2008) have been used. In 2005, researchers started to examine combinations of muscular size and/or strength with physical performance using variations of chair rise time (Capodaglio et al., 2007; Capodaglio et al., 2005; Churchward-Venne et al., 2015; Fiatarone et al., 1990; Fragala et al., 2014; Leenders et al., 2013) and gait speed (Fiatarone et al., 1990; Fragala et al., 2014). Though methodologies in chair rise, or sit-to-stand, differ from the time required to stand from a seated position once (Capodaglio et al., 2007; Fiatarone et al., 1990) to the time required to stand from a seated position five (Churchward-Venne et al., 2015; Fragala et al., 2014; Leenders et al., 2013) and ten times (Capodaglio et al., 2007; Capodaglio et al., 2005), increases in physical performance of this task are fairly consistent across studies. In one study, the overall functional capacity of participants did not increase significantly, but two of the three participants that could not rise from a chair at baseline were able to do so after eight weeks of RT (Fiatarone et al., 1990). Fiatarone et al. (1990) also observed no changes in habitual gait speed using a 6-meter course but saw a 43% increase using a tandem gait. In contrast, Fragala et al. (2014) observed a 12% increase in the habitual walk time using an eight-foot course after only six weeks of RT. It should be noted that these participants were approximately two decades young than those in the previously mentioned study, were community dwelling rather than institutionalized, and completed full body RT rather than lower body RT indicating the need for individualized prescription of interventions. Additionally, some assessments of physical performance may be more appropriate for specific populations than others, but this is difficult to compare due to the inconsistencies of reported variables and the overlapping mechanisms of loss.

Though the study of sarcopenia is not necessarily a recent trend, there are still many variables of RT, including appropriate prescription and its effect on physical performance, that are under researched and therefore novel (Chodzko-Zajko et al., 2009). To better understand the relationship of RT and functional capacity, Capodaglio et al. (2005, 2007) observed improvements in chair rise, bed rise time, functional reach, unilateral standing, timed-get-up-and-go, stair climbing, and the 6-minute walk test in healthy participants over the age of 75 following 1-year of lower body RT. Fragala et al. (2014) observed benefits of RT through a combination of strength and size, muscle quality, in a similar population but using a much different exercise prescription. Though there is no unanimous agreement on the prescription of RT to prevent or treat sarcopenia, research suggests aggressive programming of two (Churchward-Venne et al., 2015; Fragala et al., 2014; Morganti et al., 1995) to three (Binder et al., 2005; Fiatarone et al., 1990; Leenders et al., 2013; Raue et al., 2009; Slivka et al., 2008; Strasser et al., 2009) times per week for six (Fragala et al., 2014), eight (Binder et al., 2005; Fiatarone et al., 1990), twelve (Raue et al., 2009; Slivka et al., 2008; Strasser et al., 2009), twenty four (Churchward-Venne et al., 2015; Leenders et al., 2013), or fifty two weeks (Morganti et al., 1995). Though some of the benefits of RT appear to increase in magnitude between weeks 12 and 52, 40-50% of increases in strength observed by Morganti et al. (1995) occurred in the first 12 weeks. Similarly, over 50% of the increases in muscle size, strength, and performance observed by Churchward-Venne et al. (2015) occurred at 12 weeks. However, Leenders et al. (2013) observed increases in muscular size after 12 weeks that did not increase after 24 weeks. Additionally, they observed improvements in muscle strength and performance after 24 weeks that were not present after 12 weeks of RT (Leenders et al., 2013). Even at a lower duration, the load required to promote muscle hypertrophy (60-80% of 1-RM) and

frequency of training may be too strenuous for older individuals indicating a need for alternative interventions (Chodzko-Zajko et al., 2009). Others observed benefits in muscle strength and physical performance by prescribing low-intensity training (≤60% of 1-RM) in lieu of traditional RT (Capodaglio et al., 2007; Capodaglio et al., 2005). Alternatives, which will be further discussed in this paper, have shown promise in promoting similar, and potentially additional cardiovascular, benefits to RT with less physiological stress to the participants (Bogaerts et al., 2009; Martín-Hernández et al., 2013; Nakajima et al., 2006; Raimundo et al., 2009; Scott et al., 2015 .

Table 1

Resistance Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 1990-2008

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Fiatarone et al. 1990	MS, MM, P	10	88	Lower Body RT	3x/wk, 8wks	LBM, ACSA, LBS, CR, GS	143-231% ↑LBS, 9% ↑ ACSA, ↓↑HGS, 43%↑ GS
Morganti et al. 1995	MS	39	60	Full Body RT	2x/wk, 12m	UBS & LBS	72-82%↑ UBS and 32-86% ↑LBS
Binder et al. 2005	MS, MM	91	≥78	Full Body RT	3x/wk, 3m	UBS, LBS, & LBM	3-43% ↑ LBS, 17% ↑ UBS, 1% ↑ LBM
Capodaglio et al. 2005	MS, P	48	>75	Lower Body LIT	3x/wk, 12m	LBS, F, BR, 6MWT, SC, TUG, B	4-23%↑ LBS, 60-85%↑ F, 8-29% ↓CR & BR, 5% ↑ 6MWT, 12% ↓ SC, 19-21% ↓ TUG, and 25-29% ↑ B
Slivka et al. 2008	MS, MM	6	≥80	Lower Body RT	3x/wk, 12wks	LBS, ACSA	41% ↑ LBS, 3% ↑ ACSA

MM= muscle mass, MS= muscle strength, P=physical performance, RT=progressive resistance training , BC=body composition, KE = knee extensor, CS= chair stand, GS= gait speed, TGS=tandem gait speed, HGS=habitual gait speed, ACSA=anatomical cross sectional area, LBM=lean body mass, FCSA= fiber cross sectional area, W=women, M=men, HG= hand grip strength, LIT = Low-intensity training, F= flexibility, CR= chair rise, BR= bed rise, 6MWT=six-minute walk test, SC=stair climb, TUG= timed get up and go, B=balance, UBS=upper body strength, LBS=lower body strength, DV=dependent variable

Table 2

Resistance Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2009-2015

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Raue et al. 2009	MS, MM	15	85	Lower Body RT	3x/wk, 12wks	LBS, ACSA, FCSA	26% ↑ LBS, ↑↓ ACSA, ↓↑ FCSA
Strasser et al. 2009	MM	29	74	Full Body RT	3x/wk, 6m	UBS & LBS	25-30% ↑UBS and 15%↑ LBS
Leenders et al. 2013	MS, MM, P	60	≥70	Upper Body RT	3x/wk, 24wks	UBS, ACSA, LBM, FCSA, CR, HG	After 12 wks: 8% ↑ ACSA After 24 wks: 3% ↑ LBM, 24-29% ↑ Type II FCSA, 26-31% ↑LBS 42-47% ↑UBS, ↑↓ HG, 18-19%↓ CR
Fragala et al. 2014	MS, MM, P	25	71	Full Body RT	2x/wk, 6 wks	LBM, HG, GS, TUG, CR, MQ	↓↑ LBM, ↑↓ HG, 12%↑GS, ↓↑ TUG, 18%↓CR, 22% ↑ MQ
Churchward-Venne et al. 2015	MS, MM, P	85	73	Lower Body RT	2-3x/wk, 24wks	LBM, FCSA, LBS, CS	After 12 wks: 2%↑ LBM, 6%, ↑ Type I FCSA, 15% ↑ Type II FCSA, 20-33% ↑ LBS, 1% ↓ STS After 24 wks: 17% ↑ Type II FCSA, 29-50% ↑LBS, 2% ↓ CR

MM= muscle mass, MS= muscle strength, P=physical performance, RT=resistance training, GS= gait speed, TGS=tandem gait speed, HGS=habitual gait speed, ACSA=anatomical cross sectional area, LBM=lean body mass, FCSA= fiber cross sectional area, W=women, M=men, HG= hand grip strength, LIT = Low-intensity training, F= flexibility, CR= chair rise, BR= bed rise, 6MWT=six-minute walk test, SC=stair climb, TUG= timed get up and go, B=balance, UBS=upper body strength, LBS=lower body strength

2.3.2.2 Aerobic Training

In addition to age-related losses in muscle size and strength, fatigability also increases due to decrements in aerobic capacity or maximal volume of oxygen uptake (VO_{2max})(Heath et al., 1981). Decrements in VO_{2max} and increased risk of sarcopenia can be attenuated by long term participation in aerobic training (AT)(Crane et al., 2013. Crane et al. (2013) examined relationships between age, physical activity, LBM, VO_{2max}, aerobic power, grip strength, and lower body strength in 74 men and women aged 20-86 years. They determined that VO_{2max}, aerobic power, grip strength, and lower body strength decreased with age but that all variables were greater in those who had participated in at least 4 hours per week of moderate to vigorous aerobic activities for ≥10 years (Crane et al., 2013). Similarly, Harber et al. (2012) observed lower baseline VO_{2max} in older when compared to young males with similar increases after training (13±3% vs. 16±2%, respectively). They also observed 5-6% increases in muscle volume regardless of age (Harber et al., 2012). Though this study did not yield significant improvements in muscular power or FCSA, other laboratories have observed increases in LBM (~1%), lower body strength (~28-55%), ACSA (~11%), and Type I FCSA (~16%) after employing similar interventions in older women (Harber et al., 2009; Konopka et al., 2010). These results indicate that muscular and cardiovascular health can be maintained, regardless of age, by incorporating AT. Aerobic training has shown to mitigate sarcopenia by altering gene expression associated with anabolic and catabolic pathways and by improving mitochondrial content and function (Broskey et al., 2014; Drake et al., 2016; Konopka et al., 2010; Pasini et al., 2012). Despite these promising results, few studies have examined the effects of AT on muscle health in older adults (Table 3).

The ACSM recommends that older adults perform 150-300 minutes of moderate intensity or 75-150 minutes of vigorous intensity AT per week (Chodzko-Zajko et al., 2009). Additionally, walking for 30 minutes per day for at least five days has been associated with a lower risk of developing sarcopenia(Kim et al. 2013). The majority of the interventions in Table 3 meet these guidelines, however, improvements of a lesser magnitude have been observed in aerobic capacity, muscle mass, and upper body strength have been observed using low intensity exercise for 45-120 minutes per week (Strasser et al., 2009). Differences in intensity and duration are not the only reason to use caution when interpreting the magnitude of results. Throughout the literature, there are inconsistencies in reported laboratory techniques as well. For instance, strength has been measured using isometric force, power, and 1-RM (Strasser et al., 2009; Harber et al., 2012; Konopka et al., 2010). Additionally, muscle mass has been reported using magnetic-resonance imaging derived CSA, muscle volume, skin caliper assessment, and DXA (Harber et al., 2009; Harber et al., 2012; Konopka et al., 2010). These methods have been reported to have 0.5-5 % error with DXA having the lowest variability (Kuriyan, 2018). Future research with consistent methodologies is needed to better comprehend the role of AT in mitigating sarcopenia. Additionally, more research is needed to explore the effects of AT on the muscle function criteria of sarcopenia. Though studies have measured aerobic performance through VO_{2max}, aerobic power, work, or metabolic cost of walking, only one has examined diagnostic criteria for sarcopenia (Crane et al., 2013; Cruz-Jentoft et al., 2014; Harber et al., 2009; Harber et al., 2012; Konopka et al., 2010; Naseeb & Volpe, 2017; Strasser et al., 2009; VanSwearingen et al., 2009). VanSwearingen et al. (2009) examined performance changes in response to aerobic (treadmill speed intervals) and a combination of aerobic and resistance training, known as concurrent training, using gait speed and SPPB. Both interventions increased

GS and SPPB performance but aerobic exercise reduced the energy cost of walking more than concurrent training (VanSwearingen et al., 2009). In this study, the aerobic component of the concurrent training was low intensity, steady state recumbent cycling which may not be aggressive enough for the treatment of sarcopenia. More aggressive forms of concurrent training will be discussed in the next section.

Table 3

Aerobic Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Harber et al. 2009	MS, MM	7	71	Cycling (60-80% HRR)	3-4X/wk, 12 wks	LBM, VO_{2max}, MV, LBS, FCSA	30%↑VO_{2max}, 1% ↑LBM, 55%↑LBS, 12%↑MV, 16% ↑Type I FCSA, ↓↑ Type II FCSA
Strasser et al. 2009	MS, MM	27	76	Cycling (60 % HRR)	3x/wk, 24 wks	LBM, VO_{2max}, UBS, LBS	22%↑UBS, ↑↓ LBS, 6% ↑VO_{2max}, 4%↑LBM
Konopka et al. 2010	MS, MM	9	70	Cycling (60-80% HRR)	3-4x/wk, 12 wks	$VO_{2max,}$ ACSA, LBM, LBS	1% ↑LBM. 30%↑VO_{2max}, 11 % ↑ACSA, 28% ↑LBS
Harber et al. 2012	MS, MM	13	Y: 20 O:74	Cycling (60-80% HRR)	3-4X/wk, 12 wks	VO_{2max}, MV, MP, FCSA	Y: 16%↑VO_{2max}, 5% ↑MV, ↑↓MP, ↑↓ Type I or II FCSA O: 13%↑VO_{2max}, 6% ↑MV, ↑↓MP↑↓ Type I or II FCSA
VanSwearingen et al. 2009	P	47	77	Gait training and TM Speed Intervals	2x/wk, 12 wks	GS,C_w	15%↓C_w, 24%↑GS, %NR SPPB

MM= muscle mass, MS= muscle strength, P=physical performance, HRR=heart rate reserve, VO_{2max}= maximal volume of oxygen uptake, ACSA=anatomical cross-sectional area, FCSA= fiber cross-sectional area, LBM= lean body mass, LBS= lower body strength MV= muscle volume, GS= gait speed, SPPB= short physical performance battery, C_w= metabolic cost of walking, NR=% change not reported, Y=young group, O=older group, MP=muscular power, TM= treadmill

2.3.2.3. Concurrent Training

As previously discussed, resistance and aerobic exercises are effective at attenuating sarcopenia through different mechanisms. Thus, a combination of both types of exercises, known as concurrent training, may be the best intervention to mitigate losses in muscle size, strength, and/or function while also decreasing chronic disease risk and improving the quality of life in older adults (Strasser et al., 2018). To attenuate sarcopenia, at least 20-30 minutes of concurrent training completed three times per week has been recommended (Morley et al., 2010). With these guidelines and feasibility in mind, several researchers have shown improvement in variables of muscular strength, mass, and physical performance (Table 4 & 5). Goodpaster et al. (2008) showed that combining moderate intensity physical activity with resistance, flexibility, and balance exercises helped to mitigate losses in strength and accumulation of intramuscular adipose tissue. Though ACSA did not differ between the groups, this study shows that the concurrent training was beneficial in maintaining muscle strength and quality throughout aging (Goodpaster et al., 2008). Furthermore, these findings hold practical significance as muscle strength and quality better translate to increased longevity and improved quality of life. This is evidenced as increases in lower body strength when measured as maximum voluntary contraction of the knee extensors have correlated to 6-MWT distance following 12 months of concurrent training (Mian et al., 2007). In contrast, Serra-Rexach et al. (2011) showed that individuals are capable of increasing strength but not physical performance in their ninth decade of life. This study showed that lower body strength increased after eight weeks of low intensity concurrent training (30-70% 1-RM combined with aerobic exercise 12-13 RPE) and though it decreased following 4 weeks of deconditioning (mobility training only) the final result was 9% above baseline suggesting that strength can be increased and maintained by nonagenarians.

The observation that strength can be increased at that age but not physical performance might be due to a blunted response to exercise occurring after age 90 but it is difficult to understand with the variation in methodologies and lack of literature on nonagenarians. Several studies have examined the effects of concurrent training on the physical performance of individuals aged 60-89 years (Binder et al., 2002; Liu et al., 2014; Rydwik et al., 2008; VanSwearingen et al., 2009; Zampieri et al., 2015). However, there is a definite need to better understand the effects of this broad type of exercise as many variables are underreported in the literature. For instance, Binder et al. (2002) used a modified SPPB which combined typical chair stand and gait speed assessments with other assessments of activities of daily living like lifting a heavy book to a shelf and putting on a laboratory coat. Though the modified SPPB score improved in the exercise training group, the authors reported that only one variable, the chair stand score, had a significant group-by-time effect (Binder et al., 2002). Unfortunately, they did not display any data regarding this variable. Similarly, VanSwearingen et al. (2009) observed improvements in some aspects of the SPPB in response to cycling or recumbent stair climbing, resistance training, flexibility training, gait and balance training but did not report total SPPB score changes between groups. The reported observations included an increase in gait speed of 17% following endurance and gait training and of 24% following the concurrent training program (VanSwearingen et al., 2009). Additionally, chair rise time decreased with endurance training (-2.08 ± 2.50s, $p < 0.01$) and concurrent training (-2.44 ± 2.12, $p < 0.01$) with no significant differences between groups (VanSwearingen et al., 2009). It is also difficult to interpret results when the methods use combined interventions. For example, Rydwik et al. (2008) examined differences in muscle strength and performance following 12 weeks of dietician led individual and group nutritional counseling, nutritional counseling combined with training,

training only, and general training and nutrition advice. Nutritional counseling was dietician/nutritionist led and focused on nutritional needs, nutrient timing, and food preparation (Rydwik et al., 2008). They observed significant improvements in upper and lower body strength, sit-to-stand time, and stair climbing in the training group with no significant difference between the training group and the nutritional counseling with training group (Rydwik et al., 2008). These results can be misleading as they indicate that training has a greater influence than diet on muscular health. However, this interpretation should be used with caution as using "general diet advice" for the nutritional intervention was a major limitation to the study. Though the results reported are not all-inclusive, these studies show that the best prescription for increased strength and physical performance is to maintain a balanced diet and get the body moving at least twice per week with the most feasible modality for the individual.

In recent years, different exercise modalities have been shown to be efficient in mitigating sarcopenia in later decades of life (Kemmler et al., 2010; Liu et al., 2014; Zampieri et al., 2015). For example, Zampieri et al. (2015) examined the effects of lifelong concurrent activity through sports participation on total muscle fiber count, muscular strength, and physical functioning in men aged 65-79 years compared to sedentary males of the same age and young active males aged 19-33 years. Though the healthy active males had significantly greater isometric leg strength (3.2±0.6 Nm/kg vs. 2.2±0.3 Nm/kg) and total fiber count (73.4±19.3μm vs. 61.2±17.1μm) than active older males, active older males had significantly greater isometric leg strength (2.2±0.3 Nm/kg vs. 1.7±0.3 Nm/kg), TUG (4.7±1.1s vs. 6.6±1.3s), chair stand (6.3±1.3s vs. 11.9±2.1s), and total fiber count (61.2±17.1μm vs. 56.2±17.9μm) than their inactive counterparts. No differences in gait speed, chair stand, TUG, or SPPB were seen between young and older active males, however, significant differences were observed between

the older active and older sedentary groups. These results suggest that incorporating consistent combinations of aerobic and resistance exercises can help attenuate sarcopenia and optimize quality of life while aging. Liu et al. examined sarcopenic and non-sarcopenic (classified by SMI) individuals following 12 months of concurrent training (Liu et al., 2014). They observed improvements in physical functioning with no significant differences between classifications indicating that general concurrent training could help to improve the loss of physical functioning associated with sarcopenia. Furthermore, it is also possible that general concurrent training could help to improve the loss of skeletal muscle mass associated sarcopenia as similar observations in SMI were indicated but not specified (Liu et al., 2014). Similarly, Kemmler et al. (2010) reported improvements in muscular strength, size, function, and aerobic fitness following 18 months of aerobic dance combined with resistance, mobility, flexibility and balance training. Significant differences in appendicular skeletal mass (0.1% increase vs. 2% decrease), total lean body mass (1% increase vs. no change), trunk strength (5% increase vs. 2% decrease), leg power (2% increase vs. 7% decrease), and TUG (5% decrease vs. 1% increase) were observed between the intervention and control groups, respectively (Kemmler et al., 2010). They also observed a 14% change in time under load, a variable used to show aerobic fitness, which was similar to previously reported results of VO_{2max} (Binder et al., 2002; Kemmler et al., 2010). These differences, though statistically significant, may not seem practically significant. However, it is important to note that the control group was still active and exercising without typical progression suggesting that some exercise is better than no exercise but that progressive training yields greater results.

Table 4

Concurrent Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2002-2008

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Age Range**	**Description**	**Frequency & Duration**	**DV**	**Results**
Binder et al. 2002	MS, P	115	79-87	Full body AT, RT, BT, FT	3x/wk, 9m	ADLs, SPPBm, VO_{2max}, LBS, FSQ, B	13-14% ↑ $VO_{2max,}$ 14% ↑FSQ, ↑↓ADLs, 19-23% ↑LBS, 12% ↑SPPBm, 6%↑B
Mian et al. 2007	MS, P	38	70-82	Full body AT, RT, FT	2x/wk, 12m	C_w, LBS, B, 6MWT	21% ↑LBS, 30%↑B, 6% ↑6MWT, ↑↓C_w
Goodpaster et al. 2008	MS,MM	42	70-89	Full body AT, RT, BT, FT	≥5x/wk,12m	LBS, ACSA, IMAT	C: 22% ↓LBS, 4% ↓ACSA, 18% ↑IMAT I: ↑↓LBS, 3%↓ACSA, ↑↓IMAT
Rydwik et al. 2008	MS, P	96	>75	Full body AT, RT, B	2x/wk, 12wks	LBS, UBS, CS, TUG, GS, SC	17%↑LBS, 10-15% ↑UBS, 16% ↑CS, 37%↑SC, ↑↓TUG, ↑↓GS

MM= muscle mass, MS= muscle strength, P=physical performance, AT= aerobic training, RT= resistance training, BT= balance training, FT= flexibility training, MT=mobility training, SMI=skeletal muscle index, SPPB= short physical performance battery, GS=gait speed, LBS= lower body strength, UPS= upper body strength, HG=hand grip strength, SC=stair climb, TUG= timed get up and go, NR= change not reported, ADLs=activities of daily living, TS=trunk strength, LBP=lower body power, SPPBm=modified short physical performance battery, VO_{2max}= maximal volume of oxygen uptake, FSQ= functional status questionnaire, B=balance, ACSA=anatomical cross-sectional area, IMAT=intramuscular adipose tissue, C=control group, I=intervention group, LBM= lean body mass, TUL=time under load, C_w= metabolic cost of walking, 6MWT=six-minute walk test, CS=chair stand, LPS= lifelong sports participation, TFC= total fiber count, C= control group, I= intervention group

Table 5

Concurrent Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2009-2015

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Age Range**	**Description**	**Frequency & Duration**	**DV**	**Results**
VanSwearingen et al. 2009	P	47	77	Nustep or Cycling, FT, RT, BT	2x/wk, 12 wks	GS,C_w	15%↓C_w, 24%↑GS, %NR SPPB
Kemmler et al. 2010	MS, MM, P	227	65-80	Full body AT, RT, MT, FT, BT	2x/wk, 18m	LBM, HG, TS, LBS, LBP, TUG, TUL	1%↑ LBM, ↑↓HG, 5%↑TS, 14%↑ LBS, 2%↑ LBP, 5%↓ TUG, 14%↑ TUL
Liu et al. 2014	MM, P	177	70-89	Full body AT, RT, BT, FT	3x/wk, 12m	SMI, SPPB, GS	6m: %NR SMI, 15-28 % ↑ SPPB, 2-9% ↑GS 12m: %NR SMI, 12-18% ↑ SPPB, 3-7% ↑ GS
Zampieri et al. 2015	MS, P	29	65-79	LSP	≥3x/wk	LBS, GS, SPPB, TUG, CS, TFC	29%↑LBS, 21%↑GS, ↑↓SPPB, 29%↓TUG, 47%↓CS, 9%↑ TFC

MM= muscle mass, MS= muscle strength, P=physical performance, AT= aerobic training, RT= resistance training, BT= balance training, FT= flexibility training, MT=mobility training, SMI=skeletal muscle index, SPPB= short physical performance battery, GS=gait speed, LBS= lower body strength, UPS= upper body strength, HG=hand grip strength, SC=stair climb, TUG= timed get up and go, NR= change not reported, ADLs=activities of daily living, TS=trunk strength, LBP=lower body power, SPPBm=modified short physical performance battery, VO_{2max}= maximal volume of oxygen uptake, FSQ= functional status questionnaire, B=balance, ACSA=anatomical cross-sectional area, IMAT=intramuscular adipose tissue, C=control group, I=intervention group, LBM= lean body mass, TUL=time under load, C_w= metabolic cost of walking, 6MWT=six-minute walk test, CS=chair stand, LPS= lifelong sports participation, TFC= total fiber count, C= control group, I= intervention group

2.3.2.4. Alternative Training Methods

Generally, “aggressive resistance exercise” or high intensity resistance training with high loads (70-80% of 1RM) is necessary to improve muscle mass and strength(Morley et al., 2010). However, some conditions like those associated with aging make exercising with these loads difficult creating a less is more phenomena (Chodzko-Zajko et al., 2009; Lees et al., 2005). Because of this, researchers have explored alternative training methods for aging populations (Tables 6-10). One example of these alternative training methods is whole body vibration training which is thought to stimulate muscular and bone adaptation through oscillations (Bogaerts et al., 2009; Raimundo et al., 2009). Raimundo et al. compared the effects of 8 months of whole body vibration training vs. walk training on muscle strength and physical functioning in post-menopausal women (Raimundo et al., 2009). Interestingly, the walking group had greater improvements than the whole body vibration group in time to walk 4-meters (15% decrease vs. 6% increase) and complete three repetitions of chair stand (17% vs. 4% decrease)(Raimundo et al., 2009). However, whole body vibration training led to a significantly greater increase in countermovement jump height (13% increase vs. 1% decrease)(Raimundo et al., 2009). Bogaerts et al. (2009) examined the differences in muscle strength and aerobic fitness following WBV training, concurrent training (CCT), and no training in 60-80 year old community dwelling adults. Aerobic fitness increased significantly in all groups (WBV: +18%, CCT:+21%, CON:+8%, $p<0.001$) with significant differences between the intervention groups and the control ($p<0.001$) and no significant difference between whole body vibration and CCT ($p=0.588$)(Bogaerts et al., 2009). Lower body strength increased significantly by 9% following whole body vibration training and by 13% following CCT with no significant differences between the groups(Bogaerts et al., 2009). Time to peak exercise (TPE) was significantly

different between groups (WBV: +9%, CCT: +14%, CON: +1%, $p<0.001$), however, unlike $VO_{2max,}$ significant differences were observed between WBV and CCT ($p=0.003$)(Bogaerts et al., 2009). Though WBV training yields similar results regarding muscle strength and VO_{2max}, it requires several months (8-12) to show significant improvements (Bogaerts et al., 2009; Raimundo et al., 2009).

Another alternative training method, blood flow restriction (BFR) training has shown promise in populations with contraindications such as the elderly and post-operative individuals with a lesser time requirement (Hackney et al., 2018; Lambert et al., 2018). BFR is applied using special cuffs worn on the limbs that are inflated to a pressure high enough to occlude venous return but low enough to allow arterial inflow (Loenneke et al. 2014). This modality is reportedly highly anabolic and may induce muscular adaptations through previously discussed mechanisms like satellite cell differentiation and/or mechano-transduction (Hackney et al., 2018; Lambert et al., 2018). BFR can be combined with a variety of low intensity exercise modalities making it not only feasible, but also tolerable to a variety of individuals. For example, in adults ages 50-85 years, BFR has been combined with: lower body callisthenic exercises (Yokokawa et al., 2008), elastic band resistance training (Thiebaud et al., 2013; Yasuda et al., 2016; Yasuda et al., 2015), low load RT (Patterson & Ferguson, 2011; Shimizu et al., 2016; Vechin et al., 2015), and walking (Abe et al., 2010; Clarkson et al., 2017; Ozaki et al., 2010; Ozaki et al., 2011). Additionally, BFR has shown to be tolerable in healthy older adults as the RPE associated with walking with BFR recorded dropped from “somewhat hard” to “fairly light” after three weeks of training (Clarkson et al., 2017). Changes in muscular strength, size, and physical performance indicative of physical functioning have been examined comparing traditional high load resistance training(Cook et al., 2017; Karabulut et al., 2010; Kim et al., 2017; Patterson & Ferguson, 2011;

Shimizu et al., 2016; Vechin et al., 2015) and walk training (Abe et al., 2010; Clarkson et al., 2017; Ozaki et al., 2010; Ozaki et al., 2011) to low load resistance training with BFR from durations of four to twelve weeks. Following low load RT with BFR, Vechnin et al. (2015) reported increases in quadriceps CSA that were similar to those observed following traditional high load training (6% vs. 7%, respectively). Though significant increases in leg press strength were not observed in the BFR group, results were trending toward a 16% increase ($p=0.067$) (Vechin et al., 2015). In contrast, Karabulut et al. (2010) observed increases in leg press strength that were comparable to traditional RT (19% vs. 20%) but increases in knee extensor strength were significantly greater in the RT (19% vs. 31%).

Though improvements in muscle size and strength following BFR training with various exercise modalities is evident, less is understood about how this type of training affects physical functioning. To better understand this, Cook et al. (2017) compared muscle strength, size, physical function and quality of life in community dwelling adults over the age of 65 following lower body exercises combined with BFR, high-load (70% of 1-RM) RT, or low-load resistance upper body RT. High load training and BFR training resulted in greater improvements of 1-RM tests and CSA than those observed in the control group (Cook et al., 2017). However, when compared to BFR exercise, high load resistance training had significantly greater 1-RM in the leg press exercise (22.5kg vs. 7.3kg, $p<0.01$) and MVC strength (20.0Nm vs. -0.4Nm, $p<0.01$) with no significant differences in quadriceps CSA (1.65cm^2 vs. 1.97cm^2) after six weeks (Cook et al., 2017). After 12 weeks, only 1-RM in the leg extension exercise showed significant difference between HL and BFR (21.2kg vs. 9.1kg, $p<0.01$) suggesting that low load resistance training with BFR promotes similar adaptations to high-load resistance training (Cook et al., 2017). Additionally, results from this study suggest that six weeks of BFR training can promote

muscle growth and increased strength more than low-load training alone (Cook et al., 2017). Similar findings suggest that a mere four weeks of BFR training could increase muscle strength and size in young and old individuals (Kim et al., 2017; Patterson & Ferguson, 2011; Shimizu et al., 2016).

BFR training also has the potential to increase muscle oxygenation and the delivery of nutrients through vascular changes but more research is needed as the response may differ between young and older participants (Iida et al., 2011; J. Kim et al., 2017; Ozaki et al., 2010; Patterson & Ferguson, 2011; Shimizu et al., 2016; Yasuda et al., 2016; Yasuda et al., 2015). In addition, authors have suggested that there may be additional benefits regarding aerobic capacity, however, conflicting findings regarding post-training VO_{2max} indicate a need for more research on the topic (Abe et al., 2010; Ozaki et al., 2011). Clarkson et al. examined the effects of six weeks of walking with BFR on 30 second sit to stand, six minute walk distance, timed get up and go, and the number of steps completed in a modified queen's step test in inactive but healthy adults (Clarkson et al., 2017). Similar improvements in muscle size, strength and physical functioning following six and ten weeks of similar exercise protocols have also been reported (Abe et al., 2010; Ozaki et al., 2010; Ozaki et al., 2011). All muscular variables significantly increased over time and were significantly different from individuals who completed walk training without BFR indicating the usefulness of this alternative modality for aging populations (Abe et al., 2010; Ozaki et al., 2010; Ozaki et al., 2011).

BFR training has also been combined with more economical forms of low intensity resistive exercise (Thiebaud et al., 2013; Yasuda et al., 2016; Yasuda et al., 2015; Yokokawa et al., 2008). Yokokawa et al. (2008) compared the effects of body weight training with BFR to balance training in community dwelling older adults. Both programs promoted improvements in

reaction time, gait speed, and balance. BFR training showed significant improvements over balance training in TUG (15% vs. 5%) and knee extensor strength (20% vs. -4%)(Yokokawa et al., 2008). Yasuda et al. (2015, 2016) examined the response of muscular and vascular indices to 12 weeks of upper body and lower body elastic band training with BFR in elderly women and men. They observed significant changes in isometric strength (8-16% dependent on muscle group) and muscle size (7-17% dependent on muscle group) (Yasuda et al., 2016; Yasuda et al., 2015). Additionally, they observed similar results when comparing low intensity elastic band training with BFR to moderate-heavy elastic band training as trending increases (8%) and significant increases (16%) in knee extensor and leg press strength were reported (Yasuda et al., 2016). Similar findings in upper (5-15% dependent on muscle group) and lower body strength (8-40% dependent on muscle group) have also been reported (Thiebaud et al., 2013). However, when muscle size was examined via ultrasound derived muscle thickness and DXA lean body mass, only the pectoralis major showed significant increases (17%) (Thiebaud et al., 2013). Thus, though the exact mechanisms are unclear, BFR training is an efficient and reasonably quick acting alternative to traditional high load training for individuals that may have difficulty with increased weight loads, e.g., older adults.

Table 6

Alternative Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2008-2009

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Yokokawa et al. 2008	MS, P	44	72	Calisthenics w/ BFR	2x/wk, 8wks	TUG, LBS, RT, GS, B, HG	15%↓ TUG, 23%↑ LBS, 5% ↓ RT, 11%↑ GS, 16%↑ B, ↑↓ HG
Raimundo et al. 2009	MS, P	27	66	WBV	3x/wk, 8m	GS, CMJ, CS, LBS	6%↑GS, 13%↑CMJ, 4%↓CS, ↑↓LBS
Bogaerts et al. 2009	MS, P	180	67	WBV	3x/wk, 12m	VO_{2max}, LBS, TPE	18%↑$VO_{2max,}$ 9%↑TPE, 9%↑LBS

MM= muscle mass, MS= muscle strength, P=physical performance, WBV=whole body vibration, GS=gait speed, CMJ=countermovement jump, CS=chair stand, LBS=lower body strength, TPE=time to peak exercise, VO_{2max}= maximal volume of oxygen uptake, BFR= blood flow restriction, RT=resistance training, UBS=upper body strength, LBM=lean body mass, CSA=cross sectional area, MT=muscle thickness, RPE=ratings of perceived exertion, EHF=endothelial health factor, MVC=maximum voluntary contraction, CBF=circulatory blood flow, mQST=modified queen's step test, LG=leg girth, MV=muscle volume, MVO=maximum venous outflow, LVC=leg venous compliance, QOL=quality of life, MVC=maximum voluntary contraction, FG=forearm girth, FBF=peak forearm blood flow, FVC=forearm vascular conductance, FMD=flow mediated dilation, CAVI=cardio-ankle vascular index, ABI=ankle-brachial pressure index, RHI=reactive hyperemia index, vWF=von Willebrand factor, TM=thrombomodulin, $tcPO_2$= transcutaneous oxygen pressure, R_{bf}= resting blood flow, PO_{bf}= post occlusion blood flow, CCG= corrected calf girth, 6MWT=six minute walk test, CAC=carotid arterial complianc

Table 7

Alternative Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2010

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Age Range**	**Description**	**Frequency & Duration**	**DV**	**Results**
Karabulut et al. 2010	MS	37	50-64	RT w/BFR	3x/wk, 6wks	LBS, UBS	19%↑LBS, 9-23%↑UBS
Ozaki et al. 2010	MM, MS	23	57-76	Walking w/BFR	4x/wk, 10wks	CSA, LBS, CAC	3%↑CSA, 9-15%↑LBS, ↑↓ CAC
Abe et al. 2010	MM, MS, P	19	60-78	Walking w/BFR	5x/wk, 6 wks	LG, CSA, LBM, LBS, CS, TUG, VO2max	1-2%↑ LG, 5-6%↑ CSA, 6%↑ LBM,7-17%↑ LBS, ↑↓ VO2max, ~12%↑ TUG, ~15%↑ CS

MM= muscle mass, MS= muscle strength, P=physical performance, WBV=whole body vibration, GS=gait speed, CMJ=countermovement jump, CS=chair stand, LBS=lower body strength, TPE=time to peak exercise, VO2max= maximal volume of oxygen uptake, BFR= blood flow restriction, RT=resistance training, UBS=upper body strength, LBM=lean body mass, CSA=cross sectional area, MT=muscle thickness, RPE=ratings of perceived exertion, EHF=endothelial health factor, MVC=maximum voluntary contraction, CBF=circulatory blood flow, mQST=modified queen's step test, LG=leg girth, MV=muscle volume, MVO=maximum venous outflow, LVC=leg venous compliance, QOL=quality of life, MVC=maximum voluntary contraction, FG=forearm girth, FBF=peak forearm blood flow, FVC=forearm vascular conductance, FMD=flow mediated dilation, CAVI=cardio-ankle vascular index, ABI=ankle-brachial pressure index, RHI=reactive hyperemia index, vWF=von Willebrand factor, TM=thrombomodulin, tcPO2= transcutaneous oxygen pressure, Rbf= resting blood flow, PObf= post occlusion blood flow, CCG= corrected calf girth, 6MWT=six minute walk test, CAC=carotid arterial compliance

Table 8

Alternative Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2011-2013

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Age Range**	**Description**	**Frequency & Duration**	**DV**	**Results**
Patterson et al. 2011	MM, MS	10	64-70	RT w/ BFR	3x/wk, 4wks	MVC, Rbf, PObf, CCG, LBS	1%↑CCG, 11-17%↑ LBS, 18%↑MVC, ↑↓Rbf, ↑PObf
Ozaki et al. 2011	MM, MS, P	18	57-73	Walking w/BFR	4x/wk, 10wks	CSA, LG, MV, LBS, TUG, CS, VO2max	3%↑ CSA, 1%↑ LG, 3-4%↑ MV, 3-22%↑ LBS, 11%↑ TUG, 21%↑ CS, ~9% ↑ VO2max
Thiebaud et al. 2013	MM, MS	16	59-62	Band RT w/BFR	3x/wk, 8wks	UBS, LBS, MT, LBM	5-15%↑UBS, 8-40%↑LBS, 17%↑MT, ↑↓LBM

MM= muscle mass, MS= muscle strength, P=physical performance, WBV=whole body vibration, GS=gait speed, CMJ=countermovement jump, CS=chair stand, LBS=lower body strength, TPE=time to peak exercise, VO2max= maximal volume of oxygen uptake, BFR= blood flow restriction, RT=resistance training, UBS=upper body strength, LBM=lean body mass, CSA=cross sectional area, MT=muscle thickness, RPE=ratings of perceived exertion, EHF=endothelial health factor, MVC=maximum voluntary contraction, CBF=circulatory blood flow, mQST=modified queen's step test, LG=leg girth, MV=muscle volume, MVO=maximum venous outflow, LVC=leg venous compliance, QOL=quality of life, MVC=maximum voluntary contraction, FG=forearm girth, FBF=peak forearm blood flow, FVC=forearm vascular conductance, FMD=flow mediated dilation, CAVI=cardio-ankle vascular index, ABI=ankle-brachial pressure index, RHI=reactive hyperemia index, vWF=von Willebrand factor, TM=thrombomodulin, tcPO2= transcutaneous oxygen pressure, Rbf= resting blood flow, PObf= post occlusion blood flow, CCG= corrected calf girth, 6MWT=six minute walk test, CAC=carotid arterial compliance

Table 9

Alternative Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2015-2016

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Yasuda et al. 2015	MM, MS	17	61-85	Band RT w/BFR	2x/wk, 12wks	MVC, CSA, FMD, CAVI, ABI	8-16%↑MVC, 17%↑CSA, ↑↓ FMD, ↑↓CAVI, ↑↓ABI
Vechin et al. 2015	MM, MS	23	59-71	RT w/ BFR	2x/wk, 12wks	LBS, CSA	↑↓ LBS, 6%↑ CSA
Yasuda et al. 2016	MM, MS	30	61/86	Band RT w/BFR	2x/wk, 12 wks	MVC, CSA, LBS FMD, CAVI, ABI	14%↑MVC, 7%↑CSA, 8-16% ↑LBS, ↑↓ FMD, ↑↓CAVI, ↑↓ABI
Shimizu et al. 2016	MS	40	>65	RT w/BFR	3x/wk, 4wks	UBS, LBS, RHI, vWF, TM, $tcPO_2$	9%↑UBS, 11-19%↑LBS, ↑ RHI, ↓vWF, ↑↓TM, ↑$tcPO_2$

MM= muscle mass, MS= muscle strength, P=physical performance, LBS=lower body strength, BFR= blood flow restriction, RT=resistance training, UBS=upper body strength, CSA=cross sectional area, MT=muscle thickness, RPE=ratings of perceived exertion, EHF=endothelial health factor, MVC=maximum voluntary contraction, CBF=circulatory blood flow, mQST=modified queen's step test, LG=leg girth, MV=muscle volume, MVO=maximum venous outflow, LVC=leg venous compliance, QOL=quality of life, MVC=maximum voluntary contraction, FG=forearm girth, FBF=peak forearm blood flow, FVC=forearm vascular conductance, FMD=flow mediated dilation, CAVI=cardio-ankle vascular index, ABI=ankle-brachial pressure index, RHI=reactive hyperemia index, vWF=von Willebrand factor, TM=thrombomodulin, $tcPO_2$= transcutaneous oxygen pressure, R_{bf}= resting blood flow, PO_{bf}= post occlusion blood flow, CCG= corrected calf girth, 6MWT=six minute walk test, CAC=carotid arterial compliance

Table 10

Alternative Training Studies to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2017

				Intervention			
Reference	**Sarcopenic Criteria**	**N**	**Age Range**	**Description**	**Frequency & Duration**	**DV**	**Results**
Clarkson et al. 2017	P	19	60-80	Walking w/BFR	4x/wk, 6wks	STS, TUG, 6MWT, mQST RPE	28%↑ STS, 9%↑ 6MWT, 12%↓ TUG, 80%↑mQST, 21%↓RPE (by wk 3)
Cook et al. 2017	MM, MS, P	36	76	RT w/BFR	2x/wk, 12wks	LBS, CSA, GS, SPPB, QOL, MVC	6wks: 12-24%↑ LBS,4%↑ CSA,↑↓MVC,↓↑ GS, ↑↓SPPB, ↓↑QOL 12wks: 25%↑ LBS, 7%↑ CSA,↑↓ GS, ↓↑SPPB, ↑↓QOL
Kim et al. 2017	MM, MS	27	Y: 19-25 O: 60-80	Grip Training w/BFR	3x/wk, 4wks	MVC, FG, FBF, FVC	Y: 16%↑ MVC, 5%↑ FG, 56%↑ FBF, 64%↑ FVC O: 8% ↑ MVC, 2%↑ FG, ↑↓ FBF, ↑↓ FVC

MM= muscle mass, MS= muscle strength, P=physical performance, GS=gait speed, LBS=lower body strength, VO_{2max}= maximal volume of oxygen uptake, BFR= blood flow restriction, RT=resistance training, UBS=upper body strength, LBM=lean body mass, CSA=cross sectional area, MT=muscle thickness, RPE=ratings of perceived exertion, EHF=endothelial health factor, MVC=maximum voluntary contraction, CBF=circulatory blood flow, mQST=modified queen's step test, LG=leg girth, MV=muscle volume, MVO=maximum venous outflow, LVC=leg venous compliance, QOL=quality of life, MVC=maximum voluntary contraction, FG=forearm girth, FBF=peak forearm blood flow, FVC=forearm vascular conductance, FMD=flow mediated dilation, CAVI=cardio-ankle vascular index, ABI=ankle-brachial pressure index, RHI=reactive hyperemia index, vWF=von Willebrand factor, TM=thrombomodulin, $tcPO_2$= transcutaneous oxygen pressure, R_{bf}= resting blood flow, PO_{bf}= post occlusion blood flow, CCG= corrected calf girth, 6MWT=six minute walk test, CAC=carotid arterial compliance

2.3.3. Nutritional and Pharmaceutical Interventions

2.3.3.1. Dietary Interventions

In the United States, the Food and Nutrition Board of the Institute of Medicine has issued the Recommended Dietary Allowance (RDA) to help provide safe and effective guidelines for dietary intake (Nutrient Recommendations: Dietary Reference Intakes [DRI], 2015). The RDA is a guideline of the average daily level of intake sufficient to meet the nutrient requirements of 97-98% of healthy people, divided by age and gender (DRI, 2015). However, research suggests that the RDA for protein is not high enough to mitigate sarcopenia (Bauer et al., 2013; Campbell et al., 2001; Ferrando et al., 2010; Naseeb & Volpe, 2017; Scott et al., 2010; Strasser et al., 2018). Campbell et al. (2001) reported significant decreases in the quadriceps CSA of healthy older people consuming a lacto-ovo-vegetarian diet meeting the current RDA for protein. Energy adjusted protein intake has also been positively associated with appendicular lean mass (aLM) over 2.6 years in a prospective cross-sectional study (Scott et al., 2010) and has led to improved physical function after 12-weeks of supplementation (Kim & Lee, 2013). Supplementation with eight grams of once daily amino acids, including: leucine, lysine, isoleucine, valine, threonine, cysteine, histidine, phenylalanine, methionine, tyrosine, and tryptophan, led to muscle protein synthesis increase and maintenance in sarcopenic individuals as measured by improvements in IGF-1, reductions in catabolic biomarkers, and improved lean body mass (Solerte et al., 2008). Similarly, supplementation with 15 grams of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine, and arginine maintained fractional synthetic rate, a marker for MPS, and muscle function in moderately active older adults (Ferrando et al., 2010). Furthermore, increased dietary protein has led to improved insulin sensitivity and increased lean body mass (Solerte et al., 2008; Yoshimura et al., 2018). Several researchers have examined increased

protein (1.0-2.0 g·kg·day^{-1}) and amino acid, particularly leucine (2.5-2.8 g·day^{-1}), intake as a means to maintain or increase muscle size, strength, and function (Bauer et al., 2013; Naseeb & Volpe, 2017; Strasser et al., 2018). Calcium beta-hydroxy-beta methylbutyrate (HMB) is a metabolite of leucine and therefor may alter muscle protein synthesis through similar mechanisms (Deutz et al., 2013). Creatine may provide additional help to mitigate sarcopenia by altering muscle protein turnover through previously discussed pathways (Evans et al., 2017) and mechanisms (Tarnopolsky et al., 2007). Though the role of diet in muscle mass maintenance and development is not well understood, supplemental protein, essential amino acids (EAA), HMB, creatine, and L-carnitine have shown effectiveness in improving strength and function (Tables 11-14).

Evans et al. (2017) explored the effects of dietary supplements on sarcopenia by comparing a once daily cocktail containing 1.5g of L-Carnitine, 2.0g of L-Leucine, and 3.0g of creatine monohydrate to 1.5g of L-Carnitine and a placebo. They observed significant increases of approximately one kilogram in DXA derived lean body mass and lower body strength (Evans et al., 2017). Additionally, they observed that L-Carnitine alone helped to maintain composite score, which accounts for losses in muscle mass and size and physical activity, while the cocktail group improved by 63.5% (Evans et al., 2017). Though Evans et al. (20017) examined a supplement containing creatine monohydrate, Stout et al. (2007) examined two weeks of supplementation with di-creatine citrate in community dwelling individuals with a mean age of 74.5 years. Handgrip strength, which has shown strong correlations to leg strength and absolute strength (Trosclair et al., 2011), increased significantly following supplementation (Stout et al., 2007). These results suggest that various forms and assumed minimum effective doses of creatine may provide more benefit to attenuating the loss of muscle strength associated with

aging than L-Carnitine. Another promising supplement, HMB, has shown to preserve lean mass in older adults following 10 days of bedrest (5% loss in control group vs. 0% loss with supplementation) (Deutz et al., 2013). Baier et al. (2009) showed small but significant increases in LBM following one year of supplementation with a cocktail of 2.0-3.0g of HMB, 5.0-7.5g L-arginine, and 1.5-2.25g of L-lysine as determined by body weight of elderly men and women. However, no differences between groups were observed in physical performance or function variables. Additionally, handgrip strength decreased in both groups over time. In contrast, Flakoll et al. (2004) showed increases in performance, strength, and bioelectrical impedance analysis derived lean body mass using a similar supplement for 12 weeks in elderly women. Similar observations have also been reported after 24 weeks of supplementation with HMB (Stout et al., 2013). Significant decreases in TUG score and increases in DXA derived lean mass of ~2% were observed in combination with increased lower body strength and muscle quality at 180°/second were reported in the seventh decade of life (Stout et al., 2013). Interestingly, only the differences in muscle strength and quality were statistically significant between groups (Stout et al., 2013). However, the practical significance between the differences in TUG between the placebo and supplement group (-4% vs. -9%, respectively) should not be ignored (Stout et al., 2013). Though these findings are promising, few studies have examined the use of HMB as a countermeasure for sarcopenia.

Dietary protein and amino acids are widely researched nutritional interventions for older adults, the elderly, and the frail. Even so, the differences in the interventions, population studied, and reported results combined with the multifaceted nature of sarcopenia make determining an individual plan of care for its treatment and/or prevention difficult. For example, Tieland et al. (2012) increased the dietary protein of 65 frail septuagenarians from 1.0 $g \cdot kg \cdot day^{-1}$ to 1.4

$g \cdot kg \cdot day^{-1}$ using a 15g milk protein supplement and observed no significant changes in DXA derived lean body mass. Improvements in lower body strength and SPPB were observed following supplementation (Tieland et al., 2012). Trending differences between the supplement and placebo groups in knee extensor strength (19% vs. 11%, p=0.059) and significant differences in SPPB values (12% vs. 0%, p=0.02) have also been observed (Tieland et al., 2012). Similarly, smaller decreases in habitual gait speed (1% vs. 11%, p=0.039), the maintenance of SPPB values (compared to -13%, p=0.039), and significant improvements in timed get up and go (p=0.38) have been reported following 12 weeks of supplementation with a cocktail containing 25g of protein and 9.4g of essential amino acids primarily consisting of leucine (Kim & Lee, 2013). These results combined indicate practical significance of the use of protein supplementation and increased protein intake to improve quality of life, but not lean mass, in individuals with poor or decreasing muscular health. In contrast, Bauer et al. (2015) supplemented the diets of non-protein-energy malnourished elderly participants twice per day with either a cocktail containing 20g of whey protein and 3g of leucine or an isocaloric placebo. Handgrip strength, gait speed, aLM, and SPPB score increased while chair stand time decreased significantly after 13 weeks. However, only aLM and chair stand time differed between groups (~2% vs. ~0% and -15% vs. -7%, respectively). More recently, Chanet et al. (2017) showed acute stimulation of MPS, an increase of ~2% aLM, and no significant differences in muscle strength or function after six weeks of supplementation with whey protein, once per day, prior to breakfast.

Solerte et al. (2008) observed significant increases in DXA derived LBM of sarcopenic adults following 8-16 months of twice per day supplementation with a cocktail containing 2.5g of L-leucine. Interestingly, no significant differences in mean LBM were reported between sarcopenic and age-matched nonsarcopenic individuals following supplementation (Solerte et al.,

2008). These data suggest that leucine can alter sarcopenic status (Solerte et al., 2008). Recently, major improvements in functional independence, LBM, and grip strength have been observed in sarcopenic stroke patients following eight weeks of supplementation with a cocktail containing 3 g of leucine (Yoshimura et al., 2018). Both the supplement and the control group participated in the same rehabilitation program had a median protein intake per day was above the RDA (1.4 $g \cdot kg^{-1}$and 1.3 $g \cdot kg^{-1}$, respectively)(Yoshimura et al., 2018). However, these findings were not observed in moderately active adults consuming 0.8 $g \cdot kg^{-1}$ of protein per day supplemented with 15g of EAAs (5.4g of which were L-leucine) during 10 days of bedrest (Ferrando et al., 2010). DXA derived LBM decreased in the supplement (-2%) and placebo (-3%) groups to a similar but not significant extent (Ferrando et al., 2010). Losses in physical function, however, appeared to be mitigated in the supplement group (Ferrando et al., 2010). For example, stair ascent power and descent power decreased and floor transfer time increased significantly more in the placebo versus the supplemented group (-14% vs. -2%, -21% vs. -8%, 51% vs. 13%, respectively) (Ferrando et al., 2010).

The timing of nutritional intervention may also play a role in its effectiveness. For instance, Bouillanne et al. (2013) compared the effects of 6 weeks of pulse feeding (e.g., 78% of daily protein in one meal) and spread feeding (e.g., nearly equal distribution across four meals) on skeletal muscle mass, strength and performance of activities of daily living in 66 hospitalized octogenarians. They observed significant higher increases of DXA derived LBM (3%↑ vs. 1%↓, $p = 0.011$) and SMI (9%↑ vs. 3%↓, $p=0.047$) in the pulse feeding group with no differences in grip strength (Bouillanne et al., 2013). Though both groups consumed 1.5 $g \cdot kg \cdot day^{-1}$ of protein, the pulse feeding group received 78% of their protein and 16g of EAAs at noon whereas the spread group only consumed 7g of EAAS and 20% of their protein intake suggesting that not

only intake amounts, but also feeding strategies, may play a key role in sarcopenia interventions (Bouillanne et al., 2013). The effect of feeding strategies in the maintenance and development of strength is less clear. Recent findings from a study examining 1.1 $g \cdot kg \cdot day^{-1}$ of protein intake using a three meal per day spread strategy or a pulse feeding strategy in which 65% of the daily protein intake was consumed at dinner show an increase in knee extensor strength in the pulse group, which the authors attributed to learned effect of 1-RM testing (Kim et al., 2018). Changes in grip strength, muscle mass, and performance were not reported for either group (Kim et al., 2018). Additionally, Kim et al. (2018) did not report the amino acid intakes during feeding making it unclear whether the findings were due to a less than optimal pulse timing, inadequate leucine content, or a lack of protein influence all together.

Table 11

Nutrition Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2001-2009

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Campbell et al. 2001	MM, MS	10	66	RDA	3 meals/day, 14 wks	LBM, CSA, UBS, LBS	↑↓LBM, 2%↓ CSA, ↑↓ UBS, , ↑↓ LBS
Flakoll et al. 2004	MM, MS, P	23	77	CaHMB/Arg/Lys cocktail	1x/day, 12wks (AM)	TUG, LBS, HG, LBM	~18% ↑TUG, 7%↑ LBS, 3%↑HG, 2%↑LBM
Solerte et al. 2008	MM	41	75	EAA	2x/day, 16m	LBM	~4-9% ↑ LBM
Baier et al. 2009	MM, MS, P	77	76	CaHMB/Arg/Lys cocktail	1x/day, 1 yr (AM)	LBM, UBS, LBS, HG, TUG	1% ↑ LBM, ↑↓ UBS, ↑↓ LBS, ↓HG, ↑↓ TUG

MM= muscle mass, MS= muscle strength, P=physical performance, CaHMB= calcium beta-hydroxy-beta-methylbutyrate, Arg=L-arginine, Lys= L-lysine, WP=whey protein, L=leucine, CHO=carbohydrate, D=vitamin D, SPBB= short physical performance battery, HG= handgrip strength, LBM=lean body mass, LBS=lower body strength, UBS= upper body strength, TUG= timed get up and go, CS=chair stand, GS=gait speed, aLM= appendicular lean mass, L-C=L-Carnitine, Cr=Creatine Monohydrate, 6MWT= six minute walk test, Cscore=composite score, CrC=Di-Creatine Citrate, PWCFT= physical working capacity at fatigue, EAA=essential amino acids, SC=stair climb, FTT=floor transfer time, FIM= functional independence measure, SMI= skeletal muscle mass index, PPD=pulse protein diet, ADL=activities of daily living, RDA=recommended dietary allowance, PRO=protein supplement

Table 12

Nutrition Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2010-2013

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Ferrando et al. 2010	MM,MS, P	22	71	EAA	3x/day, 10d	LBM, SC, FTT, LBS	↑↓ LBM, ↓2-8% SC, 13%↑ FTT
Tieland et al. 2012	MM, MS, P	65	78	15g PRO	2x/day, 24wks	LBM, LBS, HG, SPPB	↑↓ LBM, ↑↓HG, 15-19%↑ LBS, 12%↑ SPPB
Deutz et al. 2013	MM, MS, P	24	67	CaHMB	2x/day, 10d	LBM, LBS, SPPB, TUG	↑↓ LBM, ↑↓ LBS, ↑↓ SPPB, ↑↓TUG
Stout et al. 2013	MM, MS, P	43	73	CaHMB	3x/day, 24wks	LBM, HG, LBS, TUG, MQ	2%↑ LBM, ↑↓ HG, 10%↑ LBS, 9%↓ TUG, ↑MQ

MM= muscle mass, MS= muscle strength, P=physical performance, CaHMB= calcium beta-hydroxy-beta-methylbutyrate, Arg=L-arginine, Lys= L-lysine, WP=whey protein, L=leucine, CHO=carbohydrate, D=vitamin D, SPBB= short physical performance battery, HG= handgrip strength, LBM=lean body mass, LBS=lower body strength, UBS= upper body strength, TUG= timed get up and go, CS=chair stand, GS=gait speed, aLM= appendicular lean mass, L-C=L-Carnitine, Cr=Creatine Monohydrate, 6MWT= six minute walk test, Cscore=composite score, CrC=Di-Creatine Citrate, PWCFT= physical working capacity at fatigue, EAA=essential amino acids, SC=stair climb, FTT=floor transfer time, FIM= functional independence measure, SMI= skeletal muscle mass index, PPD=pulse protein diet, ADL=activities of daily living, RDA=recommended dietary allowance, PRO=protein supplement

Table 13

Nutrition Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2013-2017

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Kim et al. 2013	P	87	78	25g PRO/EAA	2x/day, 12wks	SPPB, GS, TUG, B, HG	↑↓ SPPB, 1%↓ GS,7%↑ TUG, ↑↓B, ↑↓HG,
Bouillanne et al. 2013	MM, MS	66	84	PPD	1.5g/kg/day, 6wks	LBM,SMI, HG, ADL	3%↑ LBM, 9%↑ SMI, ↑↓HG, ↑↓ ADL
Bauer et al. 2015	MM, MS, P	380	77	WP, L,CHO, D cocktail	2x/day, 13wks	HG, CS, GS, B aLM, SPPB	4% ↑ HG,15%↓ CS, 9%↑ GS,↑↓B, ~2%↑LBM 12%↑ SPPB
Chanet et al. 2017	MM, MS, P	24	71	WP, L,CHO, D cocktail	1x/day, 6wks	aLM,	~2%↑ aLM,
Stout et al. 2017	MS, P	15	75	CrC	2-4x/day, 2wks	CS, HG, PWCFT	↑↓ CS, 7%↑ HG, 16%↑ PWCFT

MM= muscle mass, MS= muscle strength, P=physical performance, CaHMB= calcium beta-hydroxy-beta-methylbutyrate, Arg=L-arginine, Lys= L-lysine, WP=whey protein, L=leucine, CHO=carbohydrate, D=vitamin D, SPBB= short physical performance battery, HG= handgrip strength, LBM=lean body mass, LBS=lower body strength, UBS= upper body strength, TUG= timed get up and go, CS=chair stand, GS=gait speed, aLM= appendicular lean mass, L-C=L-Carnitine, Cr=Creatine Monohydrate, 6MWT= six minute walk test, Cscore=composite score, CrC=Di-Creatine Citrate, PWCFT= physical working capacity at fatigue, EAA=essential amino acids, SC=stair climb, FTT=floor transfer time, FIM= functional independence measure, SMI= skeletal muscle mass index, PPD=pulse protein diet, ADL=activities of daily living, RDA=recommended dietary allowance, PRO=protein supplement

Table 14

Nutrition Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2017-2018

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Evans et al. 2017	MM, MS, P	42	61	L-C, CrM, L, D cocktail or L-C	1x/day, 8wks (AM)	LBM, LBS, UBS, 6MWT, Cscore	Cocktail:↑ LBM,↑ LBS, ↑↓ UBS, ↑↓ 6MWT, 64%↑ Cscore, L-C:↑ LBM, ↑↓ LBS, ↑↓ UBS, ↑↓ 6MWT, ↑↓ Cscore,
Yoshimura et al. 2018	MM, MS, P	44	80	EAA	1x/day, 8wks	FIM, SMI, HG	44-148%↑ FIM, 74%↑ HG, 9%↑ SMI
Kim et al. 2018	MM, MS, P	14	59	PPD	1.1 g/kg/day, 8wks	LBM, LBS, HG, CS, SC, GS	↑↓LBM, 23%↑ LBS, ↑↓ HG, ↑↓CS, ↑↓ SC, ↑↓ GS

MM= muscle mass, MS= muscle strength, P=physical performance, CaHMB= calcium beta-hydroxy-beta-methylbutyrate, Arg=L-arginine, Lys= L-lysine, WP=whey protein, L=leucine, CHO=carbohydrate, D=vitamin D, SPBB= short physical performance battery, HG= handgrip strength, LBM=lean body mass, LBS=lower body strength, UBS= upper body strength, TUG= timed get up and go, CS=chair stand, GS=gait speed, aLM= appendicular lean mass, L-C=L-Carnitine, Cr=Creatine Monohydrate, 6MWT= six minute walk test, Cscore=composite score, CrC=Di-Creatine Citrate, PWCFT= physical working capacity at fatigue, EAA=essential amino acids, SC=stair climb, FTT=floor transfer time, FIM= functional independence measure, SMI= skeletal muscle mass index, PPD=pulse protein diet, ADL=activities of daily living, RDA=recommended dietary allowance, PRO=protein supplement

2.3.3.2. Pharmaceutical Interventions

Though exercise and nutrition are effective, and even considered the gold standard, in mitigating and preventing sarcopenia, researchers have explored pharmaceuticals in the treatment of related muscle wasting diseases (i.e. cachexia related to cancer, HIV, and AIDS) (Anker et al., 2013; Morley et al., 2014; Springer et al. 2013) . With the high prevalence and disability cost associated with these diseases, testosterone, growth hormone, dihydroepiandrosterone (DHEA), selective androgen receptor molecules (SARMs), and cannabinoids have been investigated for the treatment of cachexia (Anker et al., 2016; Morley et al., 2014). Inadequate energy intake has been associated with increased sarcopenia in men and women (Jang & Bu, 2018). Some appetite stimulants, like cannabinoids, have been examined to treat cachexia associated with cancer, HIV, and AIDS (DeJesus et al., 2007; Strasser et al., 2006; Turcott et al., 2018). Dronabinol, a synthetic oral form of delta-9-tetrahydrocannabinol (THC), has shown to increase body weight and appetite with a low incidence of non-life threatening adverse events (5-8%) in HIV/AIDS patients (DeJesus et al., 2007). Niabolone, a similar oral form of THC, maintained average energy intake and improved self-reported health related quality of life in regard to role functioning, emotional, social and insomnia, but not physical functioning, in cancer patients (Turcott et al., 2018). In another study, no significant differences between cancer patients ingesting cannabis extract, THC, or a placebo were observed in body weight, appetite, or quality of life after six weeks (Strasser et al., 2006). However, THC has shown to be well tolerated and effective in promoting weight gain in individuals over 75 years of age (Wilson et al., 2007). This appears to be the only study to date examining the effect of cannabis on any form of body mass in older adults, and though the results of this small study are

somewhat promising, more clinical evidence is required to consider this as an appropriate intervention to treat sarcopenia.

Other pharmaceuticals, such as testosterone (Chapman et al., 2009; Sih et al., 1997; Storer et al., 2017; Wittert et al., 2003), growth hormone (Harman & Blackman, 2003), and DHEA (Villareal & Holloszy, 2006), have been shown to increase lean mass, strength, and performance in middle-aged to older individuals. Testosterone has led to increases in lean mass and reduced hospitalization in undernourished, community dwelling older men and women (Chapman et al., 2009). However, changes in grip strength were not observed (Chapman et al., 2009). In contrast, grip strength increased significantly more than the placebo group following one year of bi-monthly injections in middle-aged, hypogonadal men (Sih et al., 1997). In older males, one year of oral testosterone ingestion led to 2% increases in lean mass with no significant increases in strength (Wittert et al., 2003). Similar increase in lean body mass and increases in upper body strength and power and lower body power were observed in 61-73 year old men were observed following 3 years of using testosterone gel (Storer et al., 2017). Lean body mass increased by 3% following 1 year of using testosterone gel with 3 days per week of progressive resistance training (Hildreth et al., 2013). No significant differences were observed in physical function or strength between those using testosterone or placebo gels (Hildreth et al., 2013). Participants did report more serious adverse events when using testosterone (Hildreth et al., 2013). Harman et al. (2003) examined sex hormones alone, or combined with growth hormone, in older men and women. Trending increases in lean body mass were observed in the testosterone treatment group while significant increases were observed in the growth hormone (~5-6% in men and women), growth hormone and testosterone (~8%), oestrogen/progestin and growth hormone (~6%) groups (Harman & Blackman, 2003). Strength only increased in men

with growth hormone and testosterone (7%) (Harman & Blackman, 2003). This study and others have shown mild to serious adverse events following the growth hormone interventions making the risk: benefit ration difficult to determine (Cohn et al., 1993; Harman & Blackman, 2003). DHEA with and without full body resistance training was examined in 64 elderly men and women (Villareal & Holloszy, 2006). Thigh muscle volume, upper body strength measured by chest press, lower body strength measured by leg press and knee extension, and isometric knee extension strength were significantly higher in the DHEA and placebo groups following exercise and were significantly greater in the DHEA group (Villareal & Holloszy, 2006). It is also noteworthy that no adverse events were reported during this study indicating that if pharmaceuticals are to be used to mitigate sarcopenia, DHEA is a safe and effective choice that can be made more potent with exercise (Villareal & Holloszy, 2006).

In 2015, the ghrelin agonist, anamorelin, and the SARM, enobosarm, progressed to Phase III clinical testing in humans (Anker et al., 2013). In a Phase II trial of enobosarm, individuals taking 1mg/day of the drug for 86 days showed trending differences in lean body mass increase ($p=0.055$) and fat mass (FM) decrease ($p=0.085$) when compared to those taking a placebo (Dalton et al., 2011). Results were significantly different at a higher dose of 3mg/day (LBM:$p<0.001$; FM:$p=0.049$) (Dalton et al., 2011). Additionally, no serious adverse events were reported during this study (Dalton et al., 2011). Findings like these have led to the development of two Phase III studies, the POWER trials, to evaluate the safety and efficacy of the drug on cachexia in non-small cell lung cancer patients (Crawford et al., 2016). Though the final results of physical performance and LBM have not yet been published, the NIH clinical trial report shows a lower incidence of serious adverse events with enobosarm vs. with the placebo (35% vs. 37%, respectively)(NIH, 2011). Findings regarding anamorelin are further along with three phase

III studies completed (Currow et al., 2017; Temel et al., 2016). The first two studies, ROMANA 1 and 2, showed greater median increase in LBM (4% vs. 0%) ad aLM (4% vs. 2%) in the treatment group compared to the placebo group($p<0.001$) (Temel et al., 2016). Some adverse events were reported with hyperglycemia (16%), nausea (12%), and diabetes (3%) as the most reported (Temel et al., 2016). In ROMANA 3, treatment with anamorelin resulted in the maintenance of body weight, despite treatment with chemotherapy, and low incidence (0-12%) of drug-related treatment-emergent adverse events (Currow et al., 2017). Despite the effectiveness and tolerability of anamorelin, these drugs require further investigation and have yet to be approved by the U.S. food and drug administration (FDA, 2018; Dunne et al., 2017; Garber, 2016; Graf & Garcia, 2017; Meyer, 2017).

2.3.4. Combined Interventions

2.3.4.1. Diet and Exercise

The effects of various supplements and dietary changes combined with low-high intensity physical exercise has been heavily examined in the literature over the last decade (Tables 15-18). Protein supplementation with resistance training has shown to increase anabolism through mTORC1 phosphorylation more than resistance training alone (Scaroni et al., 2018). Our lab has previously shown that protein intake, concurrent training, age, and gender are associated with changes in muscle size ($R^2=0.725$, $p<0.01$)(Stone et al., 2018). However, when examining the relationships between these variables and knee extensor peak torque, gender, age, and concurrent training remained related but total daily protein intake was removed from the model ($R^2=0.631$, $p<0.01$) (Stone et al., 2018). Additionally, acute responses to a combination of resistance training and whey protein or resistance training and three grams of essential amino acids containing ~ 1.2 grams of leucine have been observed in women over 65 (Bukhari et al., 2015). This combination

of exercise and nutrition stimulated microvascular blood flow regardless of nutrition intervention (Bukhari et al., 2015). Additionally, the nutrition interventions alone stimulated MPS for up to two hours where the nutrition interventions combined with exercise stimulated MPS for up to four hours indicating the usefulness of combined exercise and nutrition in the mitigation and possibly treatment of sarcopenia (Bukhari et al., 2015).

These data are supported by recent work showing significantly higher increases in DXA derived LBM and aLM following traditional resistance training combined with a low-dose (10.5 $g \cdot day^{-1}$) protein supplementation compared to participants completing resistance training only (Seino et al., 2018). However, in a recent study of 70 year old males, a twice daily bolus of 20g of leucine-enriched whey protein failed to increase lower body strength (20% vs. 18%), aLM(2% vs. 4%), and quadriceps CSA (6% vs. 8%) more than resistance training alone (Holwerda et al., 2018). Studies examining exercise and the leucine metabolite, HMB, more so convolute this understanding as the two components have showed promise separately but no added benefit is present when the interventions are combined (Stout et al., 2013; Vukovich et al., 2001). Still, recent studies have reported greater significant changes following resistance training and protein supplementation in SMI, LBM, appendicular mass and knee extensor strength than resistance training without additional protein indicating a need for more research on these combined interventions (Mori & Tokuda, 2018; Tieland et al., 2012).

The addition of approximately 0.1g creatine monohydrate per kg of body weight in combination with resistance exercise has been shown to improve DXA derived lean body mass, knee extensor, leg press, and plantar flexor strength in older men and women (Brose et al., 2003; Chrusch et al., 2001). However, conflicting findings have been reported following one year of concurrent training with creatine monohydrate supplementation as any increases in muscle mass,

strength, or aerobic fitness were attributed to the training regimen only (Eijnde et al., 2003). When creatine is combined with protein the results are inconclusive. For example, Candow et al. (2008) reported significantly higher lean mass and relative bench press strength with a combination of creatine monohydrate and protein than with creatine or a placebo (LBM: 6% vs. 2% vs. 1%; UBS:25% vs. 12% vs. 13%, respectively). Additionally, muscle thickness was greater in the creatine supplemented groups than the placebo group (Candow et al., 2008). These findings were not supported in men completing progressive resistance training combined with placebo, 5g of creatine monohydrate, 35g whey protein, and 5g of creatine monohydrate/ 35g of whey protein ingestion as significant time, but not group, effects were observed (Bemben et al., 2010).

Knowledge of how alternative training methods combined with protein supplementation effect muscle health and function in older adults is extremely limited. Thus, potential benefits are indicated but convoluted. In one study, frail individuals increased energy and protein intake by 20% and 30%, respectively in combination with a nine month long moderate exercise regimen using bands and dumbbells (Bonnefoy et al., 2003). After 3 months, increases in muscular power were observed in the supplement group with trending differences in doubly labelled water derived lean body mass ($p=0.09$) (Bonnefoy et al., 2003). There were no significant differences in other performance variables and differences did not remain significant after nine months of training (Bonnefoy et al., 2003). In another study, time effects for lower body strength and physical performance were observed in older adults who ingested a leucine-enriched protein supplement and completed “moderate” resistance training using bands (Oesen et al., 2015). Significantly higher chair stand scores following resistance training and/or resistance training with supplementation when compared to cognitive therapy were reported, however, there was no

added benefit of the supplementation (Oesen et al., 2015). In sarcopenic octogenarians, twice daily supplementation with three grams of an essential amino acid cocktail containing a high percentage of leucine (48%) in combination with moderate resistance, gait, and balance training showed significantly higher leg mass, habitual gait speed, maximum gait speed, and knee extensor strength when compared to similar individuals completing the same exercise regimen (Kim et al., 2012). However, changes in gait speeds were likely due to the exercise and not the amino acid supplementation (Kim et al., 2012). A similar regimen combined with a whey protein and micronutrient supplement led to significant increases in lean body mass, relative skeletal muscle mass, grip strength, and activities of daily living scores that were significantly higher than those observed in the placebo group partaking in the same exercises (Rondanelli et al., 2016). The increases in relative skeletal muscle mass and grip strength due to supplementation improved 68% of the individuals' sarcopenic status suggesting that moderate intensity exercise combined with protein supplementation can not only mitigate, but also reverse sarcopenia (Rondanelli et al., 2016). Additional studies examining novel combinations of exercise and protein as interventions are slowly emerging in the literature (Daly et al., 2015; Vojciechowski et al., 2018). For example, promising findings of improved strength, rate of force development, and physical function following the use of "exergames", like Nintendo's Wii Fit Plus, have led to the development of a protocol to examine their use with a leucine enriched protein supplement in older adults (Jorgensen et al., 2013; Vojciechowski et al., 2018). Additionally, a study yielding comparable results in the knee extensor and plantar flexor cross sectional area between adults in a BFR exercise combined with 16 g of dairy protein group and adults in a traditional resistance training group has led to the development of a study examining

the combination of BFR and blended protein supplementation in older adults (Hackney et al., 2016). These progressions indicate a novel niche for research.

Table 15

Combined Dietary and Exercise Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2001-2003

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Chrusch et al. 2001	MM, MS	30	70	RT + CrM	3x/wk, 12 wks	LBM, UBS, LBS	6%↑ LBM,↑↓ UBS, ~4%↑ LBS
Vukovich et al. 2001	MM, MS	31	70	RT + CaHMB	2x/wk, 8wks (tid)	UBS, LBS, LBM	↑↓ UBS, ↑↓ LBS,↑↓ LBM
Brose et al. 2003	MM, MS, P	28	69	RT + CrM	3x/wk, 14wks	LBM, LBS, HG, CS, GS, SC	3-6%↑ LBM, 18-46%↑ LBS, ↑↓ HG, 25%↑ CS, ↑GS, 15%↓ SC

MM= muscle mass, MS= muscle strength, P=physical performance, RT= resistance training, DP=dairy protein, MiN=micronutrients, LBM=lean body mass, aLM=appendicular lean mass, GS=gait speed, TUG=timed get up and go, CS=chair stand, WP=whey protein, P-Ex=post-exercise, h.s.=bedtime, LBS=lower body strength, CSA=cross sectional area, Leu-E=leucine enriched, HG= handgrip strength, 6MWT=six minute walk test, TBD=to be determined, CrM=creatine monohydrate, UBS= upper body strength, E=energy, SC=stair climb, LBP=lower body power, IMAT=intramuscular adipose tissue, MT= muscle thickness, CT=concurrent training, VO_{2peak}= peak oxygen uptake, bid=twice daily, LM=leg mass, PRO=protein supplement, SMI=skeletal muscle mass index, QD=once daily, RSMM=relative skeletal muscle mass, ADL=activities of daily living, CaHMB= calcium beta-hydroxy-beta methylbutyrate, tid=three times daily

Table 16

Combined Dietary and Exercise Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2003-2008

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Bonnefoy et al. 2003	MM, P	57	83	RT+ PRO + E	2x/day, 9m (AM & PM)	LBM, GS, SC, CS, LBP	**3m:**↑↓ LBM, ↑↓GS, ↑↓SC, ↑↓CS, 57%↑ LBP **9m: :**↑↓ LBM, ↑↓GS, ↑↓SC, ↑↓CS, ↑↓LBP
Eijnde et al. 2003	MM, MS, P	46	65	CT + CrM	**Sup:** 3x/day or 2x/day w/training **Ex:**2-3x/wk, 12m	VO_{2peak}, LBS, LBM	**6m:** 9%↑ VO_{2peak}, ↑↓ LBS, 2%↑ LBM **12m:** ↑↓ VO_{2peak}, ↑↓ LBS, 2%↑ LBM
Candow et al. 2008	MM, MS	35	67	RT + CrM + PRO	3x/wk, 10wks	LBM, MT, LBS, UBS	6%↑ LBM, 10%↑ MT, 20%↑ LBS, 25%↑ UBS

MM= muscle mass, MS= muscle strength, P=physical performance, RT= resistance training, DP=dairy protein, MiN=micronutrients, LBM=lean body mass, aLM=appendicular lean mass, GS=gait speed, TUG=timed get up and go, CS=chair stand, WP=whey protein, P-Ex=post-exercise, h.s.=bedtime, LBS=lower body strength, CSA=cross sectional area, Leu-E=leucine enriched, HG= handgrip strength, 6MWT=six minute walk test, TBD=to be determined, CrM=creatine monohydrate, UBS= upper body strength, E=energy, SC=stair climb, LBP=lower body power, IMAT=intramuscular adipose tissue, MT= muscle thickness, CT=concurrent training, VO_{2peak}= peak oxygen uptake, bid=twice daily, LM=leg mass, PRO=protein supplement, SMI=skeletal muscle mass index, QD=once daily, RSMM=relative skeletal muscle mass, ADL=activities of daily living, CaHMB= calcium beta-hydroxy-beta methylbutyrate, tid=three times daily

Table 17

Combined Dietary and Exercise Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2010-2013

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Bemben et al. 2010	MS	42	60	RT + CrM + WP	3x/wk, 14wks (P-Ex)	LBS, UBS	~30-78%LBS,~32-55%↑UBS
Tieland et al. 2012	MM, MS, P	62	78	RT + DP	2x/wk, 24wks (bid)	LBM, aLM, LBS, HG, SPPB, GS, CR	3%↑ LBM, 5%↑ aLM, 36-37% ↑ LBS, ↑↓ HG, ↑ 19%↑ SPPB, ↑↓ GS, 13%↓ CR
Kim et al. 2012	MM, MS,P	155	80	RT + EAA	2x/wk, 3m (bid)	LBM, aLM, LG, GS, LB	↑↓ LBM,↑↓ aLM, 3%↑ LM,13%↑ GS,7%↑ LBS
Stout et al. 2013	MM, MS, P	43	73	RT + CaHMB	3x/day, 21wks (bid)	LBM, HG, LBS, TUG, MQ	3%↑ LBM, 10%↑ HG, 10%↑ LBS,12% ↓ TUG, -7-9%↑ MQ

MM= muscle mass, MS= muscle strength, P=physical performance, RT= resistance training, DP=dairy protein, MiN=micronutrients, LBM=lean body mass, aLM=appendicular lean mass, GS=gait speed, TUG=timed get up and go, CS=chair stand, WP=whey protein, P-Ex=post-exercise, h.s.=bedtime, LBS=lower body strength, CSA=cross sectional area, Leu-E=leucine enriched, HG= handgrip strength, 6MWT=six minute walk test, TBD=to be determined, CrM=creatine monohydrate, UBS= upper body strength, E=energy, SC=stair climb, LBP=lower body power, IMAT=intramuscular adipose tissue, MT= muscle thickness, CT=concurrent training, VO_{2peak}= peak oxygen uptake, bid=twice daily, LM=leg mass, PRO=protein supplement, SMI=skeletal muscle mass index, QD=once daily, RSMM=relative skeletal muscle mass, ADL=activities of daily living, CaHMB= calcium beta-hydroxy-beta methylbutyrate, tid=three times daily

Table 18

Combined Dietary and Exercise Interventions to Mitigate Loss of Muscle Size, Strength, and/or Function in Older Adults 2015-2018

				Intervention			
Author	**Sarcopenic Criteria**	**N**	**Mean Age**	**Description**	**Frequency & Duration**	**DV**	**Results**
Oesen et al. 2015	MS, P	117	83	Band RT + Leu-E PRO	2x/day, 6m (AM & P-Ex)	LBS, HG, CS, GS, 6MWT	10-15%↑ LBS, ↑↓ HG, 25%↑ CS, 8%↑ GS, 10%↑ 6MWT
Rondanelli et al. 2016	MM, MS, P	130	80	RT + WP/MiN Cocktail	5x/wk, 12 wks (QD)	LBM, RSMM, HG, ADL	4%↑ LBM, 3%↑ RSMM, 20%↑ HG, 14%↑ ADL
Seino et al. 2018	MM, P	82	74	RT + DP/MiN Cocktail	1x/day, 12wks (P-Ex)	LBM, aLM, GS, TUG, CS	↑ LBM, ↑ aLM, ↑↓ GS, ↑↓ TUG, ↑↓ CS
Holwerda et al. 2018	MM, MS	41	70	RT + Leu-E WP	2x/day, 12 wks (P-Ex & h.s.)	LBS, aLM, CSA	20%↑ LBS, 2%↑ aLM, 6%↑ CSA
Mori et al. 2018	MM, MS, P	75	71	RT + WP	2x/wk. 24 wks (P-Ex)	SMI, HG, GS, LBS	3%↑ SMI, 6%↑ HG,~6% ↑ GS,11%↑ LBS

MM= muscle mass, MS= muscle strength, P=physical performance, RT= resistance training, DP=dairy protein, MiN=micronutrients, LBM=lean body mass, aLM=appendicular lean mass, GS=gait speed, TUG=timed get up and go, CS=chair stand, WP=whey protein, P-Ex=post-exercise, h.s.=bedtime, LBS=lower body strength, CSA=cross sectional area, Leu-E=leucine enriched, HG= handgrip strength, 6MWT=six minute walk test, TBD=to be determined, CrM=creatine monohydrate, UBS= upper body strength, E=energy, SC=stair climb, LBP=lower body power, IMAT=intramuscular adipose tissue, MT= muscle thickness, CT=concurrent training, VO_{2peak}= peak oxygen uptake, bid=twice daily, LM=leg mass, PRO=protein supplement, SMI=skeletal muscle mass index, QD=once daily, RSMM=relative skeletal muscle mass, ADL=activities of daily living, CaHMB= calcium beta-hydroxy-beta methylbutyrate, tid=three times daily

2.3.5. Summary

Several countermeasures for sarcopenia and other muscle wasting diseases have been examined over time, however, there is currently no all-inclusive prescription for the treatment. The effects of sarcopenia are highly debilitating and individualized. In addition, various conditions may limit older adults' ability to participate in some countermeasures. Of these, concurrent exercise, resistance exercise, and dietary supplements, especially protein containing essential amino acids, have shown efficacy separately and in combination. Researchers should continue to study various exercise modalities, particularly with low intensities and loads, combined with protein supplementation to develop the most tolerable interventions. Special care and consideration should be taken to maintain ethical practices when working with middle-aged to older adults.

2.4. Ethics

2.4.1. Introduction

Research ethics is an ever-changing aspect of scientific exploration with each adaption an attempt at making the experience better for those involved. From 1932-1972, the Tuskegee syphilis study, Nazi medical research studies, and the Milgram experiments of obedience give way to the Nuremberg Code and the National Research Act of 1974 (Ghooi, 2011; Hoyle et al., 2002). Even prior to these horrific experiments, Germany had a loose set of guidelines that may have been adapted by the U.S. after the Nuremberg trials (Ghooi, 2011). In 1964, the World Medical Association developed a set of guidelines known as the Declaration of Helsinki with the purpose of creating a balance between protecting human rights and scientific discovery (World Medical Association [WMA], 2013). Though this declaration is primarily addressed toward medical doctors, it has been amended nine times and is used in combination with the Common

Rule by Institutional Review Boards (IRBs) worldwide to maintain the ethical treatment of human subjects (WMA, 2013). In fact, the National Research Act of 1974 made IRB approval a requirement for all federally funded human studies (Hoyle et al., 2002). Also, this act gave way to an additional set of guidelines for ethical treatment of subjects known as the Belmont Report (Hoyle et al., 2002). Using the Declaration of Helsinki and the Belmont Report, IRBs are better able to complete a risk-benefit analysis and researchers are better able to create informed consents that include respect for persons, beneficence, and justice (WMA, 2013; Hoyle et al., 2002). Under these principles, researchers must exercise caution to avoid coercion while creating more benefit than risk to the participants, choose a representative sample that has access to the treatment or intervention after the study's completion, and maintaining the participants anonymity or confidentiality (WMA, 2013). Researchers must make ethical decisions when deciding to complete a study and throughout the study (Hoyle et al., 2002). This is especially true in sarcopenia research as the high prevalence of the disease and the risk of mortality associated with sarcopenia and frailty make not completing research to find the best countermeasure arguably unethical (Cruz-Jentoft & Morley, 2012 ; Cruz-Jentoft et al., 2010; Cruz-Jentoft et al., 2014; Izquierdo et al., 2016; von Haehling et al., 2010). Additionally, the researcher must be prepared for possible adverse events to determine the risk of the participants. Research in aging as well as randomized control trials carry innate risks, benefits, and ethical considerations that will be discussed throughout this chapter.

2.4.2. Ethical Considerations for Research of Elderly and Frail Individuals

As previously discussed, the benefits of exercise and nutritional interventions for the treatment of sarcopenia to older adults are substantial and in healthy adults provide a low net risk (Izquierdo et al., 2016; Monroe et al., 2013; Reidy & Rasmussen, 2016). However, these

interventions are not without risk and researchers must be thoughtful their experiments. As with all participants, it is important that older subjects understand: who the researcher(s) is/are and the affiliations, research aims, what is required of them, funding sources of the research, any conflicts of interest, risks and benefits associated with participation, potential discomfort, their right to refuse/withdrawal without consequence, compensation if applicable, contact information for potential questions, and the name of the IRB that approved the study (WMA, 2013; Harriss et al., 2017). It is also pertinent to the ethical treatment of these subjects that the researcher protect person identifiable information and that, if it is to be used, explicit written or oral and witnessed consent or assent must be obtained (WMA, 2013).

Though older adults are not defined as a vulnerable population based on age-alone, aging can be associated with environmental, mental, physical, and socioeconomic conditions that could cause them to fall into this category (WMA, 2013; US Department of Health and Human Services [HHS], 1979; Ilgili et al., 2014) . Justice should be considered when working with older adults, particularly when there is a possibility of cognitive decline and institutionalization (Cruz-Jentoft & Morley, 2012 ; Cruz-Jentoft et al., 2010; Cruz-Jentoft et al., 2014; Ilgili et al., 2014). To maintain justice while including respect for persons, individuals who are legally incompetent and unable to give consent must only be studied if the results would provide direct and/or indirect benefits to legally incompetent elders, the net risk is low, the study cannot be completed in a non-vulnerable population, and assent has been obtained (Monroe et al., 2013). It has been suggested that declines in cognitive function may cause an individual to need more time to process what is being asked of them and that is the responsibility of a well-trained researcher (Ilgili et al., 2014). It has also been suggested that exercise can help improve cognitive functioning and functional capacity in the elderly (Izquierdo et al., 2016). Care should be given

by the researcher to ensure that the participant, or legal guardian in the case of assent and proxy/surrogate consent, understand the risks and benefits associated with participation (WMA, 2013; HHS, 1979; Ilgili et al., 2014; Monroe et al., 2013). The recent update to the Declaration of Helsinki states that consent/assent should be obtained in written or witnessed oral form and should be displayed in language and formatting that is appropriate for the target population (WMA, 2013; Harriss et al., 2017). For a researcher to fully comply with these guidelines, they must understand the risks and benefits to the study population regarding the experiment intervention.

2.4.3. Ethical Considerations for Specific Study Design

Several ethical challenges should be considered when developing a study to examine an exercise and/or nutritional intervention to mitigate sarcopenia. Though exercise is a minimal risk activity, specific equipment used to develop a protocol may carry increased risks to the participant. Additionally, though most dietary supplements are harmless to healthy people, they may be associated with increased risk in other populations. To maintain ethical research practices, it is as important to be educated and thoughtful in the evaluation of exclusion and inclusion criteria. One example of this is the DXA scanner as it uses a low dose of radiation (0.0004mSv or 25% of the recommended radiation levels in the United States) to determine the muscle and bone mass and adiposity with great precision and minimal risks (National Council on Radiation Protection and Measurments [NCRP], 2004; Toombs et al., 2012). For the typical American, this would be considered a very low risk. However, pregnant women would be considered a vulnerable population as the low risk does not necessarily mean no risk (McCollough et al., 2007). This suggests that it would be unethical to exclude middle-aged to older adults who may benefit from a greater understanding of their body composition with little

risk; however, it is ethical to require a pregnancy test and exclude those with positive results prior to completing the scan. Other examples are evidenced in the use of blood flow restriction devices as adverse events such as: discomfort, pain, bruising, cardiovascular incidents, and rhabdomyolysis have been reported (Nakajima et al., 2006). However, the risks for this have been reviewed as relatively low <1-13% with the highest incidence being the lowest concern, bruising at the site of the cuff (Hackney et al., 2018; Nakajima et al., 2006). Even the more serious adverse cardiovascular events (deep vein thrombosis (DVT), pulmonary embolism, coronary artery disease) have relatively low risks, are not known to increase with age, and could potentially improve with the exercise (Clark et al., 2011; Iida et al., 2011; Ozaki et al., 2011; Shimizu et al., 2016; Yasuda et al., 2015). Nevertheless, researchers must remember to act in the best interest of the participant and population by using careful consideration in the development of exclusion criteria and the monitoring of subjects (WMA, 2013). These criteria may include, but are not limited to, previous injury of the targeted muscles or joints, chronic and uncontrolled cardiovascular conditions, a history of myocardial infarction or DVT, metabolic diseases, or rhabdomyolysis. Appropriate screening tools should be used to help determine these risks. If these risks are minimized, BFR exercise can ethically provide the benefits of exercise to individuals with limited exercise abilities due to their environment, previous injuries, medical conditions, and other factors (Loenneke & Abe, 2016; Yasuda et al., 2016; Yasuda & Nakajima, 2016). Additionally, nutritional interventions, like protein supplementation, can provide risks to renal function along with gastrointestinal distress (Bauer et al., 2013; Brose et al., 2003). However, similar to other potential risks these have shown to be rare and easily controlled with proper inclusion (i.e. healthy adults) and exclusion criteria (i.e. no history of kidney disease or renal impairment) (Bauer et al., 2013; Brose et al., 2003).

Ethical consideration should also be given to study design prior to the development of intervention experiments to mitigate sarcopenia. For example, in the event that a control or placebo intervention be used, a cross-over design should be considered (WMA, 2013). In the case that cross-over design is not feasible, participants should be monitored for adverse events. Additionally, since exercise is the current gold standard in the treatment of sarcopenia, any control group should have access to it after the conclusion of the study (WMA, 2013; Morley et al., 2014). As training each individual at the end of the study is not always feasible, some researchers choose health education or cognitive training to equal the benefits to those in the intervention group (Izquierdo et al., 2016; Kim et al., 2012; Oesen et al., 2015). One alternative to this, particularly in regard to supplementation studies, is providing participant results and outreach materials after the study to educate participants on their nutritional status and needs. Lastly, because researches must take in account the risk: benefit ratio of their study population and the ethical obligation to get information to that population, the minimal sample size required as determined by power analysis should be used (Harriss et al., 2017; Ilgili et al., 2014).

2.5. Conclusion

Sarcopenia is highly prevalent in older adults and treatment can be debilitating and costly. The disease has many overlapping mechanisms that are not fully understood, but provide sufficient evidence to develop countermeasures. Of these, exercise is most effective. However, the exact prescription remains unknown as abilities and limitations to physical activity and exercise are highly variable amongst individuals. The use of alternative methods of exercise is may be more appropriate for older populations and those who may be limited by their environment or conditions. There may be additional benefits to exercise when protein intake is increased. With the rising number of older adults, finding feasible countermeasures for

sarcopenia is essential to helping Americans live longer more quality lives while decreasing the overall cost of healthcare.

3. METHODOLOGY

Special populations such as middle-aged individuals progressing through the processes of aging and astronauts living and working in microgravity are at risk for losing muscle mass and function. These losses in muscle quantity or quality due to aging are referred to as sarcopenia and increasing severity includes low muscle strength, quantity or quality, and function (Alfonso J. Cruz-Jentoft et al., 2019). Sarcopenia is generally associated with old age, however, a prevalence of 19.2% has been reported in those as young as 20 years and this value increases at age 60 and beyond (Bae & Kim, 2017). The population of those over 60 years currently makes up over 420 million adults worldwide and is expected to reach over 2.1 billion between 2030 and 2050 (UN, 2015; Ilgili et al., 2014). This increase in older adults creates vulnerability to sarcopenia and will likely lead to reduced quality of life and increased healthcare costs making early intervention essential (Chodzko-Zajko et al., 2009; Janssen et al., 2004).

Recent studies have examined the effect of total protein supplementation and combinations of whey protein, essential amino acids (EAAs), antioxidants, and vitamin D on muscle quality and function (Rondanelli et al., 2016; van Dijk et al., 2016). There is also an obvious beneficial relationship between physical activity and muscle health in those over the age of 60 years is evident and it appears that the type of PA (e.g., leisure time, structured exercise, and occupational) is of little importance (Beaudart et al., 2017; Steffl et al., 2017). Exercise is widely accepted as the gold standard for increasing and maintaining muscle function and additional benefits from leucine-rich protein supplementation have been suggested (Chodzko-Zajko et al., 2009; Chodzko-Zajko et al., 2009; Cruz-Jentoft et al., 2017; Morley et al., 2010). Concurrent training (e.g., a mixture of resistive and aerobic exercises) includes resistance

training using 60-80% of an individual's 1-RM which may be too physiologically demanding for older adults (Chodzko-Zajko et al., 2009; Cook et al., 2017; Garber et al., 2011).

Blood flow restriction (BFR) exercise may be an efficient alternative as it has shown similar improvements in muscle size and strength to traditional resistance training while using only 20-30% of 1-RM (Cook et al., 2017; Hackney et al., 2016; Karabulut et al., 2010). Furthermore, BFR exercise may simulate concurrent exercise as it has previously improved vascular function in healthy older adults (Shimizu et al., 2016). Only two studies have reported increases in the muscle size following BFR training and protein or amino acid supplementation in middle-aged and older adults indicating promise as a countermeasure for sarcopenia or atrophy in response to disuse associated with microgravity (Centner et al., 2019; Hackney et al., 2016).

3.1. Investigation 1: Moderate to Vigorous Physical Activity, Leucine Intake, and Protein Intake Contributions to Muscle Health in Middle Age

The purpose for this study was to identify contributors to differences in the muscle size and strength of sedentary and active young and middle-aged adults. This study was an observational cross-sectional study. It was guided by the following research question: Are there differences in muscle strength between sedentary and active young and middle-aged adults and how does dietary intake and habitual physical activity relate to muscular health?

3.1.1. Participants

A total of 98 participants volunteered to participate in this study. Participants were recruited via flyers, word of mouth, and email. They were between the ages of 20-35 and 50-65 years. Participants included in this study were generally healthy as determined by the Physical Activity Readiness Questionnaire (PAR-Q) and a detailed health history questionnaire (Physical

Activity Readiness Questionnaire [PAR-Q] and You, 1997). Active individuals engaged in aerobic and resistive exercises 3-5 times per week at a moderate to vigorous intensity for at least three months prior to participation.

3.1.2. Documentation

Prior to data collection, this research was approved by the North Dakota State University (NDSU) and Sanford Health (Fargo, ND) Institutional Review Boards. After completion of the informed consent, Par-Q, and health history questionnaire, participants were screened for this study. Participants were excluded from this study if: 1) they were pregnant or believed they could be pregnant; 2) they had metal fragments, devices, implants, or ink from tattoos that may be affected by an MRI scan; 3) they were claustrophobic; 4) they used tobacco in any form; 5) they had a previous diagnosis of metabolic, cardiovascular, cancer; 6) they had significant mobility limitations; 6) they were taking medications that were known to directly influence muscle protein metabolism; or 7) they were third shift workers given alternative daily schedules.

3.1.3. Procedures

Participants' completed two testing sessions. Anthropometric and muscle strength tests were completed during initial subject visit to the Human Performance Lab. Body weight was measured using a digital scale to the nearest 0.1 kg (Denver Instruments DA-150, Denver, Colorado), height to the nearest 0.5 cm using a stadiometer (Seca 703 scale, Chino, CA), and waist circumferences were completed using a Gulick measuring tape to the nearest 0.1 cm. At this session, participants were sent home with an accelerometer and dietary log to be completed and returned at their follow-up session one week later. The final session took place at Sanford Health and included a magnetic resonance imaging (MRI) scan and return of materials.

3.1.4. Measures

3.1.4.1. Muscle Size

Serial axial plane MRI scans from a 3.0 T Siemens Skyra Intera whole body scanner (Siemens Healthcare Headquarters, Erlangen, DE) were obtained at Sanford Medical Center Fargo. Images were obtained by licensed radiology technologists in collaboration with researchers. Participants were positioned with elevated heels and knees to minimize the distortion of the muscle to be analyzed. The MRI settings were as follows: repetition time = 3730 m/s, 10 mm slice-to-slice interval, 420-500 mm x 328-390 mm field of view (Dicks et al., 2020). Image J version 1.42 (National Institutes of Health, Bethesda, MD, US) was used to analyze MRI-derived muscle CSA. Quadriceps CSA (CSAq) was determined for the rectus femoris and vastii by using the free-hand tool. Subcutaneous fat of the right upper and lower leg was also determined using the free-hand tool. Muscle CSA analyses were performed by three different researchers, reliability had been previously reported (Streeter et al, 2016; Stone et al, 2016).

3.1.4.2. Muscle Strength and Endurance

Muscle function of the upper and lower right leg was assessed using a Biodex Pro4 System dynamometer (Biodex Medical Systems, Shirley, NY, US). To examine the isokinetic strength and endurance of the knee flexors and extensors the participants were seated in an upright position and would move the leg through flexion and extension at angular velocities of 60 and 180 degrees/second, respectively. For both assessments, the upper leg moved through a range of motion of 95° flexion and 20° extension. In two instances, participants were limited to 25° extension due to self-reported tension in the hamstrings. A back pad was used to achieve a trunk angle of 90° when necessary. The center of the dynamometer was aligned with the subjects' lateral epicondyle and the shin pad was placed approximately 3-5 cm above the tongue

of the participants' shoe, just above the lateral malleolus. After being provided with consistent, verbal instruction participants completed a linked protocol. The protocol consisted of one warm-up set of four repetitions at 60 degrees/second with the participants contributing no more than 85% effort on the final repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during extension and flexion. After another rest period of 30 seconds, participants completed one warm-up set of five repetitions at 180° degrees/second followed by 30 seconds of rest and 21 maximal effort repetitions to determine total work (J). Upon completion of this protocol, participants were released from the chair while the researchers set up for assessment of the lower leg. To examine the strength and endurance of the ankle dorsiflexors and plantar flexors the participants were seated in an upright position at 70° tilt with the hamstrings supported. Participants would move the ankle at angular velocities of 30 and 60 degrees/second to assess strength and endurance, respectively. The center of the dynamometer was aligned with the individuals' lateral malleolus. The heel was supported by a heel cup and the foot was strapped tightly to the foot plate. After being provided with consistent, verbal instruction participants completed a second linked protocol. The protocol consisted of one warm-up set of four repetitions at 30 degrees/second with the participant contributing no more than 85% effort on the final repetition followed by 60 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during plantarflexion and dorsiflexion. After another rest period of 60 seconds, participants completed a warm-up set of 5 repetitions at 60 degrees/second followed by 60 seconds of rest and then 21 maximal effort repetitions to determine total work (J).

3.1.4.3. Dietary Intake

To examine dietary intake, participants completed a 3-day food diary. Participants were asked to log everything they ingested on two typical days (i.e. weekdays) and one atypical day (i.e. weekend day) in the week following muscle function testing. Once completed, RDs analyzed the protein (both grams per subject and g·kg−1 per subject), other nutrients, and the within-day-distribution of protein intake using Food Processor Nutrition Analysis software (ESHA, Salem, OR).

3.1.4.4. Physical Activity

Habitual PA was assessed using an Actigraph GT3X+ accelerometer (Actigraph, Pensacola, FL) for seven consecutive days. Participants were instructed to wear the accelerometer on their right hip during all waking hours except for water activities (bathing or swimming), and to keep a sleep log to record the time that the accelerometer was removed at night and put back on in the morning. The accelerometers were initialized to collect activity counts in 60 second epochs, and activity counts data was converted into the amount of time (min/day) spent in sedentary (<100 counts/min), light (100-1951 counts/min), and moderate-to-vigorous (>1952 counts/min) intensities using previously validated cutpoints (Freedson et al., 1998). Non-wear time was defined as intervals of at least 90 minutes of zero counts with allowance of two-minute interval of non-zero counts with 30-minute window (Choi et al., 2011). A minimum wear time of 4 days with 10 hrs/day was required in order to be included in the statistical analysis (Gorman et al., 2013).

3.1.5. Statistical Analysis

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY) and SAS version 9.4 (SAS Institute; Cary, NC). Descriptive statistics are reported as mean ± SD.

Statistical significance was set at $\alpha = 0.05$. Mahalanobis distance was used to remove outliers for total leucine intake, energy intake per kg of body weight, and protein intake per kg of body weight. Separate one way analysis of variance (ANOVA) with Bonferroni adjustments were used to examine group differences in protein intake per kg of body weight, energy intake per kg of body weight, CSAq, knee extensor peak torque, knee flexors peak torque, plantar flexors peak torque, and dorsi flexors peak torque. Simple linear regression was used to evaluate relationships between physical activity and nutrient intake and muscle size and strength. Sedentary behavior, light physical activity, moderate physical activity, vigorous physical activity, and MVPA were used as independent variables for physical activity. Protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), and total leucine intake (g) were used as independent variables for nutrient intake. The outcome variable for muscle size was CSAq. For muscle strength knee extensor peak torque, knee flexors peak torque, plantar flexors peak torque, and dorsi flexors peak torque were used. These models were adjusted for age, body mass index, and biological sex. Stepwise regression models were used to examine the relationship of age, protein intake, energy intake, total leucine intake, and physical activity with CSAq, KEPT, KFPT, DFPT, and PFPT.

3.2. Investigation 2: Intermittent BFR Rapidly Improves Muscular and Cardiovascular Health in Middle Age without Additional Benefits from Protein Supplementation

The purposes of this study were to determine if BFR combined with a leucine-rich protein supplement could: 1) improve muscular strength, function, or quality; 2) favorably alter body composition; 3) simulate concurrent exercise by improving indices of cardiovascular health. This was a randomized, double-blind, placebo-controlled trial. It was guided by the following research question: Does a leucine-rich protein supplement combined with BFR

exercise result in favorable changes to body composition, muscular health, and cardiovascular health in only four weeks?

3.2.1. Participants

A total of 16 healthy volunteers were recruited via word of mouth, flyers, and email. Participants were generally healthy as determined by the 2018 Physical Activity Readiness Questionnaire (PAR-Q) and recreationally active but not participating in regular structured exercise (2018 PAR-Q+ The Physical Activity Readiness Questionnaire for Everyone [PARQ+], 2018). Participants' activity was evaluated using the 2002 International Physical Activity Questionnaire. Those participating in up to 420 minutes of moderate to vigorous physical activity were considered recreationally active (International Physical Activity Questionnaire [IPAQ], 2002).

3.2.2. Documentation

Prior to data collection, this research was approved by the North Dakota State University (NDSU) and Sanford Health (Fargo, ND) Institutional Review Boards. After completion of the informed consent, PAR-Q+, IPAQ, Illinois State Medical Society deep vein thrombosis (DVT) Questionnaire and additional DVT questions, participants were screened for this study (Illinois State Medical Society [ISMS], 2013). Participants were excluded from this study if: 1) they were pregnant or believed they could be pregnant; 2) they used tobacco in any form; 3) they had a previous diagnosis of a metabolic disease, cardiovascular disease, neuromuscular disorder, renal disease, exertional rhabdomyolysis, sickle cell anemia/trait, or were being treated for cancer; 4) they had significant mobility limitations or previous injuries that limit the ability to exercise safely; 5) they were taking medications that were known to directly influence muscle

protein metabolism; 6) they were diagnosed with class III obesity; 7) were using hormonal contraceptives; or 8) they were at an increased risk for DVT.

3.2.3. Procedures

Two groups completed this double-blind, randomized, placebo-controlled study. Following a general 5-minute warmup, participants completed the following BFR exercises three times per week for four weeks: leg extension, leg curl, biceps curl, and triceps extension. Prior to training, all participants completed two weeks of familiarization to exercise in which they came to the lab three times per week. During week one of familiarization, participants completed 10-repetition maximum testing for all exercise to determine initial training load (20% of 1-RM for upper body and 30% of 1-RM for lower body) and two exercise sessions without BFR (Cook et al., 2017; Kim et al., 2017; Vechin et al., 2015). During week two of familiarization, they completed three exercise sessions with 40% occlusion applied during the last set of each exercise. Exercises were completed to a tempo of 30bpm. Each exercise was comprised of four sets and participants were instructed to complete as many reps as possible until they reached 30, 15, 15, and 15 repetitions or volitional failure. While the cuff was inflated, participants exercised one limb while the other was resting without inflation giving 1-2 minutes of rest between sets. Participants were given three minutes of rest between exercises. Exercise progression occurred via increasing occlusion pressure from 40%-50% for the upper body and 40%-80% for the lower body or via increasing load in 2-5 kg increments (Loenneke et al., 2015; Tennent et al., 2017; Vechin et al., 2015). Load was increased when participants could complete two additional repetitions for two sets on any exercise. Load and occlusion pressure were not increased simultaneously.

Participants were randomly assigned to consume either a daily whey/casein blend protein shake (~ 41 total g, 163 kcals, 1 g fat, 10 g carbohydrate, 28 g protein, and 3 g of leucine) or an isocaloric placebo of the same flavor for the four weeks following familiarization. Prior to familiarization, all participants completed pre-testing measures including: anthropometrics, isokinetic leg strength, STS, handgrip strength, balance, gait speed, TUG, resting heart rate and blood pressure, and were given a three-day dietary log to be completed over one week. Halfway through the study, participants were given a second three-day dietary log. Within three days of completing training, participant repeated the pre-testing measures. All supplements and placebos that were not consumed were returned for compliance measurement.

3.2.4. Measures

3.2.4.1. Anthropometrics and Body Composition

Height and weight were measured using a standard stadiometer (Seca 213, Chino, CA, US) and scale (Denver Instrument DA-150, Arvada, CO, US). Lean body mass, body fat percentage, appendicular lean body mass, and skeletal muscle index was assessed using DXA (GE Healthcare Lunar Prodigy, Chicago, IL, US). All biologically female participants took a pregnancy test prior to each scan. Upon verification, participants were asked to remove any jewelry or metal that may interfere with the scan and were positioned appropriately in the scanner. Scans took 5-12 minutes.

3.2.4.2. Muscular Strength

Muscle strength of the upper body was assessed using a Jamar handgrip dynamometer (Jamar Plus, Bollingbrook, IL, US). Participants were instructed to stand, hold the dynamometer in their dominant hand with a 90° bend at the elbow, and squeeze as hard as possible for three seconds. They completed three attempts with one-minute rest between sets. The best attempt was

used in analysis. Muscular lower body was assessed using a Biodex Pro4 System dynamometer (Biodex Medical Systems, Shirley, NY, US). To examine the isokinetic strength of the knee flexors and extensors the participants were seated in an upright position and would move the leg through flexion and extension at an angular velocity of 60 degrees/second. The upper leg moved through a range of motion of 95° flexion and 20° extension. A back pad was used to achieve a trunk angle of 90° when necessary. The center of the dynamometer was aligned with the subjects' lateral epicondyle and the shin pad was placed approximately 3-5 cm above the tongue of the participants' shoe, just above the lateral malleolus. The protocol consisted of one warm-up set of three repetitions at 60 degrees/second with the participants contributing no more than 85% effort on the final repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during extension and flexion. Upon completion of this protocol, participants were released from the chair while the researchers set up for assessment of the lower leg. To examine the strength of the ankle dorsiflexors and plantar flexors the participants were seated in an upright position at 70° tilt with the hamstrings supported. Participants would move the ankle at an angular velocity of 30 degrees/second. The center of the dynamometer was aligned with the individuals' lateral malleolus. The heel was supported by a heel cup and the foot was strapped tightly to the foot plate. The protocol consisted of one warm-up set of three repetitions at 30 degrees/second with the participant contributing no more than 85% effort on the final repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during plantar flexion and dorsiflexion. Muscle quality was assessed by dividing knee extensor peak torque (KEPT) by DXA derived lean body mass of the right leg as a measure of relative strength. Average weekly training volume (weight x sets x repetitions) was calculated for each exercise as a measure of dynamic strength.

3.2.4.3. Muscle function

Gait speed (m/s) was measured using a six-meter course and timed using the Brower TCi system (Draper, UT). Participants were instructed to walk at their normal pace. To obtain a consistent speed without acceleration or deceleration, start and stop points were marked two meters before and after the timing gates by cones. Timed up and Go was completed using the same course with a cone placed at the halfway mark, a 43cm chair, and a standard stopwatch. Participants were instructed to rise from the seat, walk to and around the cone, and return to their seat at a normal pace. Participants completed one untimed and two timed trials. Mean time was used in analyses.

Participants also performed a STS using a 43 cm chair. Participants were instructed to cross their arms and find a comfortable seat that they could continuously sit and stand from. They were allowed two repetitions of practice to adjust this position. They were then instructed to complete as many repetitions as possible in thirty seconds. The total number of repetitions completed was used in the analysis.

Balance was assessed using the BESS protocol on the Balance System SD (Biodex Medical Systems, Shirley, NY, US). Participants were instructed to remove their shoes and step onto the platform. Conditions included a double leg stance, single leg (non-dominant) stance, and a tandem stance with the non-dominant foot back. Each condition was completed with the eyes closed on a firm surface and then repeated using an Airex pad for a total of six trials. Each trial lasted 20 seconds, there was 10 seconds of rest between trials, and errors were scored by trained research assistants. Errors were only counted once and included moving hands from the hips, opening the eyes, stepping/stumbling/falling, hip abduction, or any deviation from position for more than five seconds. The composite score provided by the system was used in the analysis.

3.2.4.4. Cardiovascular Health

Following five minutes of supine rest, heart rate was assessed using a polar heart rate strap and watch (Polar, Kempele, FI). Resting blood pressure was also measured manually with a 3M Littman stethoscope (3M, Maplewood, MN, US) and Diagnostix 703 sphygmomanometer (American Diagnostic Corporation, Hauppauge, NY). If readings were over 140/90 mmHg or undetectable, participants were given an additional two minutes of rest and the measure was repeated.

3.2.4.5. Dietary Intake

To examine dietary intake, participants completed three 3-day food diaries. Participants were asked to log everything they ingested on two typical days (i.e. weekdays) and one atypical day (i.e. weekend day) in the week following muscle function testing. Once completed, RDs analyzed the protein (both grams per subject and $g \cdot kg^{-1}$ per subject), other nutrients, and the within-day-distribution of protein intake using Food Processor Nutrition Analysis software (ESHA, Salem, OR).

3.2.5. Statistical Analysis

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY) and SAS version 9.4 (SAS Institute; Cary, NC). Descriptive statistics are reported as mean ± SD. Statistical significance was set at $\alpha = 0.05$. An independent samples t-test was used to compare group differences in participant demographics. Separate 2x2 repeated measures ANOVAs assessed the differences in body fat percentage, total lean mass (LBM), appendicular lean body mass (aLM), skeletal muscle mass index (SMI), KEPT, KFPT, PFPT, DFPT, muscle quality, STS, hand grip strength, gait speed, balance composite score, TUG, resting heart rate, and resting blood pressure. Separate group (supplement vs. placebo) by time (pre-testing vs. mid-testing vs.

post-testing) mixed ANOVAs assessed the differences in average training volume for each exercise, protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), total macronutrient intake (total grams of carbohydrate, protein, and fat), and total leucine intake (g). Partial Eta Squared (η^2) was used to determine effect size. Separate linear mixed models were also conducted to evaluate the differences in appendicular lean aLM, LBM, SMI, body fat percentage, resting heart rate, resting blood pressure, protein intake ($g \cdot kg \cdot day^{-1}$), muscle quality, KEPT, KFPT, PFPT, DFPT, 30-second sit-to-stand, handgrip strength, timed up and go, gait speed, and balance composite score between the protein supplementation and placebo groups when adjusted for sex, age, and body mass index.

3.3. Conclusion

Middle-aged individuals are at risk for losses in muscle size, strength, and function from natural processes of aging. Astronauts have similar risks due to exposure to microgravity. Physical activity and nutrition may be combined as countermeasures for muscle atrophy and loss of function. In order to develop the most effective countermeasures, it is important to understand the role of physical and activity on muscular healthy and how it affects middle-aged adults. It is also important to understand what types of physical activity and exercise are feasible for these populations. BFR exercise has shown to be effective in many populations. When combined with leucine-rich protein supplementation, these benefits may be a useful countermeasure for at risk middle-aged adults.

4. MODERATE TO VIGOROUS PHYSICAL ACTIVITY, LEUCINE INTAKE, AND PROTEIN INTAKE CONTRIBUTIONS TO MUSCLE HEALTH IN MIDDLE AGE

4.1. Abstract

The purposes of this cross-sectional study were to compare the muscle health of young and middle-age adults and to examine the relationships between protein intake, leucine intake, physical activity and muscle size and strength. **Methods:** A total of 98 participants (49% female) were divided into the following groups: active young adults (n=25), active middle-aged adults (n=24), sedentary young adults (n=25) and sedentary middle-aged adults (n=24). Participants completed a strength assessment of knee extensors (KEPT), knee flexors (KFPT), plantar flexors (PFPT), and dorsiflexors (DFPT), a 3-day dietary intake log, 7-day accelerometry, and a magnetic resonance imaging (MRI) scan for muscle cross-sectional area analysis of the right quadriceps (CSAq). **Results:** There were significant group effects for protein intake ($g \cdot kg \cdot day^{-1}$) ($p<0.001$), energy intake ($kcals \cdot kg \cdot day^{-1}$) ($p=0.038$), KEPT ($p=0.010$), CSAq ($p=0.002$), PFPT ($p=0.004$) and DFPT ($p=0.003$). Moderate, vigorous, and moderate-to-vigorous physical activity, protein intake, and leucine were significantly and positively associated with CSAq, KEPT, PFPT ($R^2 = 0.28\text{-}0.71$, $p<0.05$). Moderate, vigorous, and moderate-to-vigorous physical activity and protein intake were negatively associated with DFPT ($R^2 = 0.48\text{-}0.58$, $p<0.05$). **Conclusion:** These results indicate that muscle size and strength are lower in middle age but that increased physical activity, protein intake, and leucine intake may be able to preserve muscle size and strength in larger muscle groups of the lower body.

4.2. Introduction

Age-related declines in skeletal muscle mass and strength, known as sarcopenia, affect a large percentage of women and men with women being 3.6 times more likely to become disabled

(Doherty, 2003). Reduced abilities to complete activities of daily living, impaired mobility and walking, and decrements in the get-up-and-go test, muscular strength, muscular power and muscle quality related to sarcopenia can greatly affect an individual's quality of life. (Thomas, 2012). The European Working Group on Sarcopenia in Older People suggests different categories and subcategories of sarcopenia based on causal conditions leading to development (Cruz-Jentoft et al., 2019). The group recently suggested specific causes of sarcopenia (e.g., inadequate nutrition and low physical activity), along with age-related or primary sarcopenia, might contribute to a secondary class of sarcopenia (Cruz-Jentoft et al., 2019; von Haehling, Morley, & Anker, 2010). Though the rate of muscle mass decline has been reported to be 0.65-2.0% per year in those over the age of 50 years, decreases may occur as early as age 30 for men and 40 for women (Canon & Crimmins, 2011; Cruz-Jentoft & Morley, 2012). Newman et al. (2003) suggested a similar rate of lean mass loss for older individuals and leg strength loss of 1.8 and 2.5 N-m in women and men, respectively (Newman et al., 2003). Other data have shown similar significant decreases in muscle strength accompanied by a three-fold greater decrease in strength than in muscle mass (Goodpaster et al., 2006). These data suggest a need to explore methods of preservation for mass and strength, or muscle quality, to maintain the quality of life for individuals as they age.

One such intervention stems from the concept that muscle atrophy originates from catabolism caused by increased protein breakdown, decreased protein synthesis, or a combination of the two (Francaux et al., 2016). Furthermore, previous researchers have suggested that aging affects markers for protein synthesis more than degradation through a blunted activation of mTOR in skeletal muscle following exercise and protein intake (Francaux et al., 2016). Recent studies have examined the effect of total protein supplementation and

combinations of whey protein, essential amino acids (EAAs), antioxidants, and vitamin D on muscle quality and function (Rondanelli et al., 2016; van Dijk et al., 2016). Van Dijk et al. (2016) showed that replacing casein protein with a whey protein containing additional leucine led to higher force production and improved muscle quality in rats. This suggests that the type of protein and protein turnover might be responsible for greater muscle quality (van Dijk et al., 2016). Similarly, Rondanelli et al. (2016) showed significant increases in fat-free mass, handgrip strength, quality of life, and activities of daily living in sarcopenic individuals consuming diets supplemented with a combination of whey protein and EAAs. Through a Mini Nutritional assessment, independent of physical activity levels, it was observed that 68% of the individuals studied improved their classification from sarcopenic to non-sarcopenic. Additionally, it has been stated that EAAs (e.g., leucine) mediate the stimulation of mTOR but only when total dietary protein intake is inadequate (Reidy & Rasmussen, 2016). Several studies have examined what amount of total protein is adequate for optimal skeletal muscle health but the results have been inconclusive, ranging from 0.66 $g \cdot kg \cdot day^{-1}$ to 1.8 $g \cdot kg \cdot day^{-1}$ with an additional amount of leucine greater than 2-3 $g \cdot kg \cdot day^{-1}$ (A J Cruz-Jentoft & Morley, 2012; Reidy & Rasmussen, 2016). It has been reported that protein intake higher than the current recommended dietary allowance (RDA) of 0.8 $g \cdot kg \cdot day^{-1}$ is associated with increased physical performance, lean mass, and muscle strength in elderly populations (Artaza-Artabe, Sáez-López, Sánchez-Hernández, Fernández-Gutierrez, & Malafarina, 2016; Isanejad et al., 2016). However, one-third of older adults in the United States (10% of older females) do not meet the current RDA (A J Cruz-Jentoft & Morley, 2012). Though there is still concern regarding the protein intake of elderly people, recent literature suggests examining the dietary habits of middle-aged, or “pre-elderly”, adults in comparison with young people to identify differences that may be important to

understanding interventions to mitigate age-related losses of muscle strength, size, and quality (English et al., 2016). Thus, the purpose for this study was to identify contributors to differences in the muscle size and strength of sedentary and active young and middle-aged adults.

4.3. Methods

4.3.1. Participants

From August 2015 to May 2016, a total of 98 participants were recruited and divided into the following groups: active young adults (AYA), sedentary young adults (SYA), active middle-aged adults (AMA), sedentary middle-aged adults (SMA) completed this cross-sectional study (Table 19 Young and middle-aged adults were defined as 20-35 and 50-65 years of age, respectively. Participants included in this study were generally healthy as determined by the Physical Activity Readiness Questionnaire (PAR-Q) and a detailed health history questionnaire (PAR-Q, 1997). Active individuals engaged in aerobic and resistive exercises three to five times per week at a moderate to vigorous intensity for at least three months prior to participation.

Table 19

Participant Demographics

	AYA	**SYA**	**AMA**	**SMA**
N	25	25	24	24
Age (yrs.)	23.0 ± 3.1	26.3 ± 4.7	57.3 ± 4.0	57.9 ± 4.4
Height (cm)	176.1 ± 10.1	173.1 ± 9.6	172.7 ± 9.6	174.5 ± 6.6
Weight (kg)	74.0 ± 17.7	72.7 ± 15.1	74.4 ± 17.7	81.9 ± 15.5

Mean ± SD

4.3.2. Documentation

Prior to data collection, this research was approved by the North Dakota State University (NDSU) and Sanford Health (Fargo, ND) Institutional Review Boards. After completion of the

informed consent, PAR-Q, and health history questionnaire, participants were screened for study eligibility. Participants were excluded if they reported: 1) current pregnancy or believed they could be pregnant; 2) metal fragments, devices, implants, or ink from tattoos that may be affected by an MRI scan; 3) claustrophobia; 4) tobacco use-in any form; 5) previous diagnosis of metabolic or cardiovascular co-morbidities, or cancer; 6) significant mobility limitations; 6) taking medications that were known to directly influence muscle protein metabolism; or 7) being third shift workers given alternative daily schedules.

4.3.3. Procedures

Participants' completed two testing sessions. Anthropometric and muscle strength tests were completed during the initial subject visit to the Human Performance Lab (NDSU, Fargo, ND). Body weight was measured using a digital scale to the nearest 0.1 kg (Denver Instruments DA-150, Denver, Colorado), height to the nearest 0.5 cm using a stadiometer (Seca 703 scale, Chino, CA), and waist circumferences were completed using a Gulick measuring tape to the nearest 0.1 cm (Fitness Mart, Gay Mills, WI). At this session, participants were sent home with an accelerometer and 3-day dietary log to be completed and returned at their follow-up session one week later. The final session took place at Sanford Health and included a magnetic resonance imaging (MRI) scan and return of materials.

4.3.4. Measures

4.3.4.1. Muscle Size

Serial axial plane MRI scans from a 3.0 T Siemens Skyra Intera whole-body scanner (Siemens Healthcare Headquarters, Erlangen, DE) were obtained at Sanford Medical Center Fargo. Images were obtained by licensed radiology technologists in collaboration with researchers. Participants were positioned with elevated heels and knees to minimize the

distortion of the analyzed muscle. The MRI settings were: repetition time = 3730 m/s, 10 mm slice-to-slice interval, 420-500 mm x 328-390 mm field of view (Dicks et al., 2020). Image J version 1.42 (National Institutes of Health, Bethesda, MD, US) was used to analyze MRI-derived muscle CSA. Quadriceps CSA (CSAq) was determined for the rectus femoris and vastii by using the free-hand tool. Subcutaneous fat of the right upper and lower leg was also determined using the free-hand tool. Muscle CSA analyses were performed by three different researchers, reliability had been previously reported (Streeter et al, 2016; Stone et al, 2016).

4.3.4.2. Muscle Strength and Endurance

Muscle function of the upper and lower right leg was assessed using a Biodex Pro4 System dynamometer (Biodex Medical Systems, Shirley, NY, US). To examine the isokinetic strength and endurance of the knee flexors and extensors the participants were seated in an upright position and would move the leg through flexion and extension at angular velocities of 60 and 180 degrees/second, respectively. For both assessments, the upper leg moved through a range of motion of 95° flexion and 20° extension. In two instances, participants were limited to 25° extension due to self-reported tension in the hamstrings. A back pad was used to achieve a trunk angle of 90° when necessary. The center of the dynamometer was aligned with the subjects' lateral epicondyle and the shin pad was placed approximately 3-5 cm above the tongue of the participants' shoe, just above the lateral malleolus. After being provided with consistent, verbal instruction, participants completed a linked protocol. The protocol consisted of one warm-up set of four repetitions at 60 degrees/second with the participants contributing no more than 50-75% effort on the final repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during extension and flexion. After another rest period of 30 seconds, participants completed one warm-up set of five repetitions at 180° degrees/

second followed by 30 seconds of rest and 21 maximal effort repetitions to determine total work (J). Upon completion of this protocol, participants were released from the chair while the researchers set up for assessment of the lower leg. To examine the strength and endurance of the ankle dorsiflexors and plantar flexors the participants were seated in an upright position at 70° tilt with the hamstrings supported. Participants would move the ankle at angular velocities of 30 and 60 degrees/second to assess strength and endurance, respectively. The center of the dynamometer was aligned with the individuals' lateral malleolus. The heel was supported by a heel cup and the foot was strapped tightly to the foot plate. After being provided with consistent, verbal instruction, participants completed a second linked protocol. The protocol consisted of one warm-up set of four repetitions at 30 degrees/second with the participant contributing no more than 75% effort on the final repetition followed by 60 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during plantar flexion and dorsiflexion. After another rest period of 60 seconds, participants completed a warm-up set of 5 repetitions at 60 degrees/ second followed by 60 seconds of rest and then 21 maximal effort repetitions to determine total work (J).

4.3.4.3. Dietary Intake

To examine dietary intake, participants completed a 3-day food diary. Participants were asked to log everything they ingested on two typical days (i.e. weekdays) and one atypical day (i.e. weekend day) in the week following muscle function testing. Once completed, RDs analyzed the protein (both grams per subject and $g \cdot kg^{-1}$ per subject) and other nutrients using Food Processor Nutrition Analysis software (ESHA, Salem, OR).

4.3.4.4. Physical Activity

Habitual physical activity (PA) was assessed using an Actigraph GT3X+ accelerometer (Actigraph, Pensacola, FL) for seven consecutive days. Participants were instructed to wear the accelerometer on their right hip during all waking hours except for water activities (e.g., bathing, swimming), and to keep a sleep log to record the time that the accelerometer was removed at night and put back on in the morning. The accelerometers were initialized to collect activity counts in 60-second epochs, and activity counts data was converted into the amount of time (min/day) spent in sedentary (<100 counts/min), light (100-1951 counts/min), moderate (1952-5724 counts/min), vigorous (5725-9498 counts/min), very vigorous (>9499 counts/min), and moderate-to-vigorous (>1952 counts/min) intensities using previously validated cut points (Freedson et al., 1998). Non-wear time was defined as intervals of at least 90 minutes of zero counts with allowance of two-minute interval of non-zero counts with 30-minute window (Choi et al., 2011). A minimum wear time of four days with 10 hrs/day was required to be included in the statistical analysis (Gorman et al., 2013).

4.3.5. Statistical Analysis

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY) and SAS version 9.4 (SAS Institute; Cary, NC). Descriptive statistics are reported as mean ± SD. Statistical significance was set at $\alpha = 0.05$. Mahalanobis distance was used to remove outliers for total leucine intake, energy intake ($kcals \cdot kg \cdot day^{-1}$), and protein intake ($g \cdot kg \cdot day^{-1}$). Separate one way analysis of variance (ANOVA) with Bonferroni adjustments were used to examine group differences in protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), CSAq, KEPT, KFPT PFPT, and DFPT. Simple linear regression was used to evaluate relationships between physical activity and nutrient intake and muscle size and strength. Sedentary behavior, light physical

activity, moderate physical activity, vigorous physical activity, and MVPA were used as independent variables for physical activity. Protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), and total leucine intake (g) were used as independent variables for nutrient intake. The outcome variable for muscle size was CSAq. For muscle strength knee extensor peak torque, knee flexors peak torque, plantar flexors peak torque, and dorsi flexors peak torque were used. These models were adjusted for age, body mass index, and biological sex. Stepwise regression models were used to examine the relationship of age, protein intake, energy intake, total leucine intake, and physical activity with CSAq, KEPT, KFPT, DFPT, and PFPT.

4.4. Results

Following outlier analysis a total of 97 participants were used in this study. There were significant group effects for protein intake per kg of body weight $F(3,93) = 7.598$, $p<0.01$, energy intake per kg of body weight $F(3,93) = 2.927$, $p=.038$, KEPT $F(3,93) = 3.978$, $p=0.01$, CSAq $F(3,93) = 5.196$, $p < 0.01$, PFPT $F(3,93) = 4.835$, $p < 0.01$, and DFPT $F(3,93) = 5.120$, $p < .01$. However, there was no significant group effect for KFPT $F(3,93) = .898$, $p=0.445$. Figure 2 shows results from Bonferroni pairwise comparisons regarding energy intake. Active young adults consumed more kilocalories per kg of body weight than sedentary middle-aged adults (34.3 ± 10.2 $kcals \cdot kg \cdot day^{-1}$ vs. 26.8 ± 6.8 $kcals \cdot kg \cdot day^{-1}$, $p=0.039$). Also, protein intake per kg of body weight was higher in active young adults ($1.7 \pm .5$ $g \cdot kg \cdot day^{-1}$) when compared to sedentary young ($1.2 \pm .5$ $g \cdot kg \cdot day^{-1}$, $p< 0.01$) and middle-aged adults ($1.1 \pm .3$ $g \cdot kg \cdot day^{-1}$, $p < 0.01$). In addition, the comparisons revealed that KEPT (201.1 ± 61.9 Nm vs. 140.4 ± 55.4 Nm, $p < 0.01$) and CSAq (69.8 ± 16.6 cm^2 vs. 52.5 ± 13.5 cm^2, $p < 0.01$) were significantly higher in young active adults than sedentary middle-aged adults (Figure 3). Figure 4 depicts lower PFPT in sedentary middle-aged adults (66.3 ± 26.4 Nm) when compared to active young (92.1 ± 31.8

Nm, p = 0.01) and middle-aged adults (93.6 ± 29.6 Nm, p =0.01). In contrast, figure 5 depicts lower DFPT in active middle-aged adults (21.1 ± 10.8 Nm) when compared to sedentary adults of similar age (30.2 ± 11.5 Nm, p =0.039) and sedentary young individuals (30.7 ± 9.4 Nm, p =0.023).

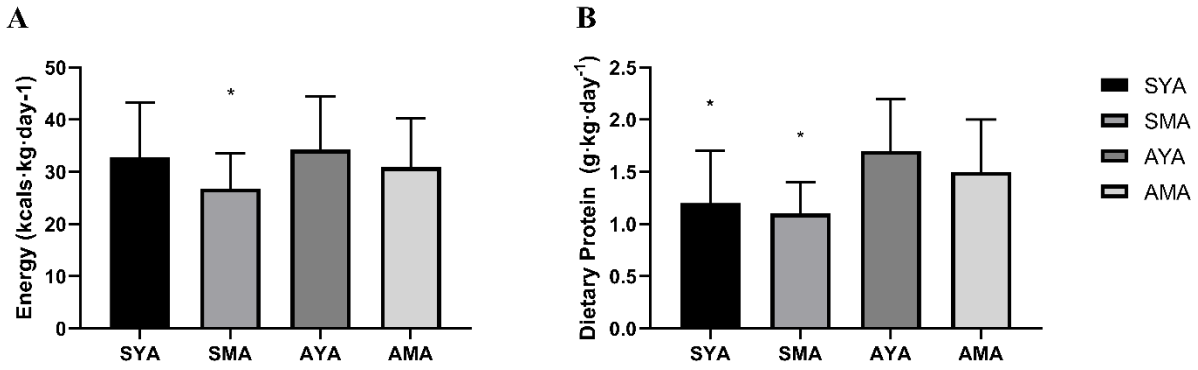

Figure 2. A) Energy Intake by Age and Activity B) Protein Intake by Age and Activity. Values are represented as mean ± SD. *denotes significance from AYA. All significance levels set at p<0.05.

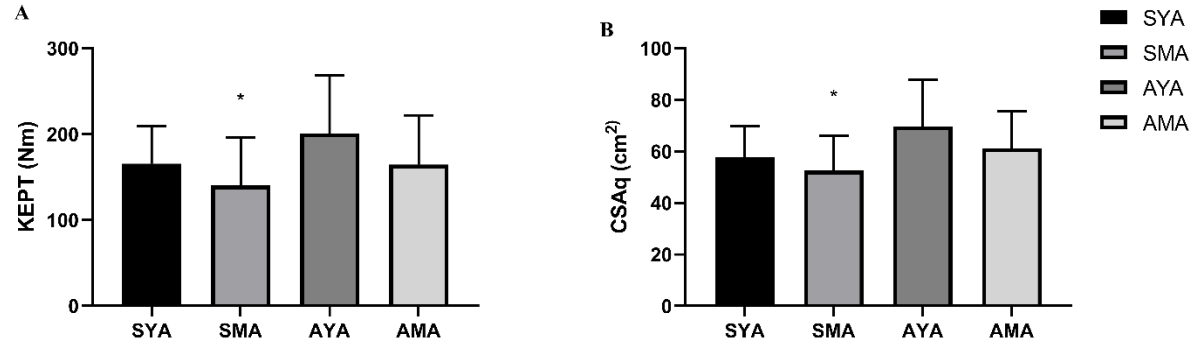

Figure 3. A) Knee Extensor Peak Torque by Age and Activity B) Quadriceps Cross-Sectional Area by Age and Activity. Values are represented as mean ± SD. *denotes significance from AYA. All significance levels set at p<0.05.

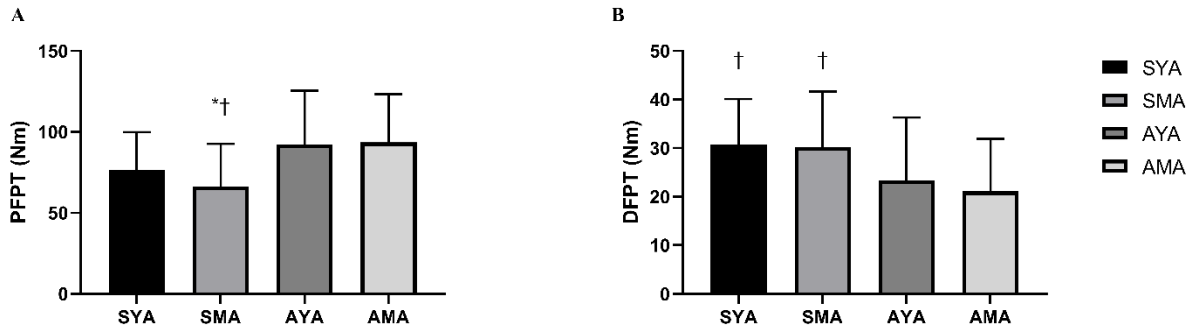

Figure 4. A) Plantar Flexors Peak Torque by Age and Activity B) Dorsi Flexors Peak Torque by Age and Activity. Values are represented as mean ± SD. *denotes significance from AYA, † denotes significance from AMA. All significance levels set at $p<0.05$.

Results of regression analysis of sedentary activity and dietary intake (Table 20) and physical activity (Table 21) on CSAq, KEPT, KFPT, DFPT, and PFPT showed several important relationships. Correlations were $R^2 \geq 0.40$, except for relationships between the independent variables and PFPT (R^2 values ranged between 0.28 and 0.71). Age and leucine significantly predicted 25% of the variance in KEPT ($F(2,94)=15.48$, $R^2=.248$, R^2adjusted=.232, $p < 0.01$) and 18% of the variance in CSAq ($F(2,94)=10.38$, $R^2=0.181$, R^2adjusted=0.163, $p < 0.01$) while only leucine was predictive for KFPT ($F(1,95)=14.72$, $R^2=0.134$, R^2adjusted=0.125, $p < 0.01$, Table 22). MVPA and protein intake ($g \cdot kg \cdot day^{-1}$) had an inverse relationship with DFPT while leucine remained positive ($F(3,93)=14.20$, $R^2=0.314$, R^2adjusted=0.292, $p < 0.01$), and leucine intake and MVPA predicted 14% of the variance in PFPT $F(2,94)=7.57$, $R^2=0.139$, R^2adjusted=0.120, $p < 0.01$, Table 23).

Table 20

Independent Associations between Nutritional Status on Muscle Size and Strength

	Protein (g·kg·day$^{-1)}$		**Energy (kcals·kg·day$^{-1)}$**		**Leucine (g/day)**	
	Estimate	**R^2**	**Estimate**	**R^2**	**Estimate**	**R^2**
CSAq	7.30*	0.70	0.12	0.65	1.25*	0.67
KEPT	17.1*	0.62	-0.09	0.60	6.36*	0.65
KFPT	5.66	0.50	-0.13	0.52	3.28*	0.54
DFPT	-4.38*	0.48	-0.26*	0.48	0.08	0.45
PFPT	11.9*	0.29	0.16	0.25	2.39*	0.28

*p<0.05
Note: estimates were adjusted for sex, age, and body mass index.

Table 21

Independent Associations between Physical Activity on Muscle Size and Strength

	Sedentary Behavior		**Light PA**		**Moderate PA**		**Vigorous PA**		**MVPA**	
	Estimate	**R^2**	**Estimate**	**R^2**	**Estimate**	**R^2**	**Estimate**	**R^2**	**Estimate**	**R^2**
CSAq	0.01	0.65	0.01	0.65	0.20*	0.69	0.78*	0.69	0.21*	0.71
KEPT	-0.02	0.60	0.01	0.60	0.63*	0.63	1.47	0.61	0.61*	0.63
KFPT	0.01	0.52	-0.01	0.80	0.06	0.52	-0.42	0.52	0.03	0.52
DFPT	0.01	0.46	-0.01	0.46	-0.24*	0.56	-0.78*	0.52	-0.25*	0.58
PFPT	0.01	0.25	0.01	0.25	0.55*	0.34	1.88*	0.31	0.56*	0.36

*p<0.05
Note: estimates were adjusted for sex, age, and body mass index.

Table 22

Stepwise Regression Determinants for Predicting Muscle Strength and Size of the Upper Leg

	Unstandardized β	**SE**	**p-value**
KEPT			
Constant	125.75	11.23	<.01
Age	-1.04	.30	<.01
Leucine	11.31	2.47	<.01
R-squared = .248			
Adjusted R-squared = .232			
KFPT			
Constant	77..98	6.69	<.01
Leucine	5.95	1.55	<.01
R-squared = .134			
Adjusted R-squared = .125			
CSAq			
Constant	50.46	2.99	<.01
Age	-.22	.08	.01
Leucine	2.63	.67	<.01
R-squared = .181			
Adjusted R-squared = .163			

Note: n = 97, KEPT = knee extensors peak torque, KFPT = knee flexors peak torque, CSAq = quadriceps cross sectional area

Table 23

Stepwise Regression Determinants for Predicting Muscle Strength of the Lower Leg

	Unstandardized β	SE	p-value
DFPT			
Constant	37.17	3.20	<.01
MVPA	-.25	.06	<.01
Protein	-7.71	2.72	<.01
Leucine	2.35	.63	<.01
R-squared = .314			
Adjusted R-squared = .292			
PFPT			
Constant	52.02	8.13	<.01
MVPA	.41	.16	.01
Leucine	3.78	1.36	.01
R-squared = .139			
Adjusted R-squared = .120			

Note: n = 97, DFPT = dorsiflexors peak torque, PFPT = plantar flexors peak torque

4.5. Discussion

This study aimed to evaluate nutrition and physical activity contributors to lower limb skeletal muscle size and strength in sedentary and active middle-aged and young adults. The main findings were that age and leucine significantly predicted 25% of the variance in KEPT and 18% of the variance in CSAq, leucine predicted 13% for KFPT, and leucine and MVPA predicted 14% of the variance in PFPT. Interestingly, MVPA and protein ($g \cdot kg \cdot day^{-1}$) had an inverse relationship with DFPT while leucine remained positive. Additionally, active young adults consumed 25% more kilocalories than sedentary middle-aged adults and 34-42% more protein ($g \cdot kg \cdot day^{-1}$) than sedentary young and middle-aged adults, respectively. They also had

33% higher KEPT, 27% higher CSAq, and 30% higher PFPT than sedentary middle-aged adults. Active middle-aged adults had 34% greater PFPT than their sedentary age matched peers. Unexpectedly, DFPT was 35-37% lower in active middle-aged adults than sedentary middle-aged and young adults, respectively. No significant differences were observed for KFPT between groups.

Adequate nutrient and energy intake from consumed foods are essential in the maintenance of muscle mass and physical function as insufficient intakes lead to catabolism (Cruz-Jentoft et al., 2017; Volpi et al., 2013). A "threshold dose" of protein and leucine of 21-40 and 3 grams per meal, respectively, may be of particular importance due to its role in muscle protein balance (Baum et al., 2016; Beaudart et al., 2017; Cruz-Jentoft et al., 2017; Kramer et al., 2017; Landi et al., 2016; Meskers et al., 2019; Verlaan et al., 2018; Volpi et al., 2013). Furthermore, it has been suggested that the current recommendation of 0.8 $g \cdot kg \cdot day^{-1}$ is not optimal for older adults (Baum et al., 2016; Landi et al., 2016; Verlaan et al., 2018; Volpi et al., 2013). Leucine had protective effects on whole body lean mass, whole body fat mass, body fat percentage, and KEPT but not DFPT following disuse in adults aged 45-60 years (English et al., 2016). Additionally, a leucine-enriched bolus of ~21 g of whey protein and 3g of leucine has shown to increase muscle protein synthesis in sarcopenic men with no difference from healthy men (Kramer et al., 2017). Our results are in line with the previous findings as leucine contributed significantly to KEPT, KFPT, PFPT, DFPT, and CSAq.

However, protein intake ($g \cdot kg \cdot day^{-1}$) was inversely associated with DFPT which was unexpected but difficult to explain given the lack of examination regarding the muscle of the lower leg. Though muscle groups of the lower extremity are associated with high individual variability particularly regarding age, height and body mass, they were examined in this study

because they are most related to functional activities (Buckinx & Aubertin-Leheudre, 2019; Harbo et al., 2012; Harris-Love et al., 2018). These associations were apparent in the knee extensors and flexors but not the ankle extensors and flexors (Buckinx et al., 2015). Additionally, strength losses of 24-30% over 12 years have been reported in the knee extensors and flexors while the elbow flexors and extensors ranged from 16-19%, respectively (Frontera et al., 2000). Furthermore, it has been suggested that the decrease in muscle quality associated with age-related decreases in strength are greater in the lower limbs when compared to the upper limbs (Buckinx & Aubertin-Leheudre, 2019). Age-related reductions in muscle size as measured by cross-sectional area have also been reported with the greatest losses reflected in the quadriceps (~16%) (Frontera et al., 2000). Though the result for dorsiflexion were not as expected and the results of the plantar flexors show promise the most commonly examined muscle groups of the leg are the knee extensors and flexors (Buckinx & Aubertin-Leheudre, 2019)

An obvious beneficial relationship between physical activity and muscle health in those over the age of 60 years is evident and it appears that the type of PA (e.g., leisure time, structured exercise, and occupational) is of little importance (Beaudart et al., 2017; Steffl et al., 2017). Furthermore, physical inactivity and disuse can negatively affect muscle protein synthesis contributing to anabolic resistance that has been observed in older adults (Landi et al., 2016). Physical activity has been associated with a 26% reduction in risk for developing functional impairments (Bradlee et al. 2018). In our study, active middle-aged adults had greater PFPT than their sedentary peers, regardless of age and MVPA and leucine were associated with greater PFPT. Previous research showed that individuals who consumed animal based proteins which are high in leucine compared to other types of protein had significantly higher skeletal muscle mass regardless of physical activity (Bradlee et al., 2018). Unsurprisingly, those who were more

active and consumed more protein had the highest percentages of skeletal muscle mass indicating a symbiotic relationship between protein intake and physical activity (Bradlee et al., 2018). After 12 weeks of walk training combined with 31g of casein protein or placebo supplementation body composition, muscle contractility and function, and VO_{2max} significantly improved in all 114 physically active sexagenarians with adequate protein intakes (ten Haaf et al., 2019). Interaction effects were observed for lean body mass and fat mass only indicating a contribution of physical activity in body composition, muscular health, cardiovascular health, and physical function with the potential of additional benefits from increased protein (ten Haaf et al., 2019). Similar improvements were observed in 60-89 year olds following 16 weeks of concurrent training, however, no additional benefits were observed from supplementing leucine-rich whey protein thrice per day (Kirk et al., 2019). Interestingly, ten Haaf et al. (2019) showed additional improvements regarding body composition in older adults who were consuming just over the RDA for protein at baseline (~0.89 $g \cdot kg \cdot day^{-1}$). At the same time, Kirk et al. (2019) did not observe additional benefits in those consuming >1.0 $g \cdot kg \cdot day^{-1}$ of dietary protein at baseline.

This study was limited by self-reported amounts and types of concurrent physical activity. In addition, though the wrist accelerometery accounts for rhythmic aerobic activity it may not include stationary activities, water activities, and resistive exercises. Nutrient intakes were also self-reported as participants were asked to estimate their food amounts using supplemental handouts equating portion sizes to the hands and common household items. Nevertheless, this study was a significant contribution to research on sarcopenia by contributing to the literature on middle-aged adults. Muscle strength has been observed to peak in early adulthood and start declining during midlife in many populations regardless of overall health (Dodds et al., 2016; Shafiee et al., 2017). Sarcopenia has been reported in individuals aged 20

and older with prevalence ranging from 19.2-423% (Bae & Kim, 2017). Regardless, most of the sarcopenia research has been performed in individuals 60 years of age or older. The need for studies that directly compare young adults and middle-aged adults such as this one has been specifically expressed (English et al., 2016). Additionally, this study contributes to the literature on aging adults by examining nutrition and physical activity together as contributors to muscle health in specific points of the human lifespan.

4.6. Conclusion

This study contributes greatly to the literature regarding symptoms of sarcopenia in middle age. Specifically, the results suggest that factors including protein intake, leucine intake, physical activity, and concurrent training interact to contribute to muscle health with aging. Additionally, these findings indicate that muscle strength can be preserved with concurrent activity regardless of age and that additional benefits may occur with adequate protein and leucine intakes.

5. INTERMITTENT BFR RAPIDLY IMPROVES MUSCULAR AND CARDIOVASCULAR HEALTH IN MIDDLE AGE WITHOUT ADDITIONAL BENEFITS FROM PROTEIN SUPPLEMENTATION

5.1. Abstract

Sarcopenia is a debilitating disease affecting many over the age of 20 years. Exercise combined with protein supplementation, is a promising countermeasure. Blood flow restricted (BFR) exercise is an effective exercise modality, but benefits of combining this therapy with protein is understudied. The purposes of this study were to determine if blended protein supplementation combined with BFR exercise can favorably alter body composition, muscle function, and cardiovascular health. **Methods:** 16 participants (female = 50%, age = 36.0 ± 10.1 yrs., BMI = 27.2 ± 5.0 kg/m^2) completed two weeks of familiarization and four weeks of BFR training for the upper and lower limbs thrice per week. The participants were randomly assigned to ingest daily shakes consisting of a 41g of whey and casein blended protein (28g protein) or an isocaloric placebo. Dual energy x-ray absorptiometry (DXA) derived body composition, muscle strength assessed via isokinetic and handgrip dynamometry, muscle function assessed via sit-to-stand, gait speed, timed up and go, and balance, and cardiovascular health assessed via resting heart rate and blood pressure were assessed pre and post-intervention. Dietary intake was recorded using 3-day food diaries and analyzed using ESHA software prior to, halfway through, and at the end of the intervention. **Results:** Mean protein intakes for the supplement and placebo groups were (1.6±.2 $g \cdot kg \cdot day^{-1}$ and 1.0±.2 $g \cdot kg \cdot day^{-1}$, respectively). Total leucine intake ($g \cdot day^{-1}$) increased by ~38% with supplementation. There were significant time effects for training volume in all exercises, sit-to-stand repetitions, gait speed, and resting heart rate ($p<0.05$). **Conclusion:** These results indicate that exercise with intermittent BFR can improve muscle

strength and function while positively influencing cardiovascular health in middle-aged adults, however there was no additional benefit to increasing protein and leucine above the recommended intakes.

5.2. Introduction

Sarcopenia is a muscle disease that is characterized primarily by low muscle strength (Cruz-Jentoft et al., 2019). Diagnosis of this disease is confirmed when low muscle quantity or quality are also present and increasing severity includes low muscle strength, quantity or quality, and function (Cruz-Jentoft et al., 2019). Sarcopenia is generally associated with old age, however, a prevalence of 19.2% has been reported in those as young as 20 years and this value increases at age 60 and beyond (Bae & Kim, 2017). The population of those over 60 years currently makes up over 420 million adults worldwide and is expected to reach over 2.1 billion between 2030 and 2050 (UN, 2015; Ilgili et al., 2014). This increase in older adults creates vulnerability to sarcopenia and will likely lead to reduced quality of life and increased healthcare costs making early intervention essential (Chodzko-Zajko et al., 2009; Janssen et al., 2004).

Sarcopenia has many overlapping mechanisms that are not fully understood but provide sufficient evidence to develop countermeasures for early intervention. Exercise is widely accepted as the gold standard for increasing and maintaining muscle function and additional benefits from leucine-rich protein supplementation have been suggested (Chodzko-Zajko et al., 2009; Chodzko-Zajko et al., 2009; Cruz-Jentoft et al., 2017; Morley et al., 2010). Concurrent exercise has been suggested for prevention of sarcopenia and the maintenance and improvement of muscular and cardiovascular health (Garber et al., 2011; Morley et al., 2010). Concurrent training includes resistance training using 60-80% of an individual's 1-RM which may be too physiologically demanding for older adults (Chodzko-Zajko et al., 2009; Cook et al., 2017;

Garber et al., 2011). Blood flow restriction (BFR) exercise may be an efficient alternative as it has shown similar improvements in muscle size and strength to traditional resistance training while using only 20-30% of 1-RM (Cook et al., 2017; Hackney et al., 2016; Karabulut et al., 2010). Furthermore, BFR exercise may simulate concurrent exercise as it has previously improved vascular function in healthy older adults (Shimizu et al., 2016).

Though several studies have shown benefits regarding muscle size, strength, and function when combining traditional high load resistance, aerobic, or concurrent training and leucine-rich protein, few have examined supplementation of these products with BFR training (Kirk et al., 2019; Mori & Tokuda, 2018; Rondanelli et al., 2011; ten Haaf et al., 2019). However, two studies have reported increases in the muscle size following BFR training and protein or amino acid supplementation in middle-aged and older adults indicating promise as a countermeasure for one diagnostic criteria of sarcopenia (Centner et al., 2019; Hackney et al., 2016). Therefore, the purposes of this study were to determine if BFR combined with a leucine-rich protein supplement could: 1) improve muscular strength, function, or quality; 2) favorably alter body composition; 3) simulate concurrent exercise by improving indices of cardiovascular health.

5.3. Methods

5.3.1. Participants

A total of 16 healthy volunteers were recruited for the investigation and randomly assigned to one of two received one of two conditions: 1) protein or 2) placebo supplementation (Table 24). Participants included in this study were generally healthy as determined by the 2018 PAR-Q+ and recreationally active but not participating in regular structured exercise (PAR-Q+, 2018). Participants' activity was evaluated using the 2002 IPAQ, those participating in up to 420 minutes of moderate to vigorous physical activity were considered to be recreationally active

(IPAQ, 2002). Participants were also given a categorical PA classification where a score of 1 equates to low PA, 2 equates to moderate PA, and 3 equates to high PA (Ara, 2005). There were no significant differences between groups for age, BMI, or PA classification at baseline ($p < 0.05$).

Table 24

Participant Demographics

	Supplement (n=8)	**Placebo (n=8)**
Female (n (%))	4 (50)	4 (50)
Age (years)	31.1 ± 8.2	40.8±10.5
BMI (kg/m^2)	28.7 ± 4.6	25.3±5.1
PA classification	2.0 ± 0.5	1.9 ± 0.6

Mean ± SD, BMI = body mass index, PA = physical activity

5.3.2. Documentation

Prior to data collection, this research was approved by the North Dakota State University (NDSU) and Sanford Health (Fargo, ND) Institutional Review Boards. After completion of the informed consent, PAR-Q, IPAQ, Illinois State Medical Society Deep Vein Thrombosis (DVT) Questionnaire and additional DVT questions participants were screened for this study (ISMS, 2013). Participants were excluded from this study if: 1) they were pregnant or believed they could be pregnant; 2) they used tobacco in any form; 3) they had a previous diagnosis of a metabolic disease, cardiovascular disease, neuromuscular disorder, renal disease, exertional rhabdomyolysis, sickle cell anemia/trait, or were being treated for cancer; 4) they had significant mobility limitations or previous injuries that limit the ability to exercise safely; 5) they were taking medications that were known to directly influence muscle protein metabolism; 6) they were diagnosed with class III obesity; 7) were using hormonal contraceptives; or 8) they were at an increased risk for DVT.

5.3.3. Procedures

Two groups completed this double-blind, randomized, placebo controlled study. Following a general 5-minute warmup, participants completed the following BFR exercises three times per week for four weeks: leg extension, leg curl, biceps curl, and triceps extension. Prior to training, all participants completed two weeks of familiarization to exercise in which they came to the lab three times per week. During week one of familiarization, participants completed 10-repetition maximum testing for all exercise to determine initial training load (20% of 1-RM for upper body and 30% of 1-RM for lower body) and two exercise sessions without BFR (Cook et al., 2017; Kim et al., 2017; Vechin et al., 2015). During week two of familiarization, they completed three exercise sessions with 40% occlusion applied during the last set of each exercise. Exercises were completed to a tempo of 30bpm. Each exercise was comprised of four sets and participants were instructed to complete as many reps as possible until they reached 30, 15, 15, and 15 repetitions or volitional failure. While the cuff was inflated, participants exercised one limb while the other was resting without inflation giving 1-2 minutes of rest between sets. Participants were given three minutes of rest between exercises. Exercise progression occurred via increasing occlusion pressure from 40%-50% for the upper body and 40%-80% for the lower body or via increasing load in 2-5 kg increments (Loenneke et al., 2015; Tennent et al., 2017; Vechin et al., 2015). Load was increased when participants could complete two additional repetitions for two sets on any exercise. Load and occlusion pressure were not increased simultaneously.

Participants were randomly assigned to consume either a daily whey/casein blend protein shake (~ 41 total g, 163 kcals, 1 g fat, 10 g carbohydrate, 28 g protein, and 3 g of leucine) or an isocaloric placebo of the same flavor for the four weeks following familiarization. Prior to

familiarization, all participants completed pre-testing measures including: anthropometrics, isokinetic leg strength, STS, handgrip strength, balance, gait speed, TUG, resting heart rate and blood pressure, and were given a three-day dietary to be completed over one week. Halfway through the study, participants were given a second three-day dietary log. Within three days of completing training, participants repeated the pre-testing measures. All supplements and placebos that were not consumed were returned for compliance measurement.

5.3.4. Measures

5.3.4.1. Anthropometrics and Body Composition

Height and weight were measured using a standard stadiometer (Seca 213, Chino, CA) and scale (Denver Instrument DA-150, Arvada, CO). Lean body mass, body fat percentage, appendicular lean body mass, and skeletal muscle index was assessed using DXA (GE Healthcare Lunar Prodigy, Chicago, IL, US). All biologically female participants took a pregnancy test (ClinicalGuard, Atlanta, GA, US) prior to each scan. Upon verification, participants were asked to remove any jewelry or metal that may interfere with the scan and were positioned appropriately in the scanner. Scans took 5-12 minutes.

5.3.4.2. Muscular Strength

Muscle strength of the upper body was assessed using a Jamar handgrip dynamometer (Jamar Plus, Bollingbrook, IL, US). Participants were instructed to hold the dynamometer in their dominant hand with a 90° bend at the elbow and squeeze as hard as possible for three seconds. They completed three attempts with one minute rest between sets. The best attempt was used in analysis. Muscular lower body was assessed using a Biodex Pro4 System dynamometer (Biodex Medical Systems, Shirley, NY, US). To examine the isokinetic strength of the knee flexors and extensors the participants were seated in an upright position and would move the leg

through flexion and extension at an angular velocity of 60 degrees/second. The upper leg moved through a range of motion of 95° flexion and 20° extension. A back pad was used to achieve a trunk angle of 90° when necessary. The center of the dynamometer was aligned with the subjects' lateral epicondyle and the shin pad was placed approximately 3-5 cm above the tongue of the participants' shoe, just above the lateral malleolus. The protocol consisted of one warm-up set of three repetitions at 60 degrees/second with the participants contributing no more than 85% effort on the final repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during extension and flexion. Upon completion of this protocol, participants were released from the chair while the researchers set up for assessment of the lower leg. To examine the strength of the ankle dorsiflexors and plantar flexors the participants were seated in an upright position at 70° tilt with the hamstrings supported. Participants would move the ankle at an angular velocity of 30 degrees/second. The center of the dynamometer was aligned with the individuals' lateral malleolus. The heel was supported by a heel cup and the foot was strapped tightly to the foot plate. The protocol consisted of one warm-up set of three repetitions at 30 degrees/second with the participant contributing no more than 85% effort on the final repetition followed by 30 seconds of rest and three maximal effort repetitions to determine peak torque (N-M) during plantarflexion and dorsiflexion. Muscle quality was assessed by dividing knee extensor peak torque (KEPT) by DXA derived lean body mass of the right leg as a measure of relative strength. Average weekly training volume (weight x sets x repetitions) was calculated for each exercise as a measure of dynamic strength.

5.3.4.3. Muscle function

Gait speed (m/s) was measured using a six meter course and timed using the Brower TCi system (Draper, UT). Participants were instructed to walk at their normal pace. To obtain a

consistent speed without acceleration or deceleration, start and stop points were marked two meters before and after the timing gates by cones. Timed up and go was completed using the same course with a cone placed at the halfway mark, a 43cm chair, and a standard stopwatch. Participants were instructed to rise from the seat, walk to and around the cone, and return to their seat at a normal pace. Participants completed one untimed and two timed trials. Mean time was used in analyses.

Participants also performed a STS using a 43 cm chair. Participants were instructed to cross their arms and find a comfortable seat that they could continuously sit and stand from. They were allowed two repetitions of practice to adjust this position. They were then instructed to complete as many repetitions as possible in thirty seconds. The total number of repetitions completed was used in the analysis.

Balance was assessed using the BESS protocol on the Balance System SD (Biodex Medical Systems, Shirley, NY, US). Participants were instructed to remove their shoes and step onto the platform. Conditions included a double leg stance, single leg (non-dominant) stance, and a tandem stance with the non-dominant foot back. Each condition was completed on a firm surface and then using an Airex pad for a total of six trials. Each trial lasted 20 seconds, there was 10 seconds of rest between trials, and errors were scored by trained research assistants. Errors were only counted once and included moving hands from the hips, opening the eyes, stepping/stumbling/falling, hip abduction, or any deviation from position for more than five seconds. The composite score provided by the system was used in the analysis.

5.3.4.4. Cardiovascular Health

Following five minutes of supine rest, heart rate was assessed using a polar heart rate strap and watch (Polar, Kempele, FI). Resting blood pressure was also measured manually with a

3M Littman stethoscope (3M, Maplewood, MN, US) and Diagnostix 703 sphygmomanometer (American Diagnostic Corporation, Hauppauge, NY). If readings were over 140/90 mmHg or undetectable, participants were given an additional two minutes of rest and the measure was repeated.

5.3.4.5. Dietary Intake

To examine dietary intake, participants completed three 3-day food diaries. Participants were asked to log everything they ingested on two typical days (i.e. weekdays) and one atypical day (i.e. weekend day) in the week following muscle function testing. Once completed, RDs analyzed the protein (both grams per subject and $g \cdot kg^{-1}$ per subject), other nutrients, and the within-day-distribution of protein intake using Food Processor Nutrition Analysis software (ESHA, Salem, OR).

5.3.5. Statistical Analysis

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY) and SAS version 9.4 (SAS Institute; Cary, NC). Descriptive statistics are reported as mean ± SD. Statistical significance was set at $\alpha = 0.05$. Independent samples t-test was used to compare group differences in participant demographics. Separate 2 x 2 repeated measures ANOVAs assessed the differences in body fat percentage, total lean mass (LBM), appendicular lean body mass (aLM), Skeletal muscle mass index (SMI), KEPT, KFPT, PFPT, DFPT, muscle quality, 30-second sit-to-stand, hand grip strength, gait speed, balance composite score, timed up and go (TUG), resting heart rate, and resting blood pressure. Separate group (supplement vs. placebo) by time (pre-testing vs. mid-testing vs. post-testing) mixed ANOVAs assessed the differences in average training volume for each exercise, protein intake ($g \cdot kg \cdot day^{-1}$), energy intake ($kcals \cdot kg \cdot day^{-1}$), total macronutrient intake (total grams of carbohydrate, protein, and fat), and

total leucine intake (g). Partial Eta Squared (η^2) was used to determine effect size. Separate linear mixed models were also conducted to evaluate the differences in aLM, LBM, SMI, body fat percentage, resting heart rate, resting blood pressure, protein intake ($g \cdot kg \cdot day^{-1}$), muscle quality, KEPT, KFPT, PFPT, DFPT, STS, handgrip strength, TUG, gait speed, and balance composite score between the protein supplementation and placebo groups when adjusted for sex, age, and body mass index.

5.4. Results

5.4.1. Compliance and Energy Intake

All participants (n=16) completed 18 ± 1 days of BFR training and were 99.1% compliant with post-exercise and non-training day consumption of the supplement or placebo shakes. Table 25 shows total energy intake and macronutrient composition. There were no significant supplement condition x time interaction effects for energy intake ($kcals \cdot kg \cdot day^{-1}$) $F(2,28) = 1.079$, $p = .354$, $\eta^2 = .072$, total protein intake ($g \cdot day^{-1}$) $F(2,28) = .795$, $p = .415$, $\eta^2 = .054$, protein intake ($g \cdot kg \cdot day^{-1}$) $F(2,28) = 1.285$, $p = .367$, $\eta^2 = .064$, carbohydrate intake $F(2,28) = .939$, $p = .403$, $\eta^2 = .063$, or fat intake $F(2,28) = 1.796$, $p = .184$, $\eta^2 = .114$. Furthermore, there were no main effects for time or condition in regard to energy intake ($kcals \cdot kg \cdot day^{-1}$), carbohydrate intake, or fat intake ($p > .05$). However, there were significant supplement condition effects for total protein intake ($g \cdot day^{-1}$) $F(1,14) = 11.726$, $p = .004$, $\eta^2 = .456$ and protein intake ($g \cdot kg \cdot day^{-1}$) $F(1,14) = 5.744$, $p = .031$, $\eta^2 = .291$. There was a significant supplement x time interaction effect for leucine intake (g/day) $F(2,28) = 7.478$, $p = .002$, $\eta^2 = .348$. Comparison of simple effects showed leucine intake was significantly higher in the supplemented group than the placebo group mid-training (6.36 ± .88 g/day vs. 1.24 ± .65 g/day, p =.001) and post-training (5.14 ± .64 g/day vs. 1.76 ± .64 g/day, p = .000). Furthermore, these

comparisons showed a significant increase from baseline to mid-training in the supplemented group ($p = .022$).

Table 25

Total Energy and Macronutrient Intake

	Supplement + BFR (n=8)			Placebo + BFR (n=8)		
	Pre	**Mid**	**Post**	**Pre**	**Mid**	**Post**
Energy (kcals·kg·day^{-1})	30.1 ± 13.0	28.1 ± 8.6	28.3 ± 8.5	27.1 ± 13.8	30.8 ± 11.1	29.9 ± 10.5
Protein (g·kg·day^{-1})†	1.6 ± .8	1.7 ± .7	1.5 ± .4	1.1 ± .3	1.0 ± .3	1.0 ± .3
Protein (g/day)†	138.9 ± 74.2	148.0 ± 61.9	135.8 ± 43.2	76.0 ± 16.4	66.3 ± 7.3	67.6 ± 25.4
Leucine (g/day)	3.5 ± 3.2	6.4 ± 3.5$^{*\#}$	5.6 ± 1.5*	2.6 ± 1.7	1.2 ± .7	1.5 ± 1.6
Carbohydrate (g/day)	239.0 ± 68.6	244.8 ± 78.8	261.8 ± 81.9	210.7 ± 113.8	258.2 ± 81.2	240.9 ± 71.3
Fat (g/day)	129.3 ± 66.4	103.2 ± 41.2	103.9 ± 48.2	74.0 ± 18.0	86.4 ± 14.1	86.3 ± 23.7

Mean ± SD. BFR= Blood Flow Restriction *significantly higher than Placebo + BFR, $p < .01$. $^{\#}$significantly higher than baseline, $p < .05$. †main effect of supplement condition, $p < .05$.

5.4.2. Body Composition

There were no significant supplement condition x time interaction effects for body fat percentage $F(1,14) = .036$, $p = .853$, $\eta^2 = .022$, LBM $F(1,14) = .800$, $p = .386$, $\eta^2 = .054$, aLM $F(1,14) = ..989$, $p = .337$, $\eta^2 = .066$, and SMI $F(1,14) = 1.978$, $p = .181$, $\eta^2 = .124$ (Table 26). Furthermore, there were no main effects for time or condition in regard to body fat percentage, LBM, aLM, or SMI ($p > .05$).

Table 26

Body Composition during Intervention

	Protein + BFR (n=8)		**Placebo + BFR (n=8)**	
	Pre	**Post**	**Pre**	**Post**
aLM (kg)	27.2 ± 5.4	27.3 ± 6.9	22.2 ± 7.3	22.8 ± 6.9
LBM (kg)	56.7 ± 10.7	56.8 ± 10.3	47.6 ± 12.6	48.7 ± 11.6
SMI (kg/m^2)	9.2 ± 2.4	9.2 ± 2.4	7.7 ± 2.8	8.0 ± 2.8
Body Fat (%)	31.7 ± 11.9	31.6 ± 12.1	30.7 ± 5.1	30.5 ± 5.4

Mean ± SD, aLM = appendicular lean body mass, LBM = lean body mass, SMI = skeletal muscle index

5.4.3. Muscle Strength

There were no significant supplement condition x time interaction effects for KEPT $F(1,14) = .472$, $p = .503$, $\eta^2 = .033$, KFPT $F(1,14) = .941$, $p = .349$, $\eta^2 = .063$, PFPT $F(1,14) = .100$, $p = .756$, $\eta^2 = .007$, and DFPT $F(1,14) = .668$, $p = .427$, $\eta^2 = .046$. Furthermore, there were no main effects for time or condition in regard to KEPT, PFPT, or DFPT ($p > .05$). However, there was a significant time effect for KFPT (g/day) $F(1,14) = 6.061$, $p = .027$, $\eta^2 = .302$ (Figure 6).

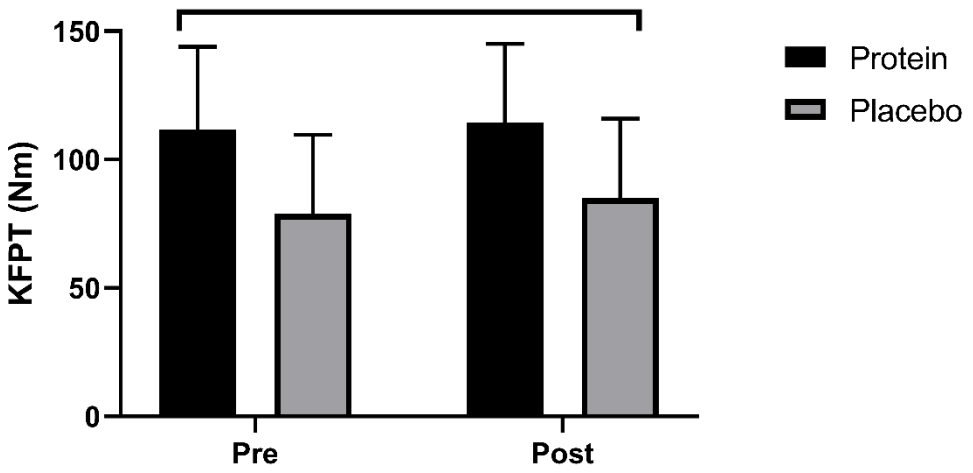

Figure 5. Knee Flexors Peak Torque by Group and Time. Values are represented as mean ± SD. Brackets denote significant time effect ($p<0.05$).

There was no significant supplement condition x time interaction effect $F(1,14) = .006$, $p = .941$, $\eta^2 = .000$, supplement effect $F(1,14) = 1.317$, $p = .270$, $\eta^2 = .086$, or time effect $F(1,14) = 1.762$, $p = .206$, $\eta^2 = .112$ for muscle quality. There was also no significant supplement condition x time interaction effect $F(1,14) = .561$, $p = .466$, $\eta^2 = .039$, supplement effect $F(1,14) = 2.276$, $p = .154$, $\eta^2 = .140$, or time effect $F(1,14) = .286$, $p = .601$, $\eta^2 = .020$ for handgrip strength. Additionally, there were no significant supplement condition x interaction time effects for leg extension volume $F(2,28) = 1.451$, $p = .251$, $\eta^2 = .094$, leg curl volume $F(2,28) = 3.804$, $p = .057$, $\eta^2 = .214$, biceps curl volume $F(2,28) = 2.244$, $p = .146$, $\eta^2 = .138$, and triceps extension volume $F(2,28) = .492$, $p = .617$, $\eta^2 = .034$. Furthermore, there were no significant supplement effects for leg extension volume $F(1,14) = 4.218$, $p = .059$, $\eta^2 = .232$, leg curl volume $F(1,14) = .264$, $p = .615$, $\eta^2 = .019$, biceps curl volume $F(1,14) = .990$, $p = .337$, $\eta^2 = .066$, and triceps extension volume $F(1,14) = .078$, $p = .785$, $\eta^2 = .006$. However, there were significant time effects for leg extension volume $F(2,28) = 19.908$, $p < .001$, $\eta^2 = .587$, leg curl volume $F(2,28) = 18.354$, $p < .001$, $\eta^2 = .567$, biceps curl volume $F(2,28) = 41.493$, $p < .001$, $\eta^2 = .748$, and triceps extension volume $F(2,28) = 60.834$, $p < .001$, $\eta^2 = .813$. Figure 7 displays significant differences for

training volume from pre-mid training for the following exercises: leg extension (2213.43 ± 223.61 vs. 2826.44 ± 290.20, $p < .01$), leg curl (1734.60 ± 234.80 vs. 2973.65 ± 394.36, $p < .01$), biceps curl (609.10 ± 79.56 vs. 1117.65 ± 82.37, $p < .01$), and triceps extension (412.12 ± 35.79 vs. 879.41 ± 74.30, $p < .01$). Triceps extension was the only exercise to have significant differences in training volume from mid-post-training (879.41 ± 74.30 vs. 1114.92 ± 71.67, $p < .01$). Pairwise comparisons also showed significant differences in training volume from pre-testing to post-testing for the following exercise: leg extension (2213.43 ± 223.61 vs. 3040.23 ± 260.90, $p <.01$), leg curl (1734.60 ± 234.80 vs. 3324.18 ± 435.4, $p < .01$), biceps curl (609.10 ± 79.56 vs. 1183.27 ± 78.62, $p < .01$), and triceps extension (412.12 ± 35.791114.92 ± 71.67, $p < .01$).

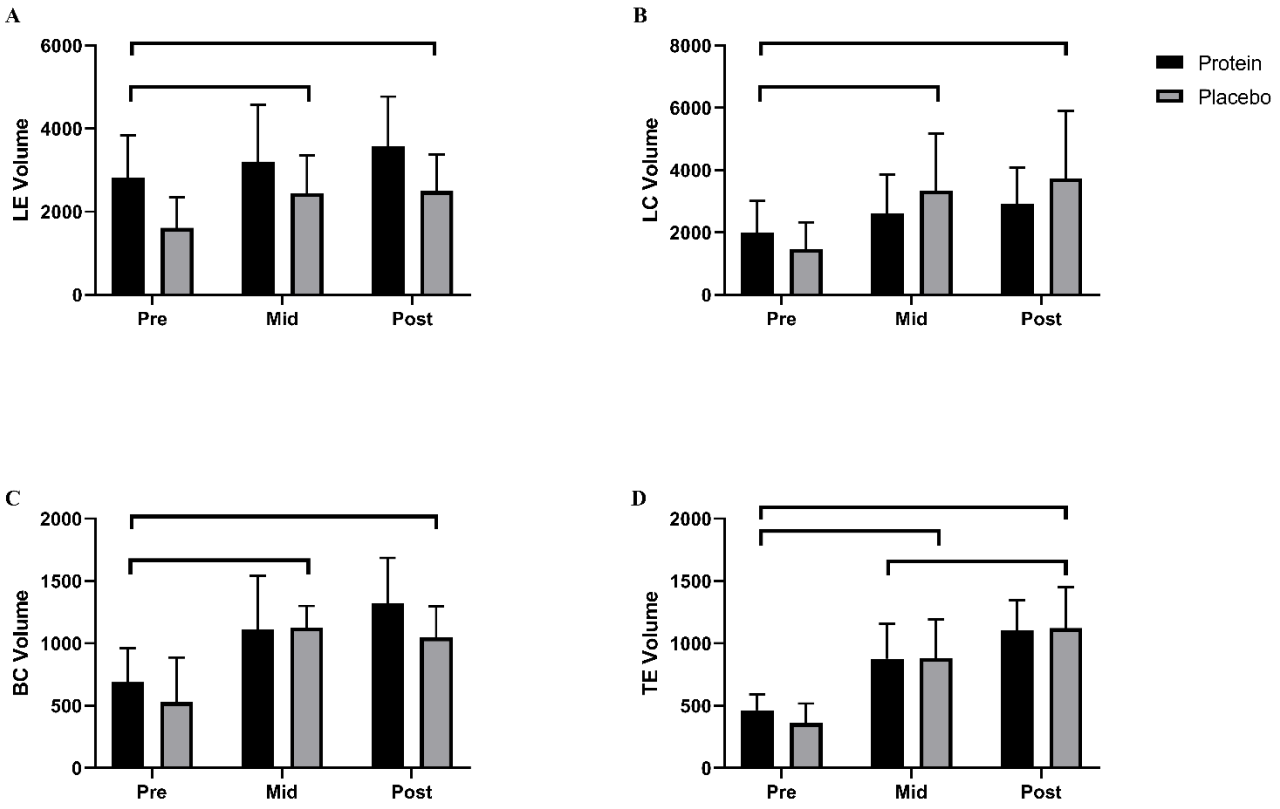

Figure 6. A) Leg Extension Training Volume by Group and Time. B) Leg Curl Training Volume by Group and Time. C) Biceps Curl Training Volume by Group and Time. D) Triceps Extension Training Volume by Group and Time. Values are represented as mean ± SD. Brackets denote significant time effect ($p<0.01$).

5.4.4. Muscular Function

There were no significant supplement condition x time interaction effects for STS F(1,13) = .233, p = .637, η^2 = .018, gait speed F(1,14) = .044, p = .837, η^2 = .003, balance composite score F(1,14) = 1.369, p = .262, η^2 = .089, or timed up and go F(1,14) = .225, p = .642, η^2 = .016. Furthermore, there were no significant supplement effects for STS F(1,13) = .275, p = .609, η^2 = .021, gait speed F(1,14) = .044, p = .837, η^2 = .003, balance composite score F(1,14) = .554, p = .469, η^2 = .038, or TUG F(1,14) = .225, p = .642, η^2 = .016. There were also no significant time effects for balance composite score F (1,14) = 2.115, p = .168, η^2 = .131 or timed up and go F (1,14) = 2.098, p = .170, η^2 = .130. However, there were significant time effects for STS F(1,13) = 10.937, p = .006, η^2 = .457 and gait speed F(1,14) = 5.162, p = .039, η^2 = .269 (Figure 8).

5.3.5. Cardiovascular Health

There were no significant supplement condition x time interaction effects for resting heart rate F(1,14) = 4.256, p = .058, η^2 = .233, resting systolic blood pressure F(1,14) = .423, p = .526, η^2 = .029, or resting diastolic blood pressure F(1,14) = .026, p = .875, η^2 = .002. Furthermore, there were no significant supplement effects for resting heart rate F(1,14) = .180, p = .678, η^2 = .013, resting systolic blood pressure F(1,14) = .423, p = .526, η^2 = .029, or resting diastolic blood pressure F(1,14) = .026, p = .875, η^2 = .002. There were also no significant time effects for resting systolic blood pressure F(1,14) = .883, p = .363, η^2 = .059 or resting diastolic blood pressure F(1,14) = 1.256, p = .281, η^2 = .082. However, there was a significant time effect for resting heart rate F(1,14) = 5.840, p = .030, η^2 =.294 (Figure 9).

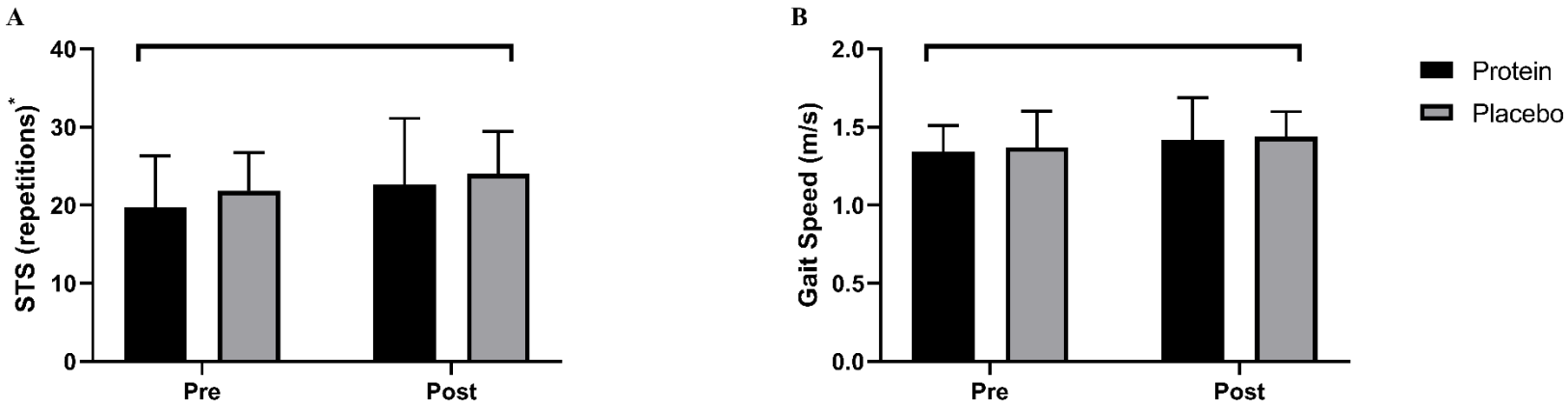

Figure 7. A) 30 second sit-to-stand by Group and Time. *n = 15 B) Gait Speed by Group and Time. Values are represented as mean ± SD. Brackets denote significant time effect ($p<0.05$).

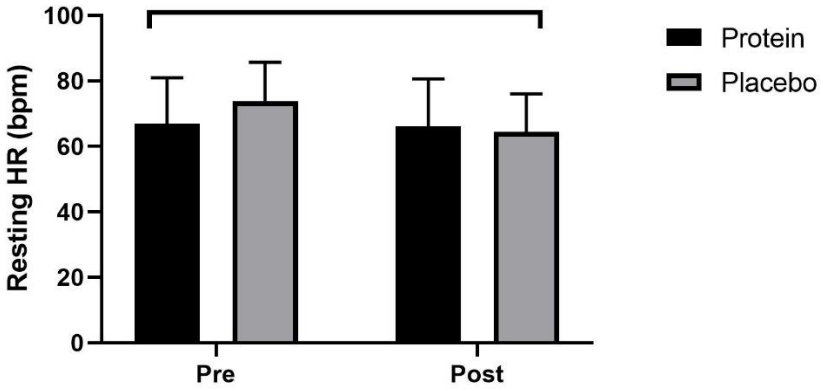

Figure 8. Resting Heart Rate by Group and Time. Values are represented as mean ± SD. Brackets denote significant time effect ($p<0.05$).

5.3.5. Linear Mixed Models: Associations in the Differences of Body Composition, Muscle Function, and Cardiovascular Health between the Protein Supplementation and Placebo Groups

After adjustment for age, sex, and BMI no significant associations were found between group assignment and aLM, LBM, SMI, BF %, resting heart rate, blood pressure, protein intake, muscle quality, KEPT, KFPT, PFPT, DFPT, sit-to-stand, handgrip strength, TUG, gait speed, or balance composite score (Table 27).

Table 27

Associations in the Differences of Lean Muscle Mass, Body Composition, Muscle Function, and Cardiovascular Health Between the Protein Supplementation and Placebo Groups

Protein Supplement Group Vs. Placebo Group	**Difference**	**95% Confidence Interval**
Appendicular Lean Body Mass (kg)	-0.26	-1.67, 1.13
Total Lean Mass (kg)	-0.48	-3.18, 2.20
Skeletal Muscle Mass Index (kg/m^2)	-0.16	-0.73, 0.40
Body Fat (%)	-0.60	-1.92, 0.72
Heart Rate (BPM)	7.76	-1.89, 17.4
Systolic Blood Pressure (mmHg)	-0.06	-9.69, 9.56
Diastolic Blood Pressure (mmHg)	1.04	-17.22, 19.31
Protein/Body Weight (kg)	0.03	-0.59, 0.67
Muscle Quality	-0.60	-3.07, 1.87
Knee Extensor Peak Torque (Nm)	-4.29	-37.04, 28.44
Knee Flexor Peak Torque (Nm)	-4.51	-14.39, 5.36
Plantar Flexors Peak Torque (Nm)	2.99	-14.09, 20.08
Dorsi Flexors Peak Torque (Nm)	4.12	-2.242, 10.49
30-Second Sit-to-Stand (Repetitions)†	-0.45	-4.09, 3.18
Handgrip Strength (kg)	2.45	-3.72, 8.63
Timed Up and Go	0.44	-0.75, 1.64
Gait Speed (m/s)	-0.03	-0.19, 0.12
Balance Composite	0.12	-0.54, 0.80

†n=15 *Note*: Each model was adjusted for sex, age, and body mass index.

5.5. Discussion

The purposes of this study were to determine if a leucine-rich blended protein supplementation combined with BFR exercise can favorably alter body composition, muscle function, and cardiovascular health. The main findings from this study were that those consuming the protein supplement significantly increased their total leucine intake (g·day^{-1}) at mid-study by ~83% with non-significant increases of ~60% at post study, while those on the

placebo showed a non-significant 42-54% reduction in total leucine intake over the course of the study. Additionally, there were significant main effects of supplementation in regard to dietary intakes of protein. Mean total protein intakes were 140.91±14.7 g/day and 70.0±14.7 g/day in the supplement and placebo groups at baseline, respectively. Similarly, mean relative protein intakes were 1.6±.2 $g \cdot kg \cdot day^{-1}$ in the supplement group and 1.0±.2 $g \cdot kg \cdot day^{-1}$ in the placebo group. Though no differences between groups were observed regarding body composition, muscular health, or cardiovascular health, the training improved strength as measured by training volume in all exercises, muscle function as measured by sit-to-stand and gait speed, and cardiovascular fitness as measured by resting heart rate.

Insufficient intakes of nutrients or energy leads to catabolism and can negatively affect the maintenance of muscle mass and physical function (Cruz-Jentoft et al., 2017; Volpi et al., 2013). Furthermore, a "threshold dose" of protein and leucine of 21-40 and 3 grams per meal, respectively, has been suggested for older adults as it has positively influenced muscle protein balance (Baum et al., 2016; Beaudart et al., 2017; Cruz-Jentoft et al., 2017; Kramer et al., 2017; Landi et al., 2016; Meskers et al., 2019; Verlaan et al., 2018; Volpi et al., 2013). Previous research has shown that a combination of low-intensity resistance training and supplementation of ~20g whey protein with 3g of leucine was able to shift sarcopenic status from pathological to non-pathological (Molnár et al., 2016). In 12 weeks, supplementation with 31g of casein protein improved body composition, muscle contractility and function, and VO_{2max} significantly in all 114 physically active sexagenarians with adequate protein intakes (ten Haaf et al., 2019). Body composition improvements were significantly more favorable in those consuming additional protein when compared to those consuming the placebo (ten Haaf et al., 2019). In another study examining concurrent training with thrice daily supplementation of leucine-rich whey protein,

exercise improved muscle strength and function with no additional benefits from supplementation (Kirk et al., 2019). Interestingly, the participants in the first study were consuming just over the RDA for protein at baseline (~0.89 $g \cdot kg \cdot day^{-1}$) while those in the second study were consuming >1.0 $g \cdot kg \cdot day^{-1}$ of dietary protein at baseline (Kirk et al., 2019; ten Haaf et al., 2019). The supplement in our study provided ~28 g of protein sourced from the whey/casein blend and significantly increased leucine and protein in the supplemented group above 3.5 ± 3.2g and 1.5 ± .4 $g \cdot kg \cdot day^{-1}$, respectively.

Few studies have examined the combination of BFR exercise and protein supplementation on muscle size; however, in men over 50 years of age, eight weeks of BFR exercise and supplementation with 15g of collagen peptides led to significant improvements in thigh muscle size but not strength when compared to a supplement only control group (Centner et al., 2019). In the aforementioned study, the supplement group had slightly higher, but not significant, improvements in muscle size (~1%) when compared to the placebo group indicating that the hypertrophic effect was likely in response to the exercise stimulus only. Similarly, our study did observed 0.2-0.4% improvement in total and appendicular lean body mass that were not significant. Nevertheless, these results in combination with the burden of disease and lack of literature indicate a practical significance and therefore warrant further exploration.

Though intermittent BFR has previously resulted in a lower training volume compared to traditional resistive exercise, our study was the first to examine training volume as a measurement of strength following an individualized ramp BFR exercise regimen (Baker et al., 2019; Freitas et al., 2020; Neto et al., 2017; Plaza-Florido & Molina-Garcia, 2019). We reported a significant time effect for average weekly training volume for all exercises indicating adaptation in response to the novel training stimulus. Ruaro et al. (2019) reported improved wrist

flexion and sit-to-stand following the addition of low intensity BFR wrist flexion exercise to traditional high load training in 60-year-old women. These results could be a result of increased overall training volume, however, that variable was not explicitly examined. Another marker for muscular adaptation is functional ability. Previously, handgrip strength, functional capacity (including sit-to-stand and TUG), and equation estimated appendicular muscle mass and muscle mass index significantly increased following 16-weeks of BFR training in 23 sarcopenic women over the age of 60 years; however, improvements in muscle mass index did not indicate a change in sarcopenic status (Letieri et al., 2019). Using a similar age group, Letieri et al. (2018) also reported improvements in isokinetic strength following 16 weeks of BFR exercise with high pressure accounting for thigh circumference and moderate pressure (e.g., ~80% of vascular occlusion) that were comparable to traditional high-load training with a greater preservation of strength following detraining in the high pressure group (Δ%: -1.26, -1.44, and -1.67, respectively). Another group of researchers reported improvements in sit-to-stand repetitions and timed up and go following eight weeks of intermittent BFR exercise and dynamic strength (e.g., 1-RM) and CSAq after 16 weeks in women over the age of 60 years (Moura et al., 2019). Though our study was much shorter, we also reported significant time effects for sit-to-stand repetitions and gait speed. Given that Letieri et al. (2018) reported improvements in muscle size occurring at 16 weeks, it is possible that the current study design did not allow enough time for these adaptations to occur. Furthermore, it is possible that additional benefits from protein supplementation were not observed because they exist only in frail or institutionalized adults (Thomas et al., 2016).

Intermittent and continuous BFR exercises have led to acute increases in heart rate, double product (heart rate x systolic blood pressure), and mean arterial pressure immediately

following exercise and significant reductions in resting heart rate, systolic blood pressure, diastolic blood pressure, and mean arterial pressure within 10-60 minutes post-exercise in young adults (Farías et al., 2019; Neto et al., 2017; Neto et al., 2016). Similar results in heart rate and blood pressure were reported in young adults (e.g., 22-30 years of age) participating in eccentric resistive exercise with BFR though mean arterial pressure and rate pressure product (double product/100) were only elevated following training without BFR (Bazgir et al., 2016). These results are consistent even when the exercise modality is not (Renzi et al., 2010). Regardless of these processes, studies are generally aimed at determining safety of BFR exercise in special populations like older adults and those at risk for cardiovascular diseases so long term adaptation have not be reported. Our study showed significant reductions in resting heart rate following progressive resistance training with BFR regardless of supplementation. In combination, these results support the use of resistance training and BFR as an effective concurrent training modality.

This study is novel in many ways. Firstly, few studies have examined intermittent BFR. Those that have reported improvements in strength and similar or higher motor unit recruitment than was observed following low intensity exercise without occlusion (de Castro et al., 2019; Freitas et al., 2020). Furthermore, it has been reported that there are no significant differences between continuous BFR and intermittent BFR regarding hypertrophic stimuli via metabolic stress and muscle swelling and that releasing the cuff pressure during rest can make exercise more tolerable (Freitas et al., 2020; Neto et al., 2017). Secondly, most studies have examined those younger than thirty or fifty years of age and older (Plaza-Florido & Molina-Garcia, 2019). Other at risk populations (e.g., those 30-50 years) should be examined not only in regard to sarcopenia, but also because of the large number of occupational athletes in this age range,

specifically astronauts, who may benefit from effective nutrition and exercise interventions to mitigate decrements in muscle health (Baker et al., 2019; Behringer & Willberg, 2019; Conceição & Ugrinowitsch, 2019; Hackney et al., 2015; Ladlow et al., 2017). Lastly, though BFR exercise has notable effects on cardiovascular function, few studies have examined it as a modality of concurrent training.

Though this study has many strengths and contributes greatly to the literature on this novel exercise modality, it is not without limitations. Firstly, this study had a very small volunteer sample. Secondly, though participants were asked to avoid any structured exercise and limit recreational participation in sports to once per week or less, we were not able to completely control for lifestyle physical activity from household duties and commuting. Lastly, this sample was limited to healthy, non-sarcopenic individuals, normobaric/normogravity environments, and predominantly white individuals (~94%). To truly extend these findings to sarcopenic individuals, those of other races and ethnicities, or occupational athletes would require replication of this study in other populations and environments.

5.6. Conclusion

Supplementing the diet with a whey-casein blended protein shake once per day for four weeks can improve leucine and protein intake in the diet. Intermittent BFR can improve muscle strength and function while positively influencing cardiovascular health in middle-aged adults. However, additional benefit to increasing protein and leucine intake above the recommended dietary allowance was not observed. Future studies should examine this intervention in frail or institutionalized individuals, individuals from various occupational, racial and ethnic backgrounds, and at microgravity.

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APPENDIX A. IRB APPROVAL #HE15191

NDSU NORTH DAKOTA STATE UNIVERSITY

September 9, 2015 **REVISED Research Team**

Dr. Kyle Hackney
Department of Health, Nutrition & Exercise Sciences
BBFH 1

IRB Approval of Protocol #HE15191, "Protein Intake and Muscular Health with Aging"
Co-investigator(s) and research team: Sherri Stastny, Shannon David, Wonwoo Byun, Steven Mitchell, Kara Stone, Chris Kotarsky, Jill Keith, Allison Barry, Dan Streeter

Approval period: 8/17/2015 to 8/16/2016
Continuing Review Report Due: 7/1/2016

Research site(s): NDSU Funding Agency: Sanford Health
Review Type: Expedited category # 4
IRB approval is based on the original submission, with revised: protocol form, recruitment materials and consent form (received 8/13/2015).

Additional approval is required:
o prior to implementation of any changes to the protocol (Protocol Amendment Request Form).
o for continuation of the project beyond the approval period (Continuing Review/Completion Report Form). A reminder is typically sent 4-6 weeks prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:
o any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (Report of Unanticipated Problem or Serious Adverse Event Form).
o any significant new findings that may affect risks to participants.
o closure of the project (Continuing Review/Completion Report Form).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,
Kristy Shirley
Kristy Shirley, CIP, Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult www.ndsu.edu/irb. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

INSTITUTIONAL REVIEW BOARD
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8995 | Fax 701.231.8098 | ndsu.edu/irb
Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

APPENDIX B. INFORMED CONSENT #HE15191

NDSU North Dakota State University
Health, Nutrition, & Exercise Sciences
NDSU Dept 2620
PO Box 6050
Fargo, ND 58108-6050
701.231.7479

Title of Research Study: Protein Intake and Muscular Health with Aging

This study is being conducted by:
Kyle Hackney, PhD, Sherri Stastny, PhD, Shannon David, PhD, and Won Byun, PhD at North Dakota State University and Steven Mitchell, MD at Sanford Health.

Why am I being asked to take part in this research study?
We are looking to recruit up to 50 participants for this research study.

You are being asked to participate in this study because you:

- Are between the ages of 20-35 or 50-65 years.
- Are generally healthy and mobile.

You should not participate in this study if you:

- Are pregnant or perceive you may be pregnant.
- Currently smoke tobacco, e-cigarettes, or used smokeless tobacco.
- Have been told by a doctor that you have diabetes, high blood pressure, or cancer.
- Have been told by a doctor that you have a neuromuscular disease.
- Have previously had a heart attack or other heart related conditions.
- Have difficulty moving without assistive devices or walking one quarter mile.
- Currently follow a structured aerobic or resistance exercise training program.
- Taking medications that influence muscle size (testosterone, growth hormone, etc).
- Have or perceive that you may have any metal fragments, devices, implants, or metal ink from tattoos.
- Have experienced claustorphobia or anxiety in small spaces.

What is the reason for doing the study?
We wish understand how dietary protein intake is associated with muscle size and strength in healthy adults across the aging process. A greater understanding of the relationship may help update guidelines for protein intake and health with aging.

What will I be asked to do? After an informational meeting, an informed consent process and form will be presented and you will be asked to sign the form for participation. Once this has been completed, the following tests or assessments.

1) **Questionnaires:** We will ask you to complete several forms to determine your overall health and the foods you generally eat.

2) **Muscle Testing:** We will measure your height, weight, body composition, and waist size. We will ask you to perform a test to determine your leg strength and endurance. Finally, a Magnetic Resonance Imaging (MRI) scan of the leg muscles will be performed at the local hospital. MRI is a non-invasive imaging technology that produces detailed anatomical images.

3) **Food Intake Journal and Physical Activity Measurement:** We will ask you to write down all of the food and beverages you consume for 3 days. We will also ask you to wear a small device that monitors your physical activity for 7 days.

Where is the study going to take place, and how long will it take? The instructional meeting, collection of questionnaires, and muscle testing will be completed at the Bentson Bunker Field house on NDSU campus (BBFH lower level, Human Performance Lab). This will take approximately 2 hours to complete. The MRI tests will be completed on a Saturday morning at Sanford Medical Center, 801 Broadway, both in Fargo, North Dakota. This will take approximately 1 hour. Food intake and Physical Activity recording will require approximately 30 minutes of effort each day for seven days. It is expected the entire study will encompass 6.5 hours of efforts.

What are the risks and discomforts? The study team has minimized the known risks by studying healthy participants. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known risks to the participant.

1. Breach of privacy and/or confidentialiy of health information (low risk of occuring).
    - Discomfort during assessments.
    - Loss of health information from questionnaires (low risk of issue)

    All assesments will be completed with only the study team present in rooms with closed doors and windows. We will keep health information confidential and locked in an office. We will also shred all health information once the study is completed.

2. Exercise related discomforts (low risk of occurring)
    - Muscle soreness and cramping.
    - Lightheadedness or an adverse cardiovascular response.
    - Repetitive stress injuries to muscle, tendon, ligaments, or bone.

    We will provide you with a warm-up prior to assessing muscle strength and we use very safe equipment for strength testing.

3. Equipment malfunction (very low risk of occurring).
    - Pinching or injury from hardware or software failures.

    All equipment will be examined prior to testing to reduce a risk of a malfunction.

4. MRI scanning (low risk of occurring).
    - General discomforts from being very still during the MRI scans.
    - Short term hearing discomfort due to the noise generated by the MRI scanner. Ear plugs and/or headphones will be provided to you to reduce the noise.
    - Claustrophobia or anxiety during the MRI scan. However, only the upper and lower legs will be placed into the MRI scanner (not the upper body), which reduces the feeling of claustrophobia in many subjects.
    - A rare injury could occur if you have metal implants such as: pacemakers, limb implants, or other medical devices in your body that may be caused to move, heat, or break by the MRI magnet.
    - A rare type of burn could occur from tattoos that contain metal ink.

    We will give you rest breaks where you will be able to move your arms and legs freely during the MRI. We will provide you with earplugs and/or head phones to reduce the noise level. Only your legs will be placed in the MRI scanner, which reduces anxiety of most subjects. A standard questionnaire from Sanford Health will be used to screen for any metal objects and tattoos.

What are the benefits to me? You may ask for a summary of your own data. This would include average intake of calories, physical activity, strength, and body composition. However, you may not get any other benefits from being in this research study.

What are the benefits to other people? We will develop educational materials intended for the general public after learning more about protein and muscle health at the conclusion of this study. These educational materials will include physical activity interactions with dietary intake.

Do I have to take part in the study? Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What are the alternatives to being in this research study? Instead of being in this research study, you can choose not to participate.

Who will see the information that I give? We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key. If you withdraw before the research is over, your information will be retained in the research record and we will not collect additional information about you.

Can my taking part in the study end early? If you fail to participate in all scheduled sessions you may be removed from the study.

Will I receive any compensation for taking part in this study? After completion of the study, you will receive compensation of $200.00.

What if I have questions?
Before you decide whether to accept this invitation to take part in the research study, please ask any questions that might come to mind now. Later, if you have any questions about the study, you can contact the Principal Investigator- Kyle Hackney, kyle.hackney@ndsu.edu, NDSU 701-231-6706 or Co-investigators- Sherri Stastny, sherri.stastny@ndsu.edu, 701-231-7479, Shannon David, shannon.david@ndsu.edu, 701-231-5686 or Won Byun, 701-231. 6738, w.byun@ndsu.edu.

What are my rights as a research participant?
You have rights as a participant in research. If you have questions about your rights, or complaints about this research you may talk to the researcher or contact the NDSU Human Research Protection Program by:

- Telephone: 701.231.8995 or toll-free 1.855.800.6717
- Email: ndsu.irb@ndsu.edu
- Mail: NDSU HRPP Office, NDSU Dept. 4000, PO Box 6050, Fargo, ND 58108-6050.

The role of the Human Research Protection Program is to see that your rights are protected in this research; more information about your rights can be found at: www.ndsu.edu/irb .

Documentation of Informed Consent:
You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

Your signature Date

_____

Your printed name

_____

Signature of researcher explaining study Date

_____

Printed name of researcher explaining study

_____

Revised: November 2012

Documentation of release of images:
You also have the choice to allow all images obtained during this study to be used by the research team in outreach materials, publications, manuscripts, poster presentations, Powerpoint presentations, and University websites. Images will only be used in a professional context when describing the study. Your name will never be associated with the images unless we obtain further permission from you at a later date.

Yes _____

No _____

_____ _____
Your signature Date

_____
Your printed name

Revised: November 2012

APPENDIX C. CONTINUING REVIEW FORM #HE15191

Date Received
6/14/2016

NDSU NORTH DAKOTA STATE UNIVERSITY

INSTITUTIONAL REVIEW BOARD
office: Research 1, 1735 NDSU Research Park Drive, Fargo, ND 58102
mail: NDSU Dept. #4000, PO Box 6050, Fargo, ND 58108-6050
p: 701.231.8995 **f:** 701.231.8098 **e:** ndsu.irb@ndsu.edu **w:** www.ndsu.edu/irb

Continuing Review or Completion Report Form

Use this form to: 1) request a continuation of IRB approval if a project is currently active (recruiting subjects, collecting data, or analysis of identifiable data), or 2) report completion of a project.

Protocol Information

Protocol #: **HE15191** Original approval date*: **8/17/2015**

Title: **Protein Intake and Muscular Health with Aging**

10/24/13

Principal investigator: **Dr. Kyle Hackney**
Department: HNES
E-Mail/Campus Address: kyle.hackney@ndsu.edu, BBFH 24

Co-investigator: Please See Appendix A
Department: Please See Appendix A
E-Mail/Campus Address: Please See Appendix A

** Complete and submit an updated protocol form & relevant attachments every 5 years following approval. Protocol records must be updated every 5 years by completing a new protocol form and any relevant attachments, and including it with this report. Use the most recent version of the forms on the IRB website at: http://www.ndsu.nodak.edu/research/institutional_review_board/forms.html.*

Project Status

☒ Ongoing and currently active, Expected end date of research: **August 2017**

☐ Complete, abandoned or inactive

Source of current funding: **Sanford Health** FAR# 0025060 ☐ Not funded
Current Funding period: Start date: 6/15/2016 End Date: 6/30/2016

Has a progress report been filed with the funding agency since last review?
☐ **No** ☒ **Yes,** **Attach copy of final grant application(s), and/or recent report to funding agency.**

Research team: *List all individuals involved in the research (project design/oversight, recruiting participants, obtaining informed consent, intervening or interacting with participants to obtain information/data, and/or handling identifiable information for research purposes). May provide as a separate attachment.*

Name, dept. or affiliation:	Specify role in research:	Email Address	Training date: (IRB Use only)
Please see appendix A			

Project Summary

1. Brief summary of results to date:

We have completed data collection on 48 individuals (25 younger adults, and 23 older adults). Data has been collected on muscle strength, endurance, and size. In addition, we have collected data on physical activity level and nutritional intake. Currently, we are in the process of trying to get our final 2 subjects. We are also organizing our data in spreadsheets to determine potential trends. Once all data is complete we will perform statistical analysis and begin the process of scientific writing.

2. List research site(s):

NDSU Rm 14 BBFH; Sanford Health Broadway Clinic: Radiology, Fargo, ND

3. List presentations or publications that have resulted from this research since the last review:

Northland Amercian College of Sports Medicine, Regional Conference, St. Catherine University, St. Paul, MN.

Intra-Reliability of ImageJ processing of MRI-derived cross-sectional area: Upper Leg Stone, K. A., Streeter, D. M., Kotarsky, C. J., Mitchell, S.L., David, S.L., Hackney, K. J. North Dakota State University, Fargo ND; Sanford Health, Fargo, ND.

Intra-Reliability of ImageJ processing of MRI-derived cross-sectional area: Lower Leg Streeter, D. M., Stone, K. A., Kotarsky, C. J., Mitchell, S.L., David, S. L., Hackney, K. J. North Dakota State University, Fargo ND Sanford Health, Fargo, ND.

Participants:

1. How many participants have completed the study since last review: 48.
2. How many participants have completed the study since first review: 48.
3. Will more participants be recruited?
   - ☐ No
   - ☒ Yes* – Indicate approximately how many: 2

Attach a copy of current consent form(s), and any recruitment materials.

4. Informed Consent: A co[illegible] of the approved informed consent fo[illegible] has been signed by each of the participants in the study, and retained for your records. Has this requirement been met?
   ☒ Yes
   ☐ N/A, waiver approved
   ☐ No – explain:

5. Have any potential participants declined to participate, or withdrawn from the research?
   ☒ No
   ☐ Yes - explain:

6. Summarize any complaints about the research (and their resolution) since the last review?
   None to my knowledge

Risk/Benefit Ratio:

1. Summarize any unanticipated problems (even if previously reported) or adverse events that have occurred since the last review:
   None

   Unanticipated problem: an unanticipated problem that involves risks to subjects or others is any incident, experience, or outcome that meets all the following criteria:
   - *is unexpected (in terms of nature, severity, or frequency) given the characteristics of the subject population and the research as described in the IRB approved protocol and consent document(s)*
   - *is related, or possibly related to participation in the research*
   - *suggests the research places subjects or others at greater risk of harm (physical, psychological, economic, or social harm) that previously known or recognized*
   - *may not have resulted in actual harm to subjects, but may only represent increased risk of harm (ie., physical, psychological, social, economic, legal).*

   Adverse event: any untoward or unfavorable medical occurrence (physical or psychological) in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to their research participation. Such events may have already been expected to occur with a certain frequency and severity, and previously identified as potential risks in the protocol form, and consent document(s).

2. Has any new information resulted from the study or any literature, that would affect the risk/benefit ratio for new subjects (or for those currently or previously enrolled)?
   ☒ No
   ☐ Yes –explain, and indicate how this has been/will be addressed with future, current, or previously enrolled participants:

Investigator's Assurance

The signature below certifies th...

- information provided in this report is complete and accurate
- each individual involved as a member of the research team possesses the necessary experience for conducting research activities in their assigned role, and is aware of and will abide by NDSU policies and procedures for the protection of research participants
- the research will be conducted according to the approved protocol
- changes will receive IRB approval prior to implementation, unless necessary to prevent immediate serious harm to participants
- all unanticipated problems involving risks to participants or others will be promptly reported to the IRB.

Kyle Hackney (email) 6/14/2016

Principal Investigator signature, date

In lieu of a written signature, submission of this report via the Principal Investigator's NDSU email constitutes an acceptable electronic signature.

----------FOR IRB USE ONLY--------

Project is: ☑ Approved for continuation ☐ Complete/Inactive ☐ Archive after _____

IRB Signature: Kristy Shirley Date: 7/25/2016

Reviewed by: ☐ Full Board - meeting date _____ ☒ Expedited review, category # 4

Current approval period expires: 7/16/2017

Next Continuing Review/Completion Report due*: 7/1/2017

Note that the IRB office will typically remind the investigator a few weeks prior to the due date; however, timely submission of the report is the PI's responsibility.

APPENDIX D. REVISED INFORMED CONSENT #HE15191

NDSU North Dakota State University
Health, Nutrition, & Exercise Sciences
NDSU Dept 2620
PO Box 6050
Fargo, ND 58108-6050
701.231.7479

Title of Research Study: Protein Intake and Muscular Health with Aging

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Kyle Hackney, PhD, Sherri Stastny, PhD, Shannon David, PhD, and Won Byun, PhD at North Dakota State University and Steven Mitchell, MD at Sanford Health.

Why am I being asked to take part in this research study?
We are looking to recruit up to 50 participants for this research study.

You are being asked to participate in this study because you:

- Are between the ages of 20-35 or 50-65 years.
- Are generally healthy and mobile.
- Are physically active and have participated in moderate and vigorous exercise 3-5 days per week for the past 3 months or more; including both resistance and cardiovascular exercises.

You should not participate in this study if you:

- Are pregnant or perceive you may be pregnant.
- Currently smoke tobacco, e-cigarettes, or used smokeless tobacco.
- Have been told by a doctor that you have diabetes, high blood pressure, or cancer.
- Have been told by a doctor that you have a neuromuscular disease.
- Have previously had a heart attack or other heart related conditions.
- Have difficulty moving without assistive devices or walking one quarter mile.
- Taking medications that influence muscle size (testosterone, growth hormone, etc).
- Have or perceive that you may have any metal fragments, devices, implants, or metal ink from tattoos.
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    - Loss of health information from questionnaires (low risk of issue)

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    - Muscle soreness and cramping.
    - Lightheadedness or an adverse cardiovascular response.
    - Repetitive stress injuries to muscle, tendon, ligaments, or bone.

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    - Pinching or injury from hardware or software failures.

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    - Short term hearing discomfort due to the noise generated by the MRI scanner. Ear plugs and/or headphones will be provided to you to reduce the noise.
    - Claustrophobia or anxiety during the MRI scan. However, only the upper and lower legs will be placed into the MRI scanner (not the upper body), which reduces the feeling of claustrophobia in many subjects.
    - A rare injury could occur if you have metal implants such as: pacemakers, limb implants, or other medical devices in your body that may be caused to move, heat, or break by the MRI magnet.
    - A rare type of burn could occur from tattoos that contain metal ink.

    We will give you rest breaks where you will be able to move your arms and legs freely during the MRI. We will provide you with earplugs and/or head phones to reduce the noise level. Only your legs will be placed in the MRI scanner, which reduces anxiety of most subjects. A standard questionnaire from Sanford Health will be used to screen for any metal objects and tattoos.

What are the benefits to me? You may ask for a summary of your own data. This would include average intake of calories, physical activity, strength, and body composition. However, you may not get any other benefits from being in this research study.

What are the benefits to other people? We will develop educational materials intended for the general public after learning more about protein and muscle health at the conclusion of this study. These educational materials will include physical activity interactions with dietary intake.

Do I have to take part in the study? Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What are the alternatives to being in this research study? Instead of being in this research study, you can choose not to participate.

Who will see the information that I give? We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key. If you withdraw before the research is over, your information will be retained in the research record and we will not collect additional information about you.

Can my taking part in the study end early? If you fail to participate in all scheduled sessions you may be removed from the study.

Will I receive any compensation for taking part in this study? After completion of the study, you will receive compensation of $200.00.

What if I have questions?
Before you decide whether to accept this invitation to take part in the research study, please ask any questions that might come to mind now. Later, if you have any questions about the study, you can contact the Principal Investigator- Kyle Hackney, kyle.hackney@ndsu.edu, NDSU 701-231-6706 or Co-investigators- Sherri Stastny, sherri.stastny@ndsu.edu, 701-231-7479, Shannon David, shannon.david@ndsu.edu, 701-231-5686 or Won Byun, 701-231. 6738, w.byun@ndsu.edu.

What are my rights as a research participant?
You have rights as a participant in research. If you have questions about your rights, or complaints about this research you may talk to the researcher or contact the NDSU Human Research Protection Program by:

- Telephone: 701.231.8995 or toll-free 1.855.800.6717
- Email: ndsu.irb@ndsu.edu
- Mail: NDSU HRPP Office, NDSU Dept. 4000, PO Box 6050, Fargo, ND 58108-6050.

The role of the Human Research Protection Program is to see that your rights are protected in this research; more information about your rights can be found at: www.ndsu.edu/irb .

Documentation of Informed Consent:
You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

Your signature Date

_____

Your printed name

_____

Signature of researcher explaining study Date

_____

Printed name of researcher explaining study

_____

Revised: November 2012

Documentation of release of images:
You also have the choice to allow all images obtained during this study to be used by the research team in outreach materials, publications, manuscripts, poster presentations, Powerpoint presentations, and University websites. Images will only be used in a professional context when describing the study. Your name will never be associated with the images unless we obtain further permission from you at a later date.

Yes _____

No _____

_____ _____
Your signature Date

_____
Your printed name

Revised: November 2012

APPENDIX E. IRB APPROVAL #HE18246

NDSU NORTH DAKOTA STATE UNIVERSITY

May 18, 2018

Kyle Hackney
Department of Health, Nutrition & Exercise Science

IRB Approval of Protocol #HE18246, "Blood Flow Restricted Exercise and Blended Protein Supplementation to Attenuate Sarcopenic Effects on Muscle Function"
Co-investigator(s) and research team: Kara Stone, Sherri Stastny, and Steven Mitchell

Approval expires: 5/10/2019 Continuing Review Report Due: 4/1/2019

Research site(s): NDSU Funding agency: n/a
Review Type: Full Board, meeting date – 5/11/2018
Risk Level: More than a minor increase over minimal risk
IRB approval is based on original submission, with revised: protocol and informed consent (received 5/18/2018). Please utilize the stamped informed consent provided.

Additional approval is required:
o prior to implementation of any proposed changes to the protocol (Protocol Amendment Request Form).
o for continuation of the project beyond the approval period (Continuing Review/Completion Report Form). A reminder is typically sent two months prior to the expiration date; timely submission of the report is your responsibility. To avoid a lapse in approval, suspension of recruitment, and/or data collection, a report must be received, and the protocol reviewed and approved prior to the expiration date.

A report is required for:
o any research-related injuries, adverse events, or other unanticipated problems involving risks to participants or others within 72 hours of known occurrence (Report of Unanticipated Problem or Serious Adverse Event Form).
o any significant new findings that may affect risks to participants.
o closure of the project (Continuing Review/Completion Report Form).

Research records are subject to random or directed audits at any time to verify compliance with IRB regulations and NDSU policies.

Thank you for cooperating with NDSU IRB procedures, and best wishes for a successful study.

Sincerely,
Kristy Shirley

Kristy Shirley, CIP
Research Compliance Administrator

For more information regarding IRB Office submissions and guidelines, please consult www.ndsu.edu/irb. This Institution has an approved FederalWide Assurance with the Department of Health and Human Services: FWA00002439.

INSTITUTIONAL REVIEW BOARD
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8995 | Fax 701.231.8098 | ndsu.edu/irb
Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

APPENDIX F. INFORMED CONSENT #HE18246

NDSU NORTH DAKOTA STATE UNIVERSITY

Health, Nutrition, and Exercise Sciences (HNES)
NDSU Dept 2620
PO Box 6050
Fargo, ND 58108-6050
701.231.7479

Blood Flow Restricted Exercise and Protein Supplementation to Rapidly Improve Muscular Health

This study is being conducted by: Kyle Hackney, PhD, Assistant Professor in HNES & Kara Trautman, MS, Graduate Research Assistant in HNES, North Dakota State University

Key Information about this study:

This consent form is designed to inform you about the study you are being asked to participate in. Here you will find a brief summary about the study; however, you can find more detailed information later on in the form.

- We are looking to recruit up to 40 men and women for this research study.
- We wish to understand how a blended protein supplement and low-intensity blood flow restricted exercise affect muscle health and the ability to complete normal tasks throughout middle age. A greater understanding of this may help to develop better diet and exercise guidelines to promote healthy aging.
- You should participate in this study if you are:
  - Between the ages of 25 and 60 years.
  - Generally healthy and mobile.
  - Not currently using tobacco products.
- You should not participate in this study if you:
  - Are pregnant or think you may be pregnant
  - Currently smoke tobacco, e-cigarettes, vapor pens, or use smokeless tobacco or use illicit drugs.
  - Have been told by a doctor that you have a neuromuscular disease, kidney disease, diabetes, high blood pressure, exertional rhabdomyolysis, sickle cell anemia/trait, or are being treated for cancer
  - Have had a heart attack or other chronic heart related condition that is not currently being controlled with medication.
  - Have difficulty moving without assistive devices or walking one-quarter mile.
  - Have previous injuries to the neck, back, arms, or legs that limit your ability to exercise.
  - Are taking medications that influence muscle size (testosterone, growth hormone, etc.)
  - Have been told by a doctor that you are obese.
  - Are currently using birth control.
  - Have an increased risk for deep vein thrombosis (DVT)

- The total time required for full participation is about 20-22 hours and will be completed over 6 weeks. Each session will range between 50-90 minutes.
- You will be paid $100 for full participation.
- All records that could be used to identify you will be kept private and accessed on a need to know basis. Your results will be published as a combination of your information and the results of others so that you cannot be identified.

Why am I being asked to take part in this study?

We wish to understand how a blended protein supplement and low-intensity blood flow restricted exercise affect muscle health and the ability to complete normal tasks as people age. A greater understanding of this may help to develop better diet and exercise guidelines to promote healthy aging.

What will I be asked to do?

I. **Screening/Information Session**
You will be asked to listen to an informational presentation about the study. If you are still interested, you will be asked to complete forms to see if you are eligible. You will be asked to complete several screening forms to determine your overall health. These forms include 4 questionnaires that will be used to determine if you are healthy enough to participate. If you are healthy enough, you will then be asked to schedule and come back for pre-testing, the training sessions, and post-testing as described below.

II. **Pre-testing/Familiarization Sessions (week 1)**
During the first week, you will be asked to complete the following pre-tests and familiarization exercises
   a. **Height and Weight:** You will be asked to stand on a digital scale and stand next to a wall with a measuring device.
   b. **Muscle Function and Activities of Daily Living:** You will perform 6 tests to determine your hand and leg strength and your ability to complete tasks of daily living. You will be asked to do a warm-up on a bike, rowing machine, or treadmill, then squeeze a hand-held device as hard as possible. You will also be asked to sit in a specialized chair and extend and curl your right leg as hard as possible. You will then be repositioned and perform another test where you will flex and extend your ankle (like you do while driving). You will also be asked to complete a sit-to-stand test where you will sit and stand as many times as possible. You will also perform a timed up and go test where you will stand from a chair and walk a 6-meter course using your usual pace. Lastly, you will complete a short physical performance battery which includes balance testing (maintaining 3 positions for 20 seconds each) and gait speed testing (similar to the timed up and go test).
   c. **Heart Rate and Blood Pressure:** You will be asked to wear a heart rate strap and we will take your blood pressure which will be used to monitor your safety throughout exercise.
   d. **Diet questionnaire:** You will be asked to estimate all of the foods that you normally eat as part of daily living using a form.
   e. **Bone Mineral Density and Body Composition Test:** A bone mineral density test will be completed while you relax on a table. Strong bones are important for your health. A

bone mineral density (BMD) test is the best way to measure your bone health. It compares your bone density, or mass, to that of a healthy person who is the same age and biological sex as you. A BMD test can provide a snapshot of your bone health. The test can be used as a screening tool for osteoporosis and broken bones. The most widely recognized BMD test is called a central dual-energy x-ray absorptiometry, or central DXA test.

You will be asked to provide a urine sample to conduct a pregnancy test before the DXA procedure. We ask that you dress in shorts and a T-shirt. For this procedure, two Velcro straps will be placed on your lower limbs to help keep the lower body in correct position during the scan. Once positioning is complete the trained researchers will guide you through the procedure. You will be asked to remain still as the scanning arm moves from your head to toes and back to your head. This takes approximately 5-13 minutes depending on your height and weight. When the scan is complete, the Velcro straps will be removed and you will be assisted off of the table.

f. **Familiarization to low-intensity exercise:** You will be asked to complete 3 sets of upper and lower body exercises with no blood flow restriction 3 days/week to become familiar with the exercises to be completed at the training sessions.

III. **Familiarization Sessions (week 2)**

During the second week, you will be asked to complete familiarization exercises with some blood flow restriction

a. **Familiarization to low-intensity exercise with blood flow restriction:** Following a bicycle, rower, or treadmill warm-up, you will be asked to complete three sets of low-intensity upper and lower body exercise with no blood flow restriction. For the fourth set, you will be asked to complete those exercises with a small amount of blood flow restriction. This will be done 3 days/week.

IV. **Training Sessions (weeks 3-5)**

V. During the third, fourth, and fifth weeks, you will be asked to complete exercise training sessions and drink a protein shake or placebo within 15 minutes of stopping exercise and as a between meals snack on each day that you are not exercising. The exercise sessions will be as follows:

a. **Week 3:** You will be asked to complete the same exercises from the familiarization session 3 days/week with the same amount of blood flow restriction for all four sets.
b. **Week 4:** You will be asked to complete the same exercises from the familiarization session 3 days/week with a slightly higher amount of blood flow restriction for all four sets. You will also be asked to repeat the diet questionnaire.
c. **Week 5:** You will be asked to complete the same exercises from the familiarization session 3 days/week with the highest recommended amount of blood flow restriction for all four sets.

VI. **Training Sessions/Post-testing (week 6)**
During the sixth week, you will be asked to complete the same training sessions as complete in week 5 with the same amount of restriction and repeat the tests from section II. You will be asked to drink a protein shake or placebo within 15 minutes of stopping all exercise sessions and as a between meals snack on each day that you are not exercising. You will also be asked to complete a final diet questionnaire.

Where is the study going to take place, and how long will it take?

The informational meeting will take about **45 minutes.** If you agree to be a part of the study and are eligible based on your health forms, a member of the research team will contact you to set up the **6** familiarization sessions and **12** training sessions.

There will be multiple training sessions (morning, afternoon, and evening) to choose from and we will do our best to accommodate your schedule. All meetings will take place at the Bentson Bunker Fieldhouse on the NDSU campus (BBFH lower level, Rooms 14, 15, and 16). It is expected that the entire study will encompass 20-22 hours.

What are the risks and discomforts?

The study team has minimized the known risks by studying a group of healthy participants. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known risks to you. If you are known to have a sensitivity to any food or food ingredient, or have had a violent allergic reaction to drugs, chemicals, or food ingredients you should not take part in this study.

- Breach of privacy and/or confidentiality of health information (low risk of occurring).
- Discomfort during assessments.
- Low dose radiation exposure. The full body DXA scan is not capable of producing high doses of radiation. For example, if you had 625 full body DXA scans in one year, you would still only be exposed to about 25% of the limit for radiation exposures. However, it is still considered good practice to test for pregnancy for all females of child bearing age before the scan is completed. Therefore, all subjects will be asked to provide a urine sample/pregnancy test upon arrival during weeks 1 and 6.
- Loss of health information from questionnaires (low risk of occurring).
    - All assessments will be completed with only the study team present in rooms with closed doors and windows. We will keep information confidential and locked in an office. We will shred all personal healthy information once the study is completed.
- Blood flow Restricted Exercise (BFR) related discomforts (low risk of occurring)
    - Muscle soreness and cramping.
    - Lightheadedness or adverse cardiovascular response.
    - Repetitive stress injuries to muscle, tendon, ligaments or bone.
    - Skin irritation, bruising, or numbness from the BFR cuff.

- We will use appropriate screening tools and check in on how you are feeling throughout the study. We will also provide you with a warm-up prior to assessing muscle strength and we use very safe equipment for strength testing. Additionally, the BFR device that we are using is the safest available and has been used in many clinical rehabilitation centers.

What are the expected benefits of this research?

Individual Benefits: You may ask for a summary of your own data. This would include average intake of calories, strength, and body composition. You will also have access to 12 personalized exercise sessions prescribed by a certified strength and conditioning specialist. However, you may not get any other benefits from being in this study.

Societal Benefits: The age-related losses in muscle size and function affect many aging people and costs the US millions of dollars in health care. The number of people and the cost of treatment is expected to rise. We will develop educational materials intended for the general public after learning more about the effects of combining protein supplementation and blood flow restricted exercise to promote better muscular health at the end of this study.

Do I have to take part in this study?

Your participation in this research is your choice. If you decide to participate in the study, you may change your mind and stop participating at any time without penalty or loss of benefits to which you are already entitled.

What are the alternatives to being in this study?

Instead of being in this research, you may choose not to participate.

Who will have access to my information?

We will keep private all research records that identify you. Your information will be combined with information from other people partaking in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under password protection. If you withdraw from the study before it is complete, your information will be retained in the research record and we will not collect additional information.

Can my participation in the study end early?
If you fail to participate in one or more scheduled sessions, you may be removed from the study.

Will I receive any compensation for participating in the study?

After completion of the entire study, you will be compensated: $25 for pre-testing and six familiarization sessions; $50 for the first six training sessions; and $25 for the last six training sessions and post-testing.

What happens if I am injured because of the study?
If you are injured during the course of this study, you should contact the principal investigator, Dr. Kyle Hackney, at 701.231.6706. Treatment for the injury will be available including first aid, emergency treatment, and follow-up care as needed. Payment for this treatment must be provided by you and your third party payer (such as health insurance or Medicaid). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a results of you participation in this research.

What if I have questions?
Before you decide whether you'd like to participate in this study, please ask any questions that come to mind now. Later, if you have questions about the study, you can contact Dr. Kyle Hackney at 701.231.6706 or kyle.hackney@ndsu.edu , or Kara Trautman at 660.238.0438 or kara.stone@ndsu.edu.

What are my rights as a research participant?
You have rights as a research participant. All research with human participants is reviewed by a committee called the *Institutional Review Board (IRB)* which works to protect your rights and welfare. If you have questions about your rights, an unresolved question, a concern or complaint about this research you may contact the IRB office at 701.231.8995, toll-free at 855-800-6717 or via email (ndsu.irb@ndsu.edu).

Documentation of Informed Consent:
You are freely making a decision whether to be in this research study. Signing this form means that

1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

You will be given a copy of this consent form to keep.

_____ _____
Your signature Date

_____ _____
Your printed name Date

You understand that the DXA examination is an X-Ray procedure. _____
(initials)

Signature of researcher explaining study

Date

Printed name of researcher explaining study