THE EFFECTS OF GRADED PROTEIN INTAKE IN CONJUNCTION WITH
RESISTANCE TRAINING ON SKELETAL MUSCLE OUTCOMES IN OLDER
ADULTS

BY

JOHN M. MICHEL

A Thesis Submitted to the Graduate Faculty of
WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE
Health and Exercise Science
May 2022
Winston-Salem, North Carolina

Approved By:
Gary D. Miller, Ph. D., Advisor
Michael J. Berry, Ph. D., Chair
Peter H. Brubaker, Ph. D.
Acknowledgements

First and foremost, I would like to thank Dr. Gary D. Miller for his mentorship, trust, and support. From the outset I was apprehensive about the process of running a study, but through it all you were nothing but supportive and put a belief in me that I was capable of rising to the demands that this project entailed. You have such a passion for helping students, and it has been abundantly evident through the past two years of mentorship. I could not have asked for a better mentor, and you taught me so many lessons that I will carry with me, not only in school and research, but in life as well. To my committee, Dr. Michael J. Berry and Dr. Peter H. Brubaker, I am very appreciative of your continued support and guidance during the thesis process. Dr. Berry, you have shown me that in the midst of stressful situations that there is always something to laugh and smile about. Dr. Brubaker, you always demonstrated kindness and attention to detail in everything that you do, and for that I am grateful. The three of you have demonstrated what it means to be a good scientist, a good educator, and a good man. Thank you for your mentorship over the past two years.

I would like to thank classmates of mine, Kristy Lievense, Sam Norton, Kathryn Alphin, Juliana Costa, and Lydia Bailey for their hard work in ensuring that this project was a success. Your work ethic and friendship throughout this process was immense and I am deeply appreciative for you all. I would also like to thank the volunteers who participated in this study. Without your hard work, this project would not have been possible. I could not have asked for a better group of participants.

I would like to thank Dr. Chris Vann for his mentorship and friendship over the past two years. You are the embodiment of hard work and have shown me what it means
to be a productive scientist. I model so much of the who I am today after you and for that I am truly thankful.

I would like to thank Dr. Miranda Orr for her mentorship over the past year, and giving me the opportunities and the freedom to develop confidence as a young scientist. You have been nothing but an excellent mentor to me and I am remarkably thankful that you allowed me to work in your lab. To all the members of the Orr Lab, thank you for your friendship and receptiveness. You have all shaped my experience in a notable way, and my experience would not have been the same without you.

Lastly, I would like to thank my parents, Michael and Judy Michel, and my girlfriend, Merrell Bowden, for providing a safe haven for me during this process. You all did so much to keep me grounded and helped shoulder my burden when I could not do it alone. I am sincerely thankful for each of you.
# Table of Contents

**List of Tables and Figures** ........................................................................................................... v

**List of Abbreviations** ................................................................................................................... vii

**Abstract** ....................................................................................................................................... viii

**Review of the Literature** ............................................................................................................. 1

Epidemiology and Background ........................................................................................................ 1

Resistance Training Interventions ................................................................................................. 3

- Effects of Body Composition and Muscle Morphology ................................................................. 3
- Effects on Strength Outcomes ....................................................................................................... 6
- Effects on the mTOR Pathway and Muscle Protein Synthesis ...................................................... 8

Protein Intake Modification Interventions ....................................................................................... 11

- Effects of Body Composition and Muscle Morphology .............................................................. 11
- Effects on Strength Outcomes ..................................................................................................... 15
- Effects on the mTOR Pathway and Muscle Protein Synthesis .................................................... 19

**Summary** ..................................................................................................................................... 23

**Purpose and Hypothesis** .............................................................................................................. 24

**Methods** ................................................................................................................................... 26

- Ethical Approval and Participants ................................................................................................. 26

- Study Design ................................................................................................................................. 27

- Resistance Training Protocol ........................................................................................................ 28
- Nutrition Intervention .................................................................................................................. 30

**Measures** .................................................................................................................................. 32

- Height, Weight, and Body Mass Index ............................................................................................ 32
- Lean/Soft Tissue Mass ..................................................................................................................... 32
- Vastus Lateralis Thickness ............................................................................................................. 33
- Peak Torque .................................................................................................................................. 33
- Muscle Quality Score and Lean/Soft Tissue Mass Index ................................................................. 34

**Analytic Plan** ............................................................................................................................... 34

**Results** ...................................................................................................................................... 36

**Tables and Figures** ...................................................................................................................... 42

**Discussion** .................................................................................................................................. 50

**References** ................................................................................................................................ 67

**Appendix A** ................................................................................................................................. 81

**Appendix B** .................................................................................................................................. 82

**Curriculum Vitae** ......................................................................................................................... 87
List of Tables and Figures

Table 1: Study Measurements Timeline (pg. 28)

Table 2: Resistance Training Progression Model (pg. 29)

Table 3: Prescribed Protein Intake for Each Group by Week (p. 31)

Table 4: Descriptive Characteristics of Participants (p. 42)

Table 5: Self-Reported Energy Intake Across Time (pg. 43)

Table 6: Self-Reported Protein Intake Across Time (pg. 44)

Table 7: Graded Protein Group Prescribed vs. Actual Protein Intake (pg. 45)

Figure 1: Lean/Soft Tissue Mass vs. Time (p. 45)

Figure 2: Appendicular Lean/Soft Tissue Mass vs. Time (p. 46)

Figure 3: Vastus Lateralis Tissue Thickness vs. Time (p. 46)

Figure 4: Knee Extensor Peak Torque at 60°/s vs. Time (p. 47)

Figure 5: Knee Flexor Peak Torque at 60°/s vs. Time (p. 47)

Figure 6: Knee Extensor Peak Torque at 120°/s vs. Time (p. 48)

Figure 7: Knee Flexor Peak Torque at 120°/s vs. Time (p. 47)

Figure 8: Muscle Quality Score at 60°/s vs. Time (p. 49)

Figure 9: Muscle Quality Score at 120°/s vs. Time (p. 49)

Figure 10: Resistance Training Timeline (p. 81)

Table 8: Exercises Performed at Weekly Training Sessions (p. 81)

Table 9: Self-Reported Carbohydrate Intake Across Time (pg. 82)

Table 10: Self-Reported Carbohydrate Intake Across Time (pg. 83)

Table 11: Protein Supplement Adherence (p. 84)

Table 12: Training Session Attendance (p. 84)
Figure 11: Training Total Volume Load vs. Time (p. 85)

Figure 12: Lean/Soft Tissue Mass Index vs. Time (pg. 85)

Figure 13: Ultrasound Reference Images at PRE and POST (pg. 86)
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBM</td>
<td>Lean Body Mass</td>
</tr>
<tr>
<td>REE</td>
<td>Resting Energy Expenditure</td>
</tr>
<tr>
<td>NHANES III</td>
<td>Third National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>SMI</td>
<td>Skeletal Muscle Index</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Classification of Diseases Volume 10</td>
</tr>
<tr>
<td>RT</td>
<td>Resistance Training</td>
</tr>
<tr>
<td>MPS</td>
<td>Muscle Protein Synthesis</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual Energy X-Ray Absorptiometry</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat Free Mass</td>
</tr>
<tr>
<td>MHC</td>
<td>Myosin Heavy Chain</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>MPB</td>
<td>Muscle Protein Breakdown</td>
</tr>
<tr>
<td>mTOR</td>
<td>Mammalian/ Mechanistic Target of Rapamycin</td>
</tr>
<tr>
<td>mTORC1</td>
<td>Mammalian/ Mechanistic Target of Rapamycin Complex 1</td>
</tr>
<tr>
<td>4EBP1</td>
<td>Eukaryotic Initiation Factor 4E-binding Protein 1</td>
</tr>
<tr>
<td>eIF4E</td>
<td>Eukaryotic Initiation Factor 4E</td>
</tr>
<tr>
<td>S6K1</td>
<td>p70 Ribosomal S6 Kinase 1</td>
</tr>
<tr>
<td>AMPKa</td>
<td>Adenosine Monophosphate-activated Protein Kinase-alpha</td>
</tr>
<tr>
<td>FSR</td>
<td>Fractional Synthetic Rate</td>
</tr>
<tr>
<td>AA</td>
<td>Amino Acid</td>
</tr>
<tr>
<td>SMD</td>
<td>Standard Mean Difference</td>
</tr>
<tr>
<td>MD</td>
<td>Mean Difference</td>
</tr>
<tr>
<td>SMDbs</td>
<td>Between Subjects Standard Mean Difference</td>
</tr>
<tr>
<td>fCSA</td>
<td>Fiber Cross-Sectional Area</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Daily Allowance</td>
</tr>
<tr>
<td>WMD</td>
<td>Weighed Mean Difference</td>
</tr>
</tbody>
</table>
Abstract

Background: The aging process is associated with a decline in skeletal muscle size and quality\(^1,2\). This process can lead to increases in comorbidities such as obesity, and can factor into survival rates in disease\(^3\)–\(^5\). There is a need for pragmatic interventions to attenuate the typical age-related loss of skeletal muscle, and as such resistance training (RT)\(^6,7\) and protein intake modification interventions\(^8\) have received much attention.

Objective: To examine the effects of graded protein intake with a conjunctive RT intervention on body composition, strength measures, and vastus lateralis thickness in older adults. **Methods:** A cohort of older adults (n=18; age=69.72±8.23 years; 11 females) were randomly assigned to either a graded protein (GP) intake or constant protein (CP) intake for 10 weeks. During this time, both groups underwent the same RT intervention. Dual energy x-ray absorptiometry (DXA) derived lean/soft tissue mass, isokinetic dynamometry derived peak torque, ultrasonography derived vastus lateralis tissue thickness, and muscle quality scores (peak torque/leg lean mass) were assessed at baseline and post-intervention, with peak torque additionally assessed at midpoint (week 5). **Results:** The RT intervention had adherence of 96% as assessed by session attendance, the CP group adhered to prescribed protein intake levels for all 10 weeks, and the GP group demonstrated no more than 5.5% variance from prescribed protein intake after week 2 of the intervention. Appendicular lean/soft tissue mass increased over time regardless of group (mean increase=0.269 kg; P=0.028). Similarly, muscle quality scores improved regardless of protein intake group at 60°/s (mean increase=1.011 N*m/kg; P=0.001) and 120°/s (mean increase=0.568 N*m/kg; P=0.015). Increases in absolute strength were only observed for knee flexion peak torque at 60°/s (mean increase= 6.167
N*m; P=0.027) with no other peak torque value increasing over time (P>0.05). No other changes were observed, and no group*time interactions were observed for any measure.

**Conclusion:** Ten weeks of RT, regardless of either a constant or graded protein intake structure, significantly improved muscle quality and appendicular lean/soft tissue mass in older adults. Results suggest that RT increases appendicular lean/soft tissue mass and muscle quality score, while protein intake level did not influence these adaptations to a significant degree.
Review of Literature

Epidemiology and Background

It is well established that the aging process is associated with the loss of both skeletal muscle size and quality\(^1,2\). The age-related loss of skeletal muscle is important for several reasons. The first being the interplay of lean body mass (LBM) and resting energy expenditure (REE) wherein a higher LBM leads to a higher REE\(^9\), since the primary modifying variable of REE is muscle protein metabolism. With a natural loss of LBM and subsequently REE, comorbidities such as obesity become more common in the older population\(^9\). Additionally, muscle proteins provide a primary reservoir for blood amino acids and can provide glucose via gluconeogenesis in the absence of sufficient intake. Indeed, it has been shown that in cardiac and cancer related cachexia (loss of skeletal muscle mass due to a severe disease), loss of muscle mass is a determinant of survival\(^4,5\). Even in the absence of cachexia, in what would be considered typical aging, the loss of skeletal muscle mass poses significant complications for older adults. Indeed, Goodpaster et al. have shown that the loss in knee extensor strength outpaces loss in skeletal muscle mass over a three-year period in older adults (n=3075, age=70-79)\(^1\). This is demonstrative of the loss in skeletal muscle quality that is typical in aging, and in many cases contributes to comorbidities such as frailty, metabolic syndrome, and insulin resistance\(^10\).

This vicious process is termed sarcopenia, and rises from the Greek “sarx” meaning flesh, and “penia” meaning loss. Sarcopenia is considered a part of the natural aging process, and is characterized by the loss of 3-8% of lean muscle mass after the age of 30\(^11\). It was seen in an analysis from the Third National Health and Nutrition
Examination Survey (NHANES III) that 59% of women and 45% of men ≥ 60 years of age were classified as Type I Sarcopenic (Dual energy x-ray absorptiometry (DXA) derived Skeletal muscle index (SMI) 1-2 SD’s below population average), while 10% of women and 7% of men ≥ 60 years were classified as Type II Sarcopenic (SMI ≥ 2 SD’s below population average)\textsuperscript{12}. More conservative estimates place the prevalence of sarcopenia at 14% for those aged between 65-70 and 50% in those people >80 years of age\textsuperscript{13}. Sarcopenia is however difficult to classify and treat, as there was no standard definition across the clinical landscape until recent developments saw the addition of sarcopenia to the International Classification of Diseases vol. 10 (ICD-10)\textsuperscript{14,15}. Even so, the application of standard cut-points for relevant sarcopenic targets (e.g. muscle mass, strength, and function) remain difficult due to variability and reliability of measurement techniques\textsuperscript{14}. The works of three consensus papers headed by the European Working Group on Sarcopenia in Older People, the European Society for Clinical Nutrition and Metabolism Special Interest Groups, and the International Working Group on Sarcopenia suggest that despite difficulties in assessing sarcopenia, it remains a significant clinical outcome and often contributes to comorbidities that infringe upon productive and healthy lives of older adults\textsuperscript{14,15,16–18}. By this notion, clinicians are urged to monitor for signs of skeletal muscle wasting and degradation of skeletal muscle quality\textsuperscript{16}.

Whether classified as sarcopenia or not, loss of skeletal muscle with aging is a harmful and ultimately debilitating process, and it is therefore vastly important to keep in mind the impact of a longer lifespan, and subsequent broadening of the elderly population. It is expected that by 2050 the world’s population that is ≥60 years of age will total 2 billion, and the population of those ≥80 years of age will be 426 million,
approximately a 2-fold and 3.5-fold increase respectively from 2015\textsuperscript{19}. With such a stark increase in the number of individuals that will compose that age group coming in the next 30 years, developing interventions that might provide effective and pragmatic solutions to the degradation of skeletal muscle mass and quality in older adults is critical.

**Resistance Training Interventions**

Resistance Training (RT) has been shown an effective intervention for those of advanced age\textsuperscript{6,7,20,21}. Indeed, RT has been repetitively and robustly shown to combat aging related skeletal muscle degradation\textsuperscript{6,22–30} and strength loss\textsuperscript{24,26–29,31–36}, as well as upregulate important cellular mechanisms as it relates to the accretion of skeletal muscle\textsuperscript{24,37,38}. Despite these promising results of RT interventions in older adults, it is typical for effects to be significantly less potent than for their younger counterparts\textsuperscript{25,37–40}. This differential effect is postulated to be due to a phenomenon termed ‘anabolic resistance’, or the reduction in overall muscle protein synthesis (MPS) in response to an anabolic stimulus\textsuperscript{24}. As a response to this blunted anabolic response, many studies have turned to nutritional modification, notably in the form of enhanced protein intake, to augment the effects of RT interventions\textsuperscript{8,11,22–24,26,28–30,32,33,39,41–50}. Notably, most all RT interventions that have been employed in these types of studies involved some form of progression in the form of either an increase in intensity, volume, or frequency. Therefore, going forward RT in the context of an intervention will refer to RT with some progressive aspect.

*Effects on Body Composition and Muscle Morphology*

Many RT interventions have assessed the measurement of different body composition variables (e.g. fat free mass, lean/soft tissue mass, fat mass, etc.)\textsuperscript{8,26,30,36}. 


Results of these interventions are varied. For example, one meta-analysis of 49 studies with 81 cohorts (n=1328) found that RT alone can increase LBM in older adults by as much as 1 kg over a 10-52 week period. This is in sharp contrast to the expected loss of ~0.18 kg of LBM over a 52 week period with no intervention. In another study with a RT intervention in a population of older individuals (age=65-91 years, n=161), lean mass was assessed via dual energy x-ray absorptiometry (DXA). Lean mass increased by 0.8 kg over a 12 week period, with significant gender differences representing gains of 1.0±1.4 kg and 0.6±1.2 kg for men and women respectively (p=0.042). In yet another study, it was observed that with 10 weeks of RT, older adults (age=59±4 years, n=16) saw significant increases to muscle tissue thickness at the vastus lateralis (p<0.05) and non-significant increases to DXA derived fat-free mass (FFM) (p=0.062). Yet another meta-analysis found in 9 studies a small effect (SMDbs=0.42; 95% CI: 0.18-0.66) of RT on measures of muscle morphology (e.g. cross-sectional area, volume, thickness). While considered a small effect, the differences seen in the effects of RT on muscle morphology measures were indeed statistically significant. This evidence suggests that despite a typical reduction in LBM with aging, it is still possible to incur gains in LBM with a RT intervention in an older population. Additionally, a study focusing on the oldest of the older population of adults found in a 12 week RT intervention among 15 older men and women (age range=85-98; mean age=88.0 yrs) type II skeletal muscle fiber size was significantly increased among the training group. Fiber type analysis indicated selective hypertrophy of type II fibers, contributing enhancement of force output capacity and potentially muscle quality as well as overall size. Muscle quality has been defined as strength per unit area of muscle, and given the enhanced contractile
properties of type II muscle fibers, enlargement of type II fibers would be expected to lead to an improvement in muscle quality\textsuperscript{54}.

Not all studies show promising results regarding the accumulation of LBM. One such study found no significant changes within group in regard to total body LBM after 12 weeks of RT (n=28; mean age=81; p>0.457)\textsuperscript{33}. Additionally, no significant within group increases in regional LBM were reported. Another such 12 week RT intervention (n=41) aiming to assess within-day protein distribution showed a decrease in whole-body and appendicular lean mass of 1.0±0.2kg and 0.7±0.1kg respectively\textsuperscript{55}. Notably however, these adults were consuming 750 kcal below equation predicted maintenance caloric intake. Additionally, the adults included in this study were younger than would typically be considered at risk for severe age-related loss of skeletal muscle (age=35±2 yrs).

Nevertheless, this finding does indicate that RT in adults of any age does not always accrue gains to LBM. Notably however, there is evidence to suggest that these gains are more likely in younger members of the elderly population\textsuperscript{6,56}. Indeed, one meta-analysis reported a relationship between aging and LBM increase such that aging was inversely related with increases in LBM (β=-0.03; P<0.01). Another meta-analysis analyzing 35 studies of older adults (age=59-88.5 years), additionally reported a negative relationship between age and change in muscle fiber size for myosin heavy chain (MHC) I and II (β=-0.33, P=0.002; β=-0.32, P=0.04)\textsuperscript{56}.

In its entirety, RT has been shown to be an effective and pragmatic strategy to combat the age-related loss of skeletal muscle mass. Many studies show that RT elicits gains in LBM, even in the oldest of the older adult population\textsuperscript{6,27,31,52,53}, however there is additionally strong evidence to support that gains are blunted in such adults\textsuperscript{6,56}. The
totality of this evidence suggests that RT can be beneficial in attenuating, if not reversing, the age-related loss of skeletal muscle mass. This evidence additionally suggests that a RT intervention/program is better served to be adopted earlier in adult life and carried on into the latter years of adulthood.

Effects on Strength Outcomes

The positive effect on strength gain in both an elderly and young healthy populations is perhaps the most well-proven adaptation associated with a RT intervention\(^8,24,27,28,31–34\). Indeed, increases to strength are exceedingly common among young populations, particularly in young healthy men\(^57–59\). While potentially not of the same magnitude, directionality of changes to strength remain when RT interventions are performed in a population of older adults\(^31,32\). It was seen in a meta-analysis of 25 studies that all studies analyzed reported positive effects on strength outcomes in regard to a RT intervention\(^31\). Furthermore, this meta-analysis reported large effects of lower (SMD\(_{bs}=1.76\)) and upper body (SMD\(_{bs}=1.61\)) 1 RM measures as well as medium effects of lower body maximum voluntary contraction strength (SMD\(_{bs}=0.76\)), as assessed via between-subject standardized mean differences. Similarly, a recent study performed in older adults (age range= 65-85 years) showed significant increases in strength measures (1 RM, isokinetic and isometric dynamometry) after 12 weeks of RT with a training intensity of 60-75% 1 RM\(^24\). Improvements seen in this study were robust, with dynamometry changes averaging a 17.18% increase in knee flexion and extension (isokinetic and isometric peak torque) and 1 RM changes averaging a 43.6% across three lifts (dumbbell chest press, latissimus dorsi vertical pull down, and leg press). Another study aimed at detailing the mechanistic differences seen in RT between age groups
found significant increases to strength measures of 3 primary lifts (knee extension, barbell back squat, leg press) in both older men and women (age=60-75 years)\textsuperscript{34}. Notably, older men averaged a 42.1\% increase across these three lifts, whereas older women averaged a 40.7\% increase across the three primary lifts. In a randomized controlled trial (RCT), older adults (mean age≥69 years) were assigned differential protein intakes while undergoing a 24 week RT intervention. Strength gains were seen homogenously across all groups where leg extension strength increased by 22±2\% in women and 23±2\% in men by midpoint (week 12), and further increased 17±1\% in women and 16±1\% in men (P<0.001) from week 12 to week 24. Strength gains in the leg press were 31±3\% in women, and 26±2\% in men (P<0.001)\textsuperscript{28}. Another RCT compared the effects of a home-based flexibility program with those of a progressive RT program on measures of strength in a population of older adults (age=70-92 years). They found that the RT group significantly outperformed the home-based flexibility group upon retest in tests of peak torque (between group difference=9.8 Nm, P=0.03), and maximal leg extension (between group difference=127.2 N, P=0.0003)\textsuperscript{60}. Notably, the participants included in this study were mobility-limited, as defined by a score on the Short Physical Performance Battery Test of ≤9. This inclusion criteria is relevant to study results, as it suggests that the benefits conferred by a RT intervention extend even to those with mobility impairments, as are typical of the older adult phenotype. Interestingly, in a meta-analysis analyzing the most effective alterations to training variables to maximize skeletal muscle adaptation found that a RT intervention using 70-79\% 1 RM is most effective for inducing such gains in strength. Additionally, this meta-analysis reported significant strength gains in 5 studies lasting ≤10 weeks\textsuperscript{31}. This is further encouraging evidence that
elucidates that meaningful strength adaptation can occur in what would typically be considered a short intervention.

Evidence of RT mediated strength gain in a variety of different study populations, designs, and timelines is robust and consistent. Given the breadth of findings regarding improvements to strength when undergoing a RT intervention, it is reasonable to expect improvements to strength after undertaking a RT program, provided that the strength measure being assessed utilizes a movement that would theoretically improve with the performance of such a RT intervention. These increases in strength are notable, as strength loss is typical in older adults due to a multitude of factors such as aforementioned skeletal muscle degradation, loss of motor neuronal efficiency and innervation, and disuse. These findings suggest that it is possible to not only impede the progress of these harmful progressions, but to in fact reverse them to a degree. That is to say, robust increases in strength shown in an older population are encouraging, as they are indicative of the fact that maladaptation can be diverted and in some cases reversed. These findings should be taken into consideration when designing a pragmatic and effective intervention to combat the age-related degradation of skeletal muscle size and quality.

Effects on the mTOR Pathway and Muscle Protein Synthesis

Muscle protein accretion is largely dependent on the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). The interplay between MPS and MPB is termed protein balance. Negative protein balance (MPB > MPS) results in a loss of skeletal muscle, whereas positive protein balance (MPS > MPB) results in a gain in skeletal muscle. Interestingly, MPS and MPB are both elevated following a
bout of RT. MPB is largely regulated by three pathways: the ubiquitin-proteasome pathway, autophagy via lysosomal degradation, and the calpain pathway. These three pathways demonstrate a unique interplay, and do not function in isolation, however the exact mechanisms of action that underpin MPB are unclear. The mechanisms of MPS are far more understood however, and rely heavily on signaling pathways induced from an anabolic stimulus.

It is well established that RT upregulates cell signaling pathways that enhance the MPS response. One such pathway of particular interest in the RT response is the mammalian target of rapamycin (mTOR) pathway. Such interest in the mTOR pathway arises for two reasons. The first is that mTOR complex 1 (mTORC1) and its associated pathway has been implicated as a powerful positive regulator of protein synthesis. The second being that this pathway has been shown to be upregulated following a bout of RT. The activities of the mTORC1 pathway and its downstream effectors are robust. One relevant downstream effect of mTORC1 is the prevention of eukaryotic initiation factor 4E-binding protein 1 (4EBP1) from binding to the eukaryotic initiation factor 4E (eIF4E). mTORC1 achieves this by phosphorylating 4E-BP1, and by inhibiting its binding to eIF4E, the translation of protein from mRNA is enhanced. Another such downstream effector of mTORC1 is p70 ribosomal S6 kinase 1 (S6K1). By the phosphorylation of S6K1 via mTORC1, mRNA production as well as ribosomal protein translation has been observed to be enhanced. The mTOR pathway as a whole, and specifically the mTORC1 pathway is complex, and contains many effectors by which it provides a stimulatory effect for overall protein synthesis, however the aforementioned downstream effectors are common targets of measurement when assessing the effects of a
RT intervention. For example, it was seen that mTOR phosphorylation as well as S6K1 phosphorylation were increased at one hour post RT in older men (age=70±2.1 years)\(^3^7\). Notably however, these effects were blunted in comparison to a young cohort (age=29.7±1.7 years). Additionally, AMP-activated protein kinase-alpha (AMPKα), a negative regulator of mTOR signaling and protein synthesis, phosphorylation was significantly elevated in the older group both from baseline and compared to the young cohort at one hour post RT\(^3^7\). In another study examining the acute effects of a RT bout, it was seen that phosphorylation of S6K1 and 4EBP1 were blunted in an older adult cohort (age=70±5 years) at one hour post exercise as compared to a young adult cohort (age=24±6 years). It was additionally seen that fractional synthetic rate (FSR), a measure of protein synthesis assessed via rates of tracer protein incorporation, was lower in the old cohort than in the young cohort\(^4^0\). This blunted anabolic signaling response is well documented\(^7^2–^7^4\), but not universal. Another similar study examining the effects of an unfamiliar RT bout found acute stimulation of relevant downstream targets of the mTORC1 pathway to be similar between young (age=27.9±1.0 years) and old (age=64.4±0.9 years) participants. Notably however, FSR was not elevated from baseline in older adults while it was in young adults\(^2^5\).

While certain findings do indicate that older adults can achieve a similar level of anabolic signaling after a bout of RT\(^2^5\), many findings indicate that after a bout of RT only, relevant signaling pathways as well as MPS show an attenuated response in older adults as compared to their young counterparts\(^3^7,^3^8,^4^0,^7^2–^7^5\). Blunted signaling as well as concomitant decreases in MPS in response to a bout of RT can perhaps provide a mechanistic basis of the overall effects of aging on skeletal muscle given that similar
basal rates of MPB and MPS have been observed in young and old adults\textsuperscript{75–78}. Despite this however, RT remains a stimulator of anabolic signaling pathways (notably the mTOR pathway) and MPS, even in older adults. This factor is important in the development and execution of practical interventions to offset the loss of skeletal muscle that occurs with aging.

**Protein Intake Modification Interventions**

In addition to RT, much attention has been given to the modification of protein intake as an intervention to negate the aging induced loss of skeletal muscle. Interest in such interventions is largely driven by a desire to maximize anabolic responses that are typically blunted in an older adult population\textsuperscript{11,41–43}, and is unsurprising given that amino acids (AA), the constituent macromolecules of protein, have been implicated as a potent anabolic stimulator\textsuperscript{76,79–81}. Indeed, several strategies have been employed to combat the phenomenon of anabolic resistance. Such strategies have focused on overall protein intake\textsuperscript{26}; acute protein supplementation\textsuperscript{8,27–29,41,44,45}, typically following a bout of RT; and overall nutrient and protein timing\textsuperscript{41,46,55}. Importantly, these interventions are often intertwined given that protein supplementation has bearing on both overall protein intake and protein timing. While conflicting results exist, some promising findings do indicate that older adults might benefit from enhancing protein intake when undergoing some type of RT\textsuperscript{23,25,,28,29,35,38,41,43,44,46,49,50}.

**Effects on Body Composition and Muscle Morphology**

Evidence suggests that RT alone can improve body composition and muscle morphology in older adults\textsuperscript{22,24,31}. This is equivocal however, as other research suggests that RT alone may not enhance body composition factors in an elderly population.\textsuperscript{6,33,55,56}
Such conflicting findings have led to the investigation of protein intake manipulation as a strategy to augment gains in body composition related variables. Indeed, the conjunctive approach of protein intake enhancement and RT is a common intervention strategy in an elderly population. A meta-analysis aimed at assessing changes in strength and body composition variables in response to ≥6 weeks of RT with or without protein supplementation found that when performing RT, conjunctive protein supplementation outperformed RT alone in fat-free mass (FFM) gain (mean difference (MD)=0.30kg; 95% CI: 0.09kg, 0.52kg; P=0.007), fat mass loss (MD: -0.41kg, 95% CI: -0.70kg, -0.13kg; P=0.007), and fiber cross-sectional area (fCSA) gain (MD: 310µm²; 95% CI: 52µm², 570µm²; P=0.02). Importantly however, this analysis included studies with both older and younger adults, and in a sub-analysis it was revealed that when age was controlled as a covariate, it influenced FFM gains in older adults (>45 years) and rendered FFM gain not significant (MD=0.06; 95% CI: -0.14-0.26). Age did not however influence fCSA gain, and the gains seen by older adults therefore remained significant. Notably, protein supplementation strategies differed among studies included in this analysis, and average protein intake was low (mean=20±18 g/d) in comparison to the ~40g bolus that is generally considered optimal for older adults undergoing RT. This could potentially provide some a basis as to why this meta-analysis found that protein supplementation does not augment FFM gains in older adults when undergoing RT, however it remains that advanced age does perhaps limit the effectiveness of protein supplementation regarding FFM gain. This analysis additionally performed a meta-regression to find optimal protein intake for both young and old participants. With protein intakes ranging from 0.9 g/kg/d to 2.4 g/kg/d, it was seen that 1.62 g of protein/kg/d (95%
CI: 1.03-2.20 g/kg/d) marked a plateau in RT induced gain in FFM. This suggests that the recommended daily allowance (RDA) of 0.8g protein/kg body weight/day recommended for healthy adults is likely too low for optimal skeletal muscle adaptation. Conversely however, a meta-analysis with similar aims found that protein supplementation had a similar effect in augmenting gains in FFM between both young (pooled estimate=0.81kg; 95% CI: 0.53, 1.1kg; P<0.00001) and old (pooled estimate=0.48kg; 95% CI: 0.10, 0.85kg; P<0.01)\textsuperscript{44}. Notably, protein supplementation did not show enhanced effects for change in fat mass, type I fCSA, or type II fCSA in older individuals (age>50 years). Importantly however, protein supplementation in this meta-analysis was higher than in the former (mean=42±30g) on training days and could potentially contribute to this more favorable finding in regard to FFM change. Notably, total dietary protein intake data are lacking from this meta-analysis given the focus only on protein supplementation. Yet another meta-analysis aimed at assessing the effects of ≥12 wks of RT with or without milk protein supplementation in those aged ≥60 years found that milk protein consumption augmented gains of FFM in participants undergoing a RT intervention (Weighted Mean Difference (WMD)=0.74 kg; 95% CI: 0.30, 1.17 kg)\textsuperscript{30}. This analysis did not however demonstrate any significant effects in regard to fat mass (WMD=0.30 kg; 95% CI: -0.25, 0.86 kg) or body weight (WMD=1.02 kg; 95% CI: -0.01, 2.04 kg). Interestingly, while not significant, this study did demonstrate that when participants received >20g/d of milk protein supplement as compared to ≤20g/d, FFM gains were increased (>20: Mean Effect=1.53 kg; 95% CI: 0.64,2.42 kg; ≤20: Mean Effect=0.69 kg; 95% CI: 0.03, 1.35 kg; P=0.273). This analysis also controlled for type of supplementation provided, and again while not significant, it was seen that whey protein
outperformed all other milk proteins (Whey Protein: Mean Effect=1.53 kg; 95% CI: 0.64, 2.42 kg; Other Milk Proteins: Mean Effect: 0.56 kg; 95% CI: 0.12, 0.99 kg; P=0.128). While neither of these effects reached significance, they are consistent with others that suggest ~40g of whey protein supplementation is advantageous in older adults41,42,49.

However, there are inconsistencies in the research findings in this area. In a RCT examining the effects of 14-weeks of RT in combination with various post-training supplements (protein only, creatine only, protein+creatine, placebo) on body composition variables in middle-to-older adults (age=48-72), no training*supplement interactions were seen82. Within group effects of training were evident for DXA derived lean/soft tissue mass (increase), arm lean/soft tissue mass (increase), and arm % fat (decrease), indicating that training alone was responsible in explaining differences seen from baseline to post-intervention. Another RCT aiming to compare the effects of RT combined with leucine enriched whey protein compared to RT + a placebo supplement (23g Maltodextrin) found no significant differences between group for total or regional LBM when change scores were compared (mean age=81 years; n=28; P≥0.088).33 Yet another RCT found that the ingestion of 20g of whey protein immediately after the completion of RT (3x/wk; 12 wks) did not augment total body or regional lean mass gains in older adults (age range=65-91; n=161; P≥0.365)27. Yet another RCT examining 24 weeks of RT with or without protein supplementation (15g/d) in older individuals (age=70±1 years; n=29) found no significant group*time interactions regarding leg lean mass (P=0.61), computed tomography derived quadriceps cross-sectional area (P=0.60), or mean fCSA (P=0.25)28.
In total, there is evidence that protein supplementation and overall enhanced protein intake in combination with RT is beneficial for body composition outcomes, especially when that supplementation approaches values close to a dose of ~40g per acute bolus post-training\textsuperscript{26,41,43,44,46}. There are contrasting findings however with a number of research studies showing no added benefit to of protein supplementation with RT in older adults regarding body composition\textsuperscript{27,28,33,82}. As has been previously mentioned, it is estimated that \(\geq 40g\) of acute protein supplementation is more ideal for skeletal muscle outcomes in older adults than is the 20g commonly reported to provide optimal anabolic stimulation in young adults\textsuperscript{41,43}. That being a consideration, it is important to note that the highest value of supplementation reported in the aforementioned studies with negative findings was 35g per acute bolus\textsuperscript{82}. Given that each of these studies failed to meet the suggested threshold value for optimal anabolic stimulation in older adults, further investigation into interventions utilizing \(\geq 40g\) of protein per acute bolus in addition to overall enhanced protein intake is warranted.

**Effects on Strength Outcomes**

The positive effects of RT alone on strength adaptation have been robustly shown\textsuperscript{24,27,28,31–33}, and attention has been given to protein intake manipulation in an attempt to maximize these strength gains\textsuperscript{8,23,26–29,32,45,55}. In a previously described meta-analysis\textsuperscript{26}, it was seen that protein intake contributed to increases in 1 RM above RT alone (MD=2.49 kg; 95% CI: 0.64, 4.33 kg). Interestingly, this analysis additionally separated effects in trained vs. previously untrained individuals and found that effects of protein supplementation effects to increase 1 RM are stronger in previously trained participants (Trained: MD=4.27 kg; 95% CI: 0.61, 7.94 kg; Untrained: MD=0.99 kg;
95% CI: -0.27, 2.25 kg). Importantly, when analyses controlled for age, efficacy of protein supplementation was not affected\textsuperscript{26}. In a RCT designed to examine the effects of whey protein, Vitamin D, and Vitamin E supplementation, it was seen in a cohort of 60 sarcopenic older adults (age range=60-85 years) that there was a group*time interaction for isokinetic dynamometer assessed handgrip strength, with the whey protein supplement group outperforming the placebo group by an average of 2.68 kg (95% CI: 0.71-4.65 kg; P=0.009)\textsuperscript{83}. Importantly, this intervention did not include a RT protocol, and was conducted in individuals fulfilling the following diagnostic criteria for sarcopenia: (1) relative skeletal muscle mass index (appendicular skeletal muscle mass (kg)/height (m)$^2$) <5.7 kg/m$^2$ or <7.0kg/m$^2$ for women and men respectively and (2) handgrip strength <18 kg and <26 kg for women and men respectively. Despite these two features, this study provides evidence that protein supplementation, even in isolation, can provide some benefit for strength gain in older adults. In another meta-analysis examining the effects of protein supplementation combined with RT vs. RT alone found in 13 RCTs that leg strength was significantly increased when protein supplementation was used (SMD=0.69 kg; 95% CI: 0.39-0.98 kg; P<0.00001) in cohorts of older adults (mean age=73.4±8.1 years)\textsuperscript{84}. Importantly however, no significant differences were seen for measures of upper body or hand grip strength in 6 RCTs. Additionally, in this meta-analysis protein supplementation strategies varied widely, with 2 RCTs using body mass to inform protein supplementation, and the remaining 15 RCTs using between 10-35 g of supplement per day. Yet another meta-analysis found that in 21 RCTs examining RT with or without protein supplementation in older adults (age>50 years) protein supplementation significantly augmented hand-grip strength (SMD=0.29 kg; 95% CI: 0.009-0.59 kg; P<0.00001).
0.08, 0.50; 6 RCTs), knee extension strength (SMD=0.27 kg; 95% CI: 0.06,0.47; 11 RCTs), and leg press strength (SMD=0.33 kg; 95% CI: 0.01-0.64; 5 RCTs)\(^29\). Finally, while these findings are statistically significant, evidence supporting clinically meaningful changes in such measures are lacking.

While there is some evidence to support the augmentation of strength gain, there also exists evidence to suggest that protein supplementation does not provide any benefit to strength gain over and above that of RT alone. One RCT seeking to examine the effects of RT with or without peanut protein supplementation in both a 6 and 10 week cohort of older adults (n=39; mean age=58.6±8.0 years) found only one group*time interaction (knee flexor peak torque; 10-week cohort; G*T P=0.032), however this effect was negated when groups were pooled together (Knee Extensor Peak Torque P=0.531; Knee Flexor Peak Torque P=0.127)\(^57\). Another RCT examining the effects of protein supplementation without a concomitant RT intervention found that two 20 g boluses of protein per day did not improve leg press strength (P=0.93), chest press strength (P=0.85), or handgrip strength in the dominant (P=0.27) or nondominant hand (P=0.99) in a cohort of older adults (n=36; age≥70 years)\(^85\). In yet another RCT, older adults (n=161; age range=65-91) completed a 12-week RT program with or without 20g of protein supplementation immediately following RT. No differences were seen in isokinetic dynamometry derived quadriceps strength when adjusted for gender, age, and baseline values (P=0.487)\(^27\). It is not only protein supplementation that has been examined however, as in a cross-sectional analysis it was seen that there was no correlation between number of meals providing ≥0.4g protein/kg body weight and number of meals providing ≥2.5g leucine with leg extensor strength and power, and
handgrip strength (P>0.05 for all Pearson Correlation Coefficients) in a cohort of healthy older adults (n=97; median age=77.0 years)\textsuperscript{86}. Similarly, in a RCT assessing a RT intervention using either an even protein distribution throughout the day (30g protein 3x/day) or a skewed protein distribution (10g, 20g, 60g consumed at breakfast, lunch, and dinner respectively) in healthy adults (mean age\textsubscript{EVEN}=33±2 years; mean age\textsubscript{SKEW}=36±2 years) found no differences in strength gain over the 12-week intervention (P=0.470)\textsuperscript{55}. Further supporting this line of evidence is a meta-analysis where the effects of RT with or without protein supplementation in older adults (mean age range=61-79 years) were examined. Herein, a total on 9 RCTs with a total of 462 participants were examined, and it was determined that protein supplementation provided no additional benefit above that of RT alone in regard to strength measures (SMD=0.13; 95% CI: -0.06, .03)\textsuperscript{8}. It is important to note however, that there were large differences in protein supplementation protocols, with 3 studies using body mass to inform the amount of supplementation provided (range=0.3-0.8 g/kg/day), and 6 trials using a supplementation total regardless of body mass (range=6-40g/day) with only one study reaching 40g of supplemental protein per day.

Taken in its entirety, there is evidence to suggest both that protein supplementation in conjunction with RT can be beneficial in augmenting strength gains, and contrastingly, protein supplementation during a RT intervention provides no additional benefit. Some important methodological factors come into the forefront when effects of protein modification are analyzed. First, few interventions achieve ~40g of protein in a single bolus in older adults which has been shown to outperform a comparative 20g of protein that is ideal for younger individuals undergoing a RT
protocol, but may be insufficient in an older cohort. This shortfall could potentially contribute to the negligible findings of protein supplementation seen in RCTs and meta-analyses alike. The second consideration is that many studies manipulate protein only via supplementation. This is a common methodology, and is useful to some ends, however it is thought that modification of overall protein is more impactful to relevant outcomes than protein intake in a single instance. Overall, there is conflicting evidence, however further investigation into supplementation with a large (~40g) bolus of protein and overall protein modification upwards of 1.6g protein/day is warranted.

Effects on the mTOR Pathway and Muscle Protein Synthesis

As previously discussed, muscle protein accretion occurs by MPS exceeding MPB over time. As such, maximizing upregulation of aforementioned cell signaling pathways that contribute to MPS is critical, particularly in an aged population. While RT alone has been examined as a strategy to enhance such signaling pathways (e.g. mTOR and its relevant effectors), attention has also been given to protein or AA intake to stimulate such events in isolation, or as a potential force multiplier for signaling events when combined with RT. Indeed, it was seen in a RCT examining different whey protein boluses (0g, 10g, 20g, 40g) after unilateral knee extensor training in older adults (n=37; mean age ≥70) that myofibrillar protein fractional synthetic rate (FSR) was significantly higher in the resting leg in participants who consumed at least 20g of whey protein (P<0.005) as compared to those who consumed 0g of whey protein. It was additionally seen that in participants’ legs that underwent RT that consumption of at least 20g of whey protein produced a higher FSR than both 0g and 10g
doses (P<0.01) and that FSR was 32% higher in those that consumed 40g than those that consumed 20g (P=0.02). Notably, trained legs exhibited higher FSR than untrained legs in all instances (P<0.05). This suggests that in older individuals undertaking RT, consumption of 40g of whey protein outperforms up to 20g of whey protein ingestion in terms of FSR, a notion suggested by others\(^{41,43,46}\). In another randomized trial examining the effects of graded protein boluses (10g, 20g, 35g) on FSR, AA concentrations, and AA kinetics in older adults (n=33; mean age=73±2 years) it was seen that only the 35g whey protein bolus produced a significant increase from basal FSR (44±16% different from basal; P<0.05)\(^50\). Notably, both net balance (determined via area under the curve of rate of disappearance of ingested phenylalanine minus area under the curve of rate of appearance of endogenous phenylalanine) of phenylalanine and muscle protein-bound L\([1^{-13}C]\)phenylalanine enrichment (determined via gas chromatography combustion isotope ratio mass spectrometry, and gas chromatography mass spectrometry) were significantly enhanced in participants consuming a 35g whey protein bolus as compared to a 10g or 20g bolus (P<0.05). Importantly, there was no RT intervention associated with this trial, suggesting that even in a resting state, 35g of whey protein can outperform up to 20g of whey protein in older adults. Yet another trial examining the effects of combined RT and AA ingestion in young (n=7; mean age=29.7±1.7 years) vs. old subjects (n=6; mean age=70.0±2.1 years) on FSR and effectors of the mTOR pathway found that FSR was diminished in older participants up to 3 hours after RT and 2 hours after AA ingestion, but increased significantly to resemble their younger counterparts from 3-6 hours post RT and AA ingestion (P<0.05)\(^37\). This study additionally found that mTOR phosphorylation and S6K1 phosphorylation significantly increased from baseline
at all time points post RT, albeit more robustly after the ingestion of AA. (P<0.05).
Notably, 4EBP1 phosphorylation status was not significantly increased until the
consumption of AA post-RT, and AMPKa phosphorylation was significantly upregulated
at both 1 (without AA consumption) and 3 hours (with AA consumption) post-RT
(P<0.05). These findings suggest that while the consumption of AA in older individuals
likely has some benefit, there remains a delay in signaling events and protein accretion
initiation in older adults as compared to young.

Protein type and AA composition has also been examined in its relevance to
anabolic signaling in older adults. The essential AA (EAA) leucine has drawn particular
interest, as it has been implicated as a strong anabolic stimulus, potentially due to its
unique ability to stimulate mTOR, 4EBP1, and S6K1 phosphorylation to a greater degree
than other EAAs\(^91,92\). Indeed, in a randomized trial aiming to compare the effects of 6.7g
of EAA with differential compositions (26% leucine vs. 41% leucine), both young (n=16;
mean age<31 years) and old (mean age>66 years) participants were examined for FSR
via isotope infusion in the post-prandial period. It was seen that both 26% leucine and
41% leucine EAA consumption significantly increased FSR in the young cohort (P<0.05)
from basal values, while only the 41% leucine EAA consumption increased FSR from
basal values in the old cohort (P<0.05)\(^39\). Another trial examining the acute effects of
6.8g of EAA supplementation (26% leucine) on FSR and relevant mTOR pathway targets
before and after 12 weeks of RT found that older adults (n=19; mean age=71±4 years)
significantly increased FSR after EAA consumption both before and after undergoing 12
weeks of RT\(^24\) (P<0.05). This increase in FSR in older participants created a more
youthful signature, as FSR rates were similar 3 hours post-EAA ingestion between old
participants ingesting 6.8g of EAA and young participants ingesting 10g of EAA. This study additionally reported a significant upregulation to mTOR and S6K1, as determined via immunoblotting (P<0.05). These findings are unusual, as 12 weeks of RT did not improve sensitivity to EAA, and EAA alone were sufficient to stimulate FSR to the degree of a young adult. While this does call into question the existence of universal anabolic resistance in an elderly population, this study does still demonstrate the effectiveness of EAA supplementation in older adults. EAA supplements are not the only supplement of interest however, as given its high EAA content in comparison to other protein sources (e.g. milk, casein, egg, soy, pea) and in particular leucine content, whey protein has become a supplement of interest in this regard. Indeed, whey protein has been shown to outperform isonitrogenous collagen peptides (reviewed in) as well as a milk supplement when examining MPS.

Overall, the effects of protein ingestion with or without concomitant RT on cellular anabolic signaling are well documented. A robust body of evidence suggests that EAA or protein ingestion is beneficial for cell signaling events as it pertains to skeletal muscle accretion in older adults. Furthermore, these effects are typically attributed primarily to leucine content within a given protein source, and thus whey protein arises as a substantial intervention strategy due to its large EAA and leucine content as compared to other protein sources. Given the lack of a true upper boundary in terms of maximal signaling effects of a protein bolus, more targeted research into the upper limit of a bolus of protein is warranted. Importantly, effectors of the mTOR pathway as well as tracer methodology driven measures such as FSR are meaningful and
elucidating marks when it comes to determining optimal strategies for protein intake with or without RT in older adults.

**Summary**

The totality of evidence suggests that RT is an effective and pragmatic intervention to combat the effects of aging on skeletal muscle loss. RT shows robust effects in terms of strength development and in some instances can attenuate or even reverse the true loss of skeletal muscle, typical in the aging phenotype. RT additionally has a large stimulatory effect on relevant MPS pathways (e.g. mTOR/mTORC1), suggesting that prolonged and persistent RT can provide sufficient MPS to produce relevant and observable effects on muscle protein accretion in an elderly population. Importantly, all RT interventions in older adults presented herein all involved some progression in regard to RT. This typically involved an increase in training volume over time, however increases in absolute intensity, relative intensity, or frequency are also common. It is also seen that in some instances, the modification of protein intake via supplementation or dietary intake can augment such gains to strength and lean tissue gain. This is not universally the case however, as findings for both strength and lean tissue gain in many cases are equivocal. Nonetheless, there remains promising evidence at the molecular and cellular level for the use of protein in conjunction with RT in the elderly. RT in conjunction with protein, particularly protein sources that contain a high proportion of LEU, typically shows enhanced anabolic signaling as compared to either intervention in isolation. Two studies have examined the acute effects of graded protein intake with and without RT in older adults. Both studies found that the maximal dose of protein provided (40 g and 35 g respectively) stimulated muscle protein synthesis to a
greater degree than any other dose. It has additionally been posited that the initial
upregulation in MPS pathways is attributable to repair of acute muscle damage, with
subsequent elevations targeted at muscle hypertrophy specifically (reviewed in⁹²). Given
the success of these acute graded protein intake trials in older adults along with the
hypertrophy driven MPS signaling and the bioenergetically expensive nature of de novo
protein synthesis, it stands to reason that skeletal muscle outcomes in older adults may
benefit from a graded protein structure wherein protein intake is matched to training
volume/intensity. However, no study has examined the effects of chronic graded protein
intake in conjunction with progressive RT in older adults on body composition, strength
measures, and muscle morphology.

**Purpose and Hypotheses**

The primary aim of the present study was to determine whether grading protein
intake promotes greater total body lean/soft tissue mass gain in older adults compared to
consuming protein at a constant level of 0.8-1.0 g of protein/kg of body weight/day
(g/kg/d) while performing progressive RT. This constant level of protein intake
consumed by the comparable group represents the RDA for protein intake in adults (0.8
g/kg/d) and allows for slight variability due to typical dietary fluctuation. The secondary
aims of the present study were to determine if grading protein intake to training
intensity/volume promotes 1) greater strength gain, 2) greater muscle tissue thickness
gain, and 3) greater muscle quality increase than consumption of protein at a constant
level while performing a progressive RT intervention. In regard to the primary aim, it is
hypothesized that grading protein to training intensity/volume will produce greater
lean/soft tissue mass gain than consuming protein at a constant level while undergoing a
progressive RT intervention. Regarding secondary aims, it is hypothesized that grading protein to training intensity/volume will produce 1) greater strength gain, 2) greater muscle tissue thickness gain, and 3) greater enhancement to muscle tissue quality than consuming protein at a constant level (0.8-1.0 g/kg/d) while undergoing a progressive RT intervention.
Methods

Ethical Approval and Participants

Prior to study initiation, protocols were reviewed and approved by the Wake Forest University Reynolda Campus Institutional Review Board and was conducted in accordance with standards set by the latest version of the Declaration of Helsinki (IRB approval number: IRB00024112). This trial was registered as a clinical trial at www.clinicaltrials.gov (ID: NCT04845282).

Healthy, community dwelling older adults (≥55 years) were recruited for the present study and were screened upon establishing interest, and consenting to participate in the study. Participants were required to be free from comorbidities that could be exacerbated by study protocols such as: cardiovascular disease, type 1 or type 2 diabetes, renal failure, liver disorders, or thyroid disorders; or were required to provide explicit written consent from a physician stating that they were medically cleared to participate in the study after review of study protocols by the participant’s primary care physician. Participants were excluded if they were: consuming an agent known to be confounding to skeletal muscle adaptation (e.g. creatine monohydrate, testosterone, or growth hormone), currently undergoing cancer treatment, used a whey protein supplement regularly over the previous three months, were pre-menopausal (women only). Participants additionally could not have adhered to a progressive RT program in the three months prior to study initiation. A progressive RT program was defined as a program in which volume, intensity, or difficulty were monitored and/or modulated. Prior to study initiation, participants were instructed to cease any other vigorous exercise outside of study protocols. Descriptive characteristics of study participants are presented in Table 1.
Study Design

Study design is depicted in Table 2. Briefly, participants underwent a testing battery 48-72 hours prior to the initiation of an acclimation and 3 RM testing period (PRE). Participant randomization occurred between PRE and acclimation protocol initiation. Following PRE, participants then underwent one week of acclimation and 3 RM testing wherein participants performed all exercises called for by study protocols to become familiar with movements required of them. Participants were additionally asked to log dietary intake for the week in order to acclimate to this process prior to initiation of RT and dietary protocols. During this acclimation week, participants also tested baseline 3 RM$s for all exercises required by the study. 3 RM$s were tested in accordance with National Strength and Conditioning Association (NSCA) protocols. Following the acclimation week, the nutrition protocol began along with 5 weeks of RT, after which participants underwent testing for a subset of measures at week 5 (MID). Following this, participants continued to adhere to nutrition protocols and performed 5 more weeks of RT, after which undergoing the full battery of testing 72 hours after the last bout of training. The battery of testing is detailed further below along with more detailed descriptions of both the RT and nutrition interventions.

Table 1: Study Measurements Timeline

<table>
<thead>
<tr>
<th></th>
<th>PRE</th>
<th>Wk 0</th>
<th>Wk 1</th>
<th>Wk 2</th>
<th>Wk 3</th>
<th>Wk 4</th>
<th>Wk 5</th>
<th>MID</th>
<th>Wk 6</th>
<th>Wk 7</th>
<th>Wk 8</th>
<th>Wk 9</th>
<th>Wk 10</th>
<th>POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA Scan</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Dynamometry</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Acclimation</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Nutrition Protocol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 RM Testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
Resistance Training Protocol

All participants performed 3 full body RT sessions per week at Wake Forest’s Health and Exercise Science Clinical Research Center. Training times ran concurrently with Wake Forest’s Healthy Exercise and Lifestyle Programs, and therefore training sessions were held on Monday/Wednesday/Friday from 6:00-9:00 AM and Monday/Tuesday/Thursday from 5:30-7:00 PM. Participants were allowed to attend any sessions they would like with the exception that they could not attend both Monday morning and evening sessions on the same day. Training was conducted in accordance with the American College of Sports Medicine’s 2009 position stand, “Progression models in resistance training for healthy adults”. The training progression model is detailed further below in Figure 1 and Table 3. This RT protocol was divided largely into two constituent mesocycles, or around one month of training with a common focus and a progression in intensity and/or volume. Mesocycles were further divided into microcycles, or one week of training sessions. Briefly, the first mesocycle began with training intensities of 60% 1 RM at week 1, progressing to 75% 1 RM by week 4 after which participants underwent a deload, or an intentional drop in training intensity and volume, to maximize recovery and performance for both the next mesocycle and midpoint testing at 50% 1 RM. The second mesocycle began at 70% 1 RM and progressed to 85% 1 RM by week 9 after which participants underwent another deload period to dissipate fatigue prior to post-testing. Exercise intensity was determined as a percentage of predicted 1 RM from pre-tested 3 RMs using NSCA protocols and conversion factors. Briefly, participants’ 3 RMs were divided by 0.93 to predict a 1 RM for each exercise. Predicted 1 RM was then used to prescribe training intensities for the
duration of the study. Additionally, exercises used in each session are detailed in Table 4 below. Exercises were included based on their common inclusion in RT programs as well as availability of the Clinical Research Center. All exercises were instructed to be completed for 8-12 repetitions. If the repetition range was unattainable for a participant, intensity was decreased by 5% per repetition missed. Adherence to training sessions was monitored throughout the study, and all training sessions were overseen by an investigator (JMM, KKL, SCN) to ensure proper technique.

Table 2: Resistance Training Progression Model

<table>
<thead>
<tr>
<th>Resistance Training</th>
<th>% 1 RM</th>
<th>Repetition Range</th>
<th>Number of Sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>60</td>
<td>8-12</td>
<td>2</td>
</tr>
<tr>
<td>Week 2</td>
<td>65</td>
<td>8-12</td>
<td>2</td>
</tr>
<tr>
<td>Week 3</td>
<td>70</td>
<td>8-12</td>
<td>3</td>
</tr>
<tr>
<td>Week 4</td>
<td>75</td>
<td>8-12</td>
<td>3</td>
</tr>
<tr>
<td>Week 5*</td>
<td>50</td>
<td>4-6</td>
<td>2</td>
</tr>
<tr>
<td>Week 6</td>
<td>70</td>
<td>8-12</td>
<td>2</td>
</tr>
<tr>
<td>Week 7</td>
<td>75</td>
<td>8-12</td>
<td>3</td>
</tr>
<tr>
<td>Week 8</td>
<td>80</td>
<td>8-12</td>
<td>3</td>
</tr>
<tr>
<td>Week 9</td>
<td>85</td>
<td>8-12</td>
<td>3</td>
</tr>
<tr>
<td>Week 10*</td>
<td>30</td>
<td>3-5</td>
<td>1</td>
</tr>
</tbody>
</table>

*Denotes a week of significant drop in volume load due to planned deload weeks (week 5 and week 10)

**Nutrition Intervention**

After PRE, participants were randomly assigned to one of two groups, graded protein intake (GP), or constant protein intake (CP). Prescribed protein intake levels per group are depicted in Table 5. Briefly, the GP group began at 0.8g of protein per kg of bodyweight per day (g/kg/d) from both a whey protein supplement and dietary sources
and increased to 2.2 g/kg/d at weeks 9 and 10. Importantly, a whey protein supplement, Combat 100% Whey (MusclePharm®, Las Vegas NV), was provided to this group to assist in achieving protein intake goals. This supplement provided 120 kcal, 25 g of protein, 1 g of fat, and 2 g of carbohydrates per serving. A whey protein supplement was chosen based on its high percentage of leucine as compared to other common protein supplements. Additionally, the protein supplement was provided as one serving (25 g) in weeks 1-5 of the study after which supplementation increased to two servings (50 g) at week 6. In the weeks thereafter, protein supplement could be increased to 75 g at any time by either choice of the participant, or failure to meet protein intake goals through the diet. For protein supplement boluses up to 50 g, all protein was consumed immediately after training, and between meals on non-training days. If protein supplement intake reached 75 g, participants were instructed to take 50 g immediately post-training and 25 g in between meals on training days, and at two separate occasions in between meals on non-training days. Conversely, the CP group was instructed to consume protein at a constant level of 0.8-1.0 g/kg/d, in accordance with the RDA, for the duration of the study. This group was not provided any sort of protein supplement. Adherence to prescribed protein intake levels were monitored throughout the study via weekly monitoring of self-reported dietary inputs. Additionally, both groups were prescribed a caloric surplus of 200-300 kcal based on the Harris-Benedict equation using the moderate activity factor in order to potentiate skeletal muscle hypertrophy⁹⁸. Participants were instructed to consume 3-5 g/kg/d of carbohydrates based on the recommendations of Slater and Phillips⁹⁹. Fats made up the remainder of calories for a given day. Importantly however, participants were instructed to focus primarily on their protein intake levels and daily energy intake.
The differentiating intervention between the two groups was the amount of protein ingested. Nutrient intake was tracked via a mobile application (MyFitnessPal, Inc.; Baltimore, MD, USA). This mobile application has been validated against paper-based food intake records. Participants were asked to track food intake 3 days per week (2 week days and 1 weekend day), a strategy that has been used previously. Nutrient tracking was monitored weekly by the research staff, and if participants were out of the desired range of protein intake and daily energy consumption, they were instructed in how to adjust intake for the following week to meet their goals.

Table 3: Prescribed Protein Intake for Each Group by Week

<table>
<thead>
<tr>
<th>Protein Intake</th>
<th>Wk1</th>
<th>Wk2</th>
<th>Wk3</th>
<th>Wk4</th>
<th>Wk5</th>
<th>Wk6</th>
<th>Wk7</th>
<th>Wk8</th>
<th>Wk9</th>
<th>Wk10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant Protein Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Intake (total) (g/kg/day)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Protein intake (supplement) (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Graded Protein Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Intake (total) (g/kg/day)</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50*</td>
</tr>
<tr>
<td>Protein intake (supplement) (g)</td>
<td>0.8</td>
<td>1</td>
<td>1.2</td>
<td>1.4</td>
<td>1.4</td>
<td>1.6</td>
<td>1.8</td>
<td>2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*Denotes an optional increase to 75g of supplement per day as determined by either participant desire to increase or failure to meet protein goal.

Measures

Height, Weight and Body Mass Index

Height was measured using a wall-mounted Seca 216 stadiometer (Hamburg, Germany) at PRE. Participants were instructed to remove their shoes and stand with their back to the wall with eyes facing straight in front. The measuring bracket was then pulled down until it laid flat against the head of participants. Height was recorded to the nearest 0.5 cm. Weight was measured using a Tanita scale (Arlington Heights, IL, USA) after the removal of all outerwear and shoes. Weight was recorded to the nearest 0.1 kg. Body
Mass Index (BMI) was calculated by using the CDC promoted equation of weight in kg/height in m².

**Lean/Soft Tissue Mass**

Lean/soft tissue mass was defined as the DXA derived body mass without bone or fat mass. Whole body and regional lean/soft tissue mass were determined via DXA scan at both PRE and POST. Participants were instructed to perform an overnight fast and were then subjected to total-body DXA testing (GE Lunar iDXA; GE Corporation, Fairfield CT, USA). Briefly, participants were instructed to wear clothing free of any metal, if this instruction was violated participants were provided a standard hospital scrubs. They were also to remove any metal objects (i.e jewelry) and were positioned in the field of view of the machine by the same trained and experienced research staff at all time points. After participants were positioned, the DXA scan commenced and lasted approximately 10 minutes per participant. Regions of interest were set to create accurate regional measurements and underwent quality control adjustments by a departmental certified bone densitometry technologist. The DXA system in our department has a coefficient of variation of 0.85% for measures of lean/soft tissue mass.

**Vastus Lateralis Thickness**

Following body composition testing, participants were tested for vastus lateralis thickness using ultrasound. Vastus lateralis thickness was determined by placing a 13-6 MHz transducer (SonoSite M-Turbo; FUJIFILM Corporation, Minato Cirtty, Tokyo, Japan) midway between the inguinal crease and the superior aspect of the patella in the transverse plane. Measurements were taken from the supine position after ≥10 minutes to account for fluid shifting, and were taken with accompanying software by aligning the
measuring calipers with the outer connective tissue (superficial fascia) and the inner connective tissue (deep fascia), thus surrounding the vastus lateralis. Measurements were taken immediately after image capture and saved according to manufacturer protocols. All images and measurements were taken by the same investigator in order to minimize variability among measurements as suggested previously\textsuperscript{102,103}. Reference images for PRE and POST are presented in Figure 13.

\textit{Peak Torque}

Knee extensor peak torque was assessed with the use of an isokinetic dynamometer (Humac Norm; Computer Sports Medicine Incorporated, Stoughton, MA, USA). The subject’s preferred leg was tested at PRE, MID, and POST at 60°/s and 120°/s moving through a 30° range of motion. Upon entry, the subject was informed of protocols and the purpose for the test, participant anthropometric information was entered into the accompanying software, after which the dynamometer was adjusted to software derived recommendations. The rotational axis was then aligned with the lateral epicondyle of the subject’s involved leg and the testing protocol began. Participants performed 3 repetitions of concentric extension and flexion at both 60°/s and 120°/s in order to practice the motion that would be required for the test. After practice repetitions were complete, participants completed 5 repetitions at 60°/s and 120°/s respectively and the peak torque achieved over 5 repetitions was calculated by the dynamometer. After the test was complete, data were saved and exported for analysis.

\textit{Skeletal Muscle Quality Score and Lean/Soft Tissue Mass Index}

Given that muscle quality has been defined as strength per unit area of muscle, a proxy measure of skeletal muscle quality has previously been defined as a strength
measure divided by lean mass of the area of interest\textsuperscript{35,104}. Given the particular interest in skeletal muscle quality in older adults, skeletal muscle quality was defined as knee extensor peak torque produced/leg lean/soft tissue mass at a given time point (PRE and POST). DXA derived lean/soft tissue mass was used to determine lean/soft tissue mass index, defined as lean/soft tissue mass in kg/height in m\textsuperscript{2} at PRE and POST.

**Analytic Plan**

All analyses were performed in SPSS v28.0 (Chicago, IL, USA) unless otherwise noted. All data were initially checked for normality by using Shapiro-Wilk tests, where a significance level of $P<0.05$ indicated non-normally distributed data. If it was determined that data were normally distributed, parametric techniques were used. If data were not normally distributed, a square-root transformation of data was performed and normality was once again assessed. If transformed data were normally distributed, transformed data were then subject to parametric analyses. For analysis using repeated measures Analyses of Variance (ANOVA) or analysis of covariance (ANCOVA), data were also tested for sphericity using Mauchly’s test of sphericity where a significance of $P<0.05$ indicated a violation of the assumption of sphericity. For those data sets for which sphericity was violated, Greenhouse-Geisser corrections were utilized. Levene’s test of equality of variance was additionally performed with a significance level of $P<0.05$ indicating an inequality of variances. Additionally, independent samples t-tests were performed on all baseline timepoints of all variables to ensure that there were no differences at baseline.

To test our primary hypothesis, a $2\times2$ (group*time) repeated measures ANOVA was performed to look for main effects of group, main effects of time, and any significant group*time interactions. Notably, this was done for both total lean soft tissue mass as
well as appendicular lean soft tissue mass. To test secondary hypotheses, those data with 2 time points (muscle tissue thickness, muscle quality score) were tested with a 2x2 (group*time) repeated measures ANOVA as described above. Data were examined for main effects of group and time as well as for significant group*time interactions. Measures of strength gain were taken at 3 time points and were analyzed with a 2x2 (group*time) ANCOVA using baseline measures as the covariate. Data were examined for main effects of group and time, as well as for group*time interaction. For all the statistical models described above, if a significant interaction was present, data were tested for simple main effects of group and time. Bonferroni post hoc comparisons were generated to compare main effects. In addition to these methods, partial eta squared effect sizes of interaction terms for primary outcomes were used for a post hoc power analysis in G*Power v3.1, a method that has been used previously. This was conducted to determine achieved power and sample sizes required to achieve significant interaction terms for primary outcomes.
Results

Results are presented initially for adherence to the interventions, including dietary intake and resistance training.

Participants

Participants did not differ significantly in any baseline descriptive characteristic (P≥0.229). Mean age for participants was 69.72±8.23 years, demonstrating a relatively aged cohort. Both the GP and the CP had the same number of participants (n=9), with the CP group having 3 males and 6 females and the GP group having 4 males and 5 females.

Self-Reported Dietary Intake

A significant group*time interaction (P<0.001) as well as a significant main effect of time (P<0.001) was observed for absolute energy intake (kcals/day), where energy intake increased over the course of the intervention in the GP group, but not in the CP group. No significant main effect of group was observed (P=0.096). A significant group*time interaction (P<0.001) as well as main effects of time (P<0.001) and group (P=0.010) were observed for relative energy intake (kcal/kg/d), where energy intake was higher in the GP group and increased over the course of the intervention, however the CP group did not show an increase over time. Mean values as well as results of pairwise comparisons are shown in Table 6. Mean prescribed energy intake was 2453 kcal/day for the GP group and 2531 kcal/day for the CP group. The GP group averaged an intake of 1888±27 kcal/day whereas the CP group averaged an intake of 1587±75 kcal/day representing a 26.6% and 46.0% deviation from prescribed values for the GP and CP groups respectively.
A significant group*time interaction (P<0.001) as well as main effects of time (P<0.001) and group (P<0.001) were observed for absolute protein intake (g/day), by study design, GP had higher protein intake than CP and GP increased protein intake over the course of the intervention, whereas this did not occur for CP. Similarly, a significant group*time interaction (P<0.001) and main effects of both time (P<0.001) and group (P<0.001) were observed for relative protein intake (g/kg/day). These data are presented in Table 7. As presented in Table 10, GP exceeded prescribed protein intake in weeks 1-2, after which GP deviated no more than ~5% from prescribed protein intakes. Furthermore, overall average self-reported protein intakes per group as compared to prescribed were 1.59 g/kg/d (actual) vs. 1.56 g/kg/d (prescribed) for the GP group and 0.96 g/kg/d (actual) vs. 0.8-1.0 (prescribed) for the CP group.

A significant group*time interaction was seen for both absolute (P=0.008) and relative (P=0.003) carbohydrate intake. No significant main effect of group or time were observed for either absolute (P_{TIME}=0.309; P_{GROUP}=0.181) or relative (P_{TIME}=0.245; P_{GROUP}=0.071) carbohydrate intake. Mean values as well as pairwise comparisons are displayed in Table 8 below.

No significant group*time interaction was observed for absolute fat intake (P=0.131). There were additionally no significant main effects of time (P=0.115) or group (P=0.617) observed for absolute fat intake. Relative fat intakes were not normally distributed, so these data were square root transformed to perform parametric analyses. After square root transformation, a significant group*time interaction (P<0.001) as well as main effects of group (P<0.001) and time (P<0.001) were observed. Means as well as pairwise comparisons are presented in Table 9.
Supplement adherence in the GP group as determined via bag return (bags returned/bags given out*100) was 81%. Supplement adherence as determined via self-report was 98%. These data are presented by week in Table 11.

**Training Attendance and Volume**

Training session attendance did not vary between group (P≥0.286). Overall training attendance was 96% (defined as (training sessions attended/total training sessions prescribed)*100). Detailed training attendance data are presented in Table 12.

No group*time interaction was observed (P=0.653) for total volume load as defined by total sets*total repetitions performed*load used. There was additionally no main effect of group for total volume load (P=0.631). There was however a main effect of time (P<0.001) for total volume load. This is unsurprising given that all weeks except for deload weeks involved a progression in intensity and/or volume. These data are presented in Figure 2, with pairwise comparisons to determine differences from Week 1 total volume load denoted.

**Lean/Soft Tissue Mass and Lean/Soft Tissue Mass Index**

Analyses of overall lean/soft tissue mass did not demonstrate a significant group*time interaction (P=0.209; F_{1,16}=1.714; η^2_p=0.097), a significant main effect of time (P=0.245; F_{1,16}=1.457; η^2_p=0.083), or a significant main effect of group (P=0.867; F_{1,16}=0.029; η^2_p=0.002). Pairwise and post-hoc comparisons were not performed given the lack of significance for main effects of group, time, or group*time interaction. Data are presented in Figure 3. Analyses of appendicular lean/soft tissue mass revealed no significant group*time interaction (P=0.634; F_{1,16}=0.236; η^2_p=0.015). These analyses did not indicate a significant main effect of group (P=0.974; F_{1,16}=0.001; η^2_p<0.001), but did
reveal a significant main effect of time (P=0.028; F1,16=5.819; ηp²=0.267) with POST having a higher mean value than PRE by 0.269 kg. These data are presented in Figure 4. 

Post hoc power analyses conducted in G*power105 showed that this study achieved power of 16.6% and 74.3% to observe a group*time interaction for appendicular lean/soft tissue mass, and whole body lean/soft tissue mass respectively when adopting the conventional α=0.05. According to this power calculation, adequate sample size to observe significant group*time interactions for appendicular and whole body lean/soft tissue mass are 132 (66/group), and 22 subjects (11/group) respectively. Analyses of lean/soft tissue mass index did not reveal a significant main effect of group (P=0.838; F1,16=0.043; ηp²=0.003) or time (P=0.281; F1,16=1.244; ηp²=0.072), nor any significant group*time interaction (P=0.207; F1,16=1.729; ηp²=0.098). These data are presented in Figure 12.

Vastus Lateralis Thickness

Analyses of ultrasonography derived vastus lateralis thickness did not indicate a significant group*time interaction (P=0.384; F=0.802; ηp²=0.048), or a significant main effect of time (P=0.455; F=0.586; ηp²=0.035). These analyses did however reveal a significant main effect of group (P=0.040; F1,16=5.003; ηp²=0.238), with GP having a higher mean vastus lateralis thickness value by 0.373 cm. These data are presented in Figure 5.

Isokinetic Dynamometry

Analyses of knee extensor peak torque at 60°/s showed no significant group*time interaction (P=0.972; F1,16=0.001; ηp²<0.001), no significant main effect of time (P=0.078; F1,16=3.573; ηp²=0.192), nor a significant main effect of group (P=0.584; F1,16=0.313; ηp²=0.02). Analyses of covariance revealed that knee extensor peak torque
values at PRE significantly adjusted the association between group and time with subsequent strength measures (P<0.001; F1,16=49.861; \( \eta_p^2=0.769 \)). These data are presented in Figure 6. Knee flexor peak torque at 60°/s demonstrated no significant group*time interaction (P=0.360; F1,16=0.891; \( \eta_p^2=0.056 \)), nor a main effect of group (P=0.373; F1,16=0.842; \( \eta_p^2=0.053 \)). These analyses did however reveal a main effect of time (P=0.027; F1,16=6.009; \( \eta_p^2=0.286 \)), with POST having a higher value than MID by an average of 6.167 N*m. Analyses of covariance demonstrated that knee flexor peak torque values at PRE significantly adjusted the association between group and time with subsequent strength measures (P<0.001; F1,16=192.918; \( \eta_p^2=0.928 \)). These data are presented in greater detail in Figure 7.

Analyses of knee extensor peak torque at 120°/s did not reveal a significant group*time interaction (P=0.341; F1,16=0.966; \( \eta_p^2=0.060 \)), a significant main effect of time (P=0.690; F1,16=0.166; \( \eta_p^2=0.011 \)), or a significant main effect of group (0.944; F1,16=0.005; \( \eta_p^2<0.001 \)). Analyses of covariance once again indicated that knee extensor peak torque values at 120°/s at PRE significantly adjusted the association between group and time with subsequent strength measures (P<0.001; F1,16=38.736; \( \eta_p^2=0.721 \)). These data are presented in Figure 8. Analyses of knee flexor peak torque at 120°/s did not reveal a significant group*time interaction (P=0.527; F1,16=0.42; \( \eta_p^2=0.027 \)), a significant main effect of time (P=0.267; F1,16=1.331; \( \eta_p^2=0.081 \)), or a significant main effect of group (P=0.906; F1,16=0.015; \( \eta_p^2=0.001 \)). Analyses of covariance again revealed that knee flexor peak torque values at 120°/s at PRE significantly adjusted the association between group and time with subsequent
strength measures (P<0.001; F1,16=168.688; ηp²=0.918). These data are presented in Figure 9.

**Skeletal Muscle Quality Score**

Analyses of muscle quality score (knee extensor peak torque values at either 60°/s and 120°/s divided by leg lean mass at the corresponding time point) at 60°/s revealed no significant group*time interaction (P=0.152; F1,16=2.263; ηp²=0.124), nor a significant main effect of group (P=0.198; F1,16=1.802; ηp²=0.101). These analyses did however reveal a significant main effect of time (P=0.001; F1,16=16.052; ηp²=0.501), with POST outperforming PRE by 1.011 N*m/kg. These data are presented in Figure 10. Analyses of muscle quality score at 120°/s revealed no significant group*time interaction (P=0.444; F1,16=0.616; ηp²=0.037), nor a significant main effect of group (P=0.271; F1,16=1.298; ηp²=0.075). These analyses did however reveal a significant main effect of time (P=0.015; F1,16=7.408; ηp²=0.316), with POST outperforming PRE by 0.676 N*m/kg. These data are presented in Figure 11.
### Table 4: Descriptive Characteristics of Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Constant Protein</th>
<th>Graded Protein</th>
<th>Total</th>
<th>P-Value for Group Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>72.11±7.17</td>
<td>67.33±8.93</td>
<td>69.72±8.23</td>
<td>0.229</td>
</tr>
<tr>
<td>Sex (number of males)</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>0.653</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.89±8.96</td>
<td>169.61±9.45</td>
<td>168.75±8.98</td>
<td>0.697</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.87±19.31</td>
<td>69.47±12.72</td>
<td>73.67±16.44</td>
<td>0.292</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>27.27±5.45</td>
<td>24.31±4.34</td>
<td>25.79±5.02</td>
<td>0.221</td>
</tr>
<tr>
<td>Total Lean/Soft Tissue Mass (kg)</td>
<td>45.32±11.24</td>
<td>46.40±8.96</td>
<td>45.86±9.88</td>
<td>0.825</td>
</tr>
<tr>
<td>Leg Press Estimated 1 RM (KG)</td>
<td>118.17±32.37</td>
<td>128.05±73.87</td>
<td>123.11±55.56</td>
<td>0.718</td>
</tr>
<tr>
<td>Leg Extension Estimated 1 RM (KG)</td>
<td>56.92±15.60</td>
<td>66.04±32.06</td>
<td>61.48±24.90</td>
<td>0.458</td>
</tr>
<tr>
<td>Week</td>
<td>Constant Protein</td>
<td>Graded Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------------------</td>
<td>----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absolute</td>
<td>SD</td>
<td>Relative</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>1717</td>
<td>359</td>
<td>22.58</td>
<td>4.11</td>
</tr>
<tr>
<td>2</td>
<td>1698</td>
<td>220</td>
<td>22.66</td>
<td>4.54</td>
</tr>
<tr>
<td>3</td>
<td>1508</td>
<td>242</td>
<td>20.17</td>
<td>4.89</td>
</tr>
<tr>
<td>4</td>
<td>1474</td>
<td>292</td>
<td>20.12</td>
<td>6.38</td>
</tr>
<tr>
<td>5</td>
<td>1555</td>
<td>309</td>
<td>20.44</td>
<td>3.63</td>
</tr>
<tr>
<td>6</td>
<td>1655</td>
<td>238</td>
<td>22.05</td>
<td>4.52</td>
</tr>
<tr>
<td>7</td>
<td>1580</td>
<td>187</td>
<td>21.15</td>
<td>4.32</td>
</tr>
<tr>
<td>8</td>
<td>1573</td>
<td>155</td>
<td>21.17</td>
<td>4.73</td>
</tr>
<tr>
<td>9</td>
<td>1484</td>
<td>342</td>
<td>19.57</td>
<td>4.26</td>
</tr>
<tr>
<td>10</td>
<td>1623</td>
<td>297</td>
<td>21.22</td>
<td>2.20</td>
</tr>
</tbody>
</table>

*Significantly different from Week 1
#Significantly different from CP group for the same time period
Table 6: Self-Reported Protein Intake Across Time

<table>
<thead>
<tr>
<th>Protein (g/d or g/kg/d)</th>
<th>Week</th>
<th>Constant Protein</th>
<th>Graded Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute (g/d)</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>73</td>
<td>16</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>16</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>15</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>15</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>16</td>
<td>0.94</td>
</tr>
<tr>
<td>6</td>
<td>79</td>
<td>21</td>
<td>1.01</td>
</tr>
<tr>
<td>7</td>
<td>72</td>
<td>15</td>
<td>0.93</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>16</td>
<td>0.94</td>
</tr>
<tr>
<td>9</td>
<td>74</td>
<td>17</td>
<td>0.96</td>
</tr>
<tr>
<td>10</td>
<td>75</td>
<td>19</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*Significantly different from Week 1
#Significantly different from CP group for same time period
Table 7: GP Group Prescribed vs. Actual Protein Intake

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescribed</td>
<td>0.8</td>
<td>1</td>
<td>1.2</td>
<td>1.4</td>
<td>1.4</td>
<td>1.6</td>
<td>1.8</td>
<td>2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Actual</td>
<td>1.17</td>
<td>1.16</td>
<td>1.25</td>
<td>1.41</td>
<td>1.42</td>
<td>1.69</td>
<td>1.81</td>
<td>1.93</td>
<td>2.20</td>
<td>2.17</td>
</tr>
<tr>
<td>%Difference</td>
<td>37.92</td>
<td>15.10</td>
<td>4.43</td>
<td>0.40</td>
<td>1.42</td>
<td>5.41</td>
<td>0.55</td>
<td>-3.45</td>
<td>-0.10</td>
<td>-1.58</td>
</tr>
</tbody>
</table>

Figure 1: Lean/Soft Tissue Mass vs. Time

Main Effect of Group: P=0.867
Main Effect of Time: P=0.245
G*T Interaction: P=0.209
Figure 2: Appendicular Lean/Soft Tissue Mass vs. Time

Main Effect of Group: P=0.974
Main Effect of Time: P=0.028 (POST>PRE)
G*T Interaction: P=0.634

Figure 3: Vastus Lateralis Tissue Thickness vs. Time

Main Effect of Group: P=0.040 (GP>CP)
Main Effect of Time: P=0.455
G*T Interaction: P=0.384
Figure 4: Knee Extensor Peak Torque at 60°/s vs. Time

Main Effect of Group: P=0.584
Main Effect of Time: P=0.078
G*T Interaction: P=0.972

Figure 5: Knee Flexor Peak Torque at 60°/s vs. Time

Main Effect of Group: P=0.373
Main Effect of Time: P=0.027 (POST>MID)
G*T Interaction: P=0.360
Figure 6: Knee Extensor Peak Torque at 120°/s vs. Time

Main Effect of Group: P=0.944
Main Effect of Time: P=0.690
G*T Interaction: P=0.341

Figure 7: Knee Flexor Peak Torque at 120°/s vs. Time

Main Effect of Group: P=0.906
Main Effect of Time: P=0.267
G*T Interaction:P=0.527
Figure 8: Muscle Quality Score at 60°/s vs. Time

Main Effect of Group: P=0.198
Main Effect of Time: P=0.001 (POST>PRE)
G*T Interaction: P=0.152

Figure 9: Muscle Quality Score at 120°/s vs. Time

Main Effect of Group: 0.271
Main Effect of Time: 0.015 (POST>PRE)
G*T Interaction: 0.444
**Discussion**

The purpose of the present study was to examine the effects of graded protein intake vs constant protein intake on skeletal muscle adaptations in older adults undergoing RT. The main findings of this study include: i) the RT and protein intake protocols were feasible among both groups, ii) there was a ubiquitous increase in appendicular lean/soft tissue mass over time, iii) muscle quality scores improved over time at two different knee extension velocities, iv) protein intake protocols of either graded or constant protein intake did not influence total body or appendicular lean/soft tissue mass, v) only peak torque attained during flexion at 60°/s improved over time, with no effect of protein protocol assignment for any strength measures. The primary hypothesis that the GP group would improve total body and appendicular lean/soft tissue mass to a greater degree than the CP group was not supported. Additionally, the secondary hypothesis that the GP group would improve vastus lateralis thickness, peak torque, muscle quality score, and lean/soft tissue mass index to a greater degree than the CP group was also not supported.

**Resistance Training and Nutrition Intervention**

The protein intake intervention was feasible among both the GP group and the CP group based on self-reported dietary records, with the CP group remaining in desired range of protein intake for the entire 10-week intervention, and the GP group deviating no more than 5.4% after week 2 of the intervention. Additionally, self-reported protein intakes were largely in line with prescribed values. Self-reported total energy consumption was however much lower than prescribed. The literature regarding self-reported dietary intake consistently demonstrate that self-reported energy intake
underreports true energy intake\textsuperscript{106–108}. It is additionally possible that self-reported protein intake was overreported, as a cross-sectional analysis indicated that both protein and carbohydrate intake were overreported when overall energy intake was underreported\textsuperscript{109}. While self-reported dietary intake is an accepted method of administering and monitoring a dietary intervention, the chronic underreporting of energy intake across many self-report measures remains an unresolved issue. Despite the well documented variance associated with these methods, based on the data attained from the present study it does appear that the protein intake protocol was feasible among participants, with minimal deviation from prescribed values after a brief acclimation period (weeks 1-2). Protein supplementation additionally showed a high degree of acceptability among participants, with 81\% of supplementation achieved when observed via bag return, and 98\% of supplementation achieved when observed via self-report.

The RT intervention showed a high degree of feasibility among participants. Both the GP group and the CP group achieved 96\% adherence to the RT program, determined by RT session attendance. Additionally, analyses demonstrated that the RT intervention was congruent between intervention groups, such that groups did not differ in total volume load for the 10-week intervention. This level of adherence is higher than has been previously seen in similar RT interventions in older adults\textsuperscript{110,111}

**Muscle Quality Score**

Despite minimal findings for changes in strength measures, it was observed that both groups improved muscle quality scores over time for leg extension at both 60°/s and 120°/s. Importantly, similar improvements were noted in the CP and GP groups over time. Several studies have examined changes in muscle quality over time with RT, and
the improvement of muscle quality with RT in older adults is well supported. It was seen in a recent meta-analysis that in 5 studies (n=195) examining muscle quality (defined identically to this study), this variable improved vs. control groups (P=0.001; SMD=0.48; 95% CI: 0.19-0.78) with some type of training intervention. Notably, the characteristics of one study included in this analysis remain unclear, however 3 of 4 clearly presented studies included in this analysis involved some form of RT\textsuperscript{112}. In another study, Brooks et al. reported that in a cohort of older (n=62; age≥55 years) adults with type 2 diabetes, those undergoing an RT intervention (16 weeks, 3 sessions/week, 60-80\% 1 RM) improved muscle quality to a greater degree than those only receiving standard care (P<0.001)\textsuperscript{113}. Notably, muscle quality in this Brooks et al. study was defined as 1 RM/DXA derived leg lean mass, a comparable method to the present study. Similarly, Tracy et al. report that in a cohort of older men and women (n=23; age≥65 years) unilateral leg training increased muscle quality in the trained leg (P<0.01) when muscle quality was defined as 1 RM/MRI derived muscle volume\textsuperscript{114}. Additionally, in a study examining the effects of dumbbell based RT vs. elastic band based RT in older adults (n=65; mean age=66.5±7.09 years), it was reported that upper body muscle quality increased to a greater degree in the dumbbell RT group than both the elastic band RT group and a control group\textsuperscript{115}.

The literature regarding supplemental and overall protein interventions and improvement in muscle quality is less abundant. Hofmann et al. report that in a cohort of elderly women (n=91; mean age=83.6 years) that lower body muscle quality increased similarly in subjects performing RT alone and performing RT with a protein supplement, with both group increasing more than the control group\textsuperscript{116}. Notably, lower body muscle
quality in this study was defined as peak power estimated via 30 second chair stand test/BIA derived muscle mass of the lower extremity. Conversely, Khanal et al. report in a recent cross-sectional analysis that in older white women (n=281; age range=65-91), those consuming protein at a level of $\geq 1.17\text{ g/kg/d}$ had a higher muscle quality$^{117}$. Importantly, muscle quality was defined as dynamometry derived handgrip strength/total body mass; and given the cross-sectional nature of this analysis, information regarding RT in this population is limited. Finally, Lemieux et al. report in a cohort of older women (n=67; age=61±6 years) that those consuming higher dietary protein intake ($\geq 1.20\text{ g/kg/d}$ vs. 0.8-1.19 g/kg/d) had a higher muscle quality score (defined as maximum isometric knee extensor strength in kg/DXA derived lean tissue mass)$^{118}$. Again, given the cross-sectional nature of this analysis, information regarding RT and other relevant external factors are not present.

There exists a great deal of incongruency in the literature regarding muscle quality in relation to protein intake, specifically as it relates to conjunctive RT interventions. Taken in its totality, it appears that RT is chiefly responsible for improvement in muscle quality over time in older adults. This is a relevant finding, given that it has been well-documented that, in the absence of RT, muscle quality declines during the aging process (reviewed in$^{119}$). The typical decline in muscle quality with age has additionally been associated with mobility impairment and muscular dysfunction in later life$^{120}$ thrusting improvements in muscle quality in older adults to relevance. Mechanistically, this finding could be attributed to selective hypertrophy of Type II fibers, which has been seen before in older adults undergoing RT$^{53}$. This is however impossible to confirm in the present study given the lack of muscular tissue collection.
Another plausible mechanism for this enhancement of muscle quality could be the neural adaptations that are characteristic of the first ~6 weeks of RT\textsuperscript{121}. Indeed, evidence to suggest enhanced motor unit recruitment, firing rate, and synchronization have been observed in older adults undergoing RT interventions (reviewed in\textsuperscript{122}). This is once again impossible to confirm given the lack of electromyography measures taken in the present study.

**Total Body and Appendicular Lean/Soft Tissue Mass**

While significant increases in appendicular lean/soft tissue mass were seen in both groups, overall lean/soft tissue mass did not change. This perhaps stands to reason given that the primary focus of the training intervention was the lower body. Gain in appendicular lean mass is of particular interest given its role in maintaining proper locomotion in older adults. Indeed, a recent cross-sectional analysis found that DXA derived appendicular lean mass was significantly associated with better dynamic balance measures (four square step test) in older adults (n=260; mean age=78±6.7 years)\textsuperscript{123}. Appendicular lean mass has additionally been seen to be associated with lower risk of mortality in a cohort of pre-frail and frail older adults (n=1487; age≥65 years; median follow up=8.9 years)\textsuperscript{124}. Indeed, this study found in a regression model accounting for demographics, behavioral, clinical, physical function, and frailty characteristics, that each increase of one standard deviation to appendicular lean mass was associated with a 50% lower risk of all-cause mortality. Additionally, appendicular lean/soft tissue mass gain has been observed in both protein intake modification\textsuperscript{125,126} as well as RT interventions\textsuperscript{127} previously. Importantly however, although an increase in appendicular lean/soft tissue
mass was observed, the mean increase was 0.269 kg, which was not robust enough to impact overall lean/soft tissue mass.

However, there are mixed findings in the literature as a lack of overall lean mass gain has also previously been observed. Avila et al. report that a group of older adults (n=15; age=60-75 years) did not significantly improve lean mass over the course of a 10-week RT protocol\textsuperscript{128}. It has additionally been reported that in a 6-week RT intervention in older adults (n=22; mean age=70.6±6.1 years) lean body mass was not significantly improved\textsuperscript{129}. Reid et al. report that older adults (age=74.2±7.0 years) performing either 12-weeks of power training (n=23) or strength training (n=22) (both: 3 sessions/week, 3 sets of 8 repetitions, 70% 1 RM; differing concentric and eccentric contraction speeds) did not significantly improve leg lean mass from baseline\textsuperscript{130}. Yet another RT intervention (2x/week, 10-weeks) in aged humans (n=16; mean age=59±4 years) did not demonstrate significant improvement in DXA derived FFM gain from baseline to post-intervention (P=0.061)\textsuperscript{52}.

One contributing factor to the lack of significant total body lean/soft tissue mass gain is likely the duration of the RT intervention. While gains in lean/soft tissue mass have been observed in as little as 6 weeks in older adults (mean age=58.6±8.0 years)\textsuperscript{57}, two meta-analyses examining RT adaptation in older adults reported a mean training period of 24.0 weeks (range: 6-52 weeks)\textsuperscript{31} and 20.5±9.1 weeks\textsuperscript{6}. Indeed, studies that demonstrate favorable muscle morphology and body composition adaptation often have durations of ≥12 weeks, with some interventions lasting ≥1 year\textsuperscript{22–24,28,34}. This contrasts with the duration of the present study. It is possible that had the study period been longer,
appendicular lean/soft tissue mass gain would have been robust enough to alter total body lean/soft tissue mass in a favorable way.

Another potential contributing factor to the lack of gain in lean/soft tissue mass is the inadequacy of energy intake throughout the intervention in both the GP and the CP groups. Nicklas et al. report that in a 5 month RT intervention with or without caloric restriction, overweight and obese older adults (n=126; age range= 65-79 years) subjected to caloric restriction (n=63; 600 kcal deficit/day) saw a significant reduction in DXA derived lean mass (-1.1±1.6 kg; P<0.0001) whereas the RT only group saw a non-significant increase in lean mass (0.3±1.3 kg). Notably these results were significant between-groups as well (P<0.0001)\textsuperscript{110}. Furthermore, a recent meta-analysis comparing RT with or without energy restriction found that subjects undergoing energy restriction while training vs. those performing RT only, gains in lean mass were impaired (effect size=-0.57; P=0.02). This analysis additionally found via meta-regression that an energy deficit of ~500 kcal/day prevented significant gain to lean mass in older individuals (mean age=60±11 years)\textsuperscript{131}. The energy deficit reported for the current study on average exceeds both the 600 kcal/day as well as the 500 kcal/day marks that the aforementioned studies found sufficient to nullify lean mass gain. While it is possible that energy intake was underreported in the present study, it remains possible that a lack of sufficient energy intake contributed to the adaptations seen in total body as well as appendicular lean/soft tissue mass. Given the high bioenergetic cost of de novo MPS over and above simple regulatory muscle protein turnover, it is unsurprising that insufficient energy intake would limit the accrual of muscle tissue and lean mass (reviewed in\textsuperscript{98}). It is possible that this high energy cost of developing muscle proteins led to selective hypertrophy of the
extremities, given that training was primarily targeted at the lower body. In an energy deficit, as reported in the present study, it is possible that subjects were not sufficiently bioenergetically positioned to accrue lean/soft tissue mass gains ubiquitously, but rather incurred selective growth in those areas that were most utilized during the study.

Yet another potential contributing factor to the overall lean/soft tissue mass results seen in this study is the advanced age of participants. While it is possible to positively alter muscle morphology in even the oldest of the older adult population\textsuperscript{53}, it has been demonstrated via meta-regression that age has an inverse relationship with lean mass gain ($\beta=-0.03; \ P=0.01$)\textsuperscript{6}. Given that subjects in this study were of an advanced age (mean age=$69.72\pm8.23$ years), it is likely that this had some influence on their achievable lean/soft tissue mass adaptation. Additionally, participants in this study were primarily female. It has been previously demonstrated that older women have a lower capacity for RT induced adaptation\textsuperscript{27}, and is therefore another consideration in the lack of observable lean/soft tissue mass gain at the total body level. Mechanistically, this might be attributable to anabolic signaling capabilities declining with age. Notably, the mTOR pathway and its downstream effectors are typically less responsive with age, and mTOR limiting pathways (e.g. AMPKa) have been shown to be more robustly stimulated in aged individuals\textsuperscript{37}. While it has been demonstrated that enhanced protein intake in conjunction with RT can combat this signaling deficiency, these findings are not universal (reviewed in\textsuperscript{11}). Additionally, it is important to note that even if enhanced signaling were achieved in the GP group, it is possible that acute increases to signaling pathways might not lead to observable gains to lean/soft tissue mass over the course of 10-weeks, as this relationship has yet to be fully elucidated\textsuperscript{47}. 
Another consideration for the results of the present study is the overall impact of training volume on gains in muscle mass and changes in body composition. The relationship between training volume and skeletal muscle hypertrophy induced lean mass gain is well established in young adults\textsuperscript{12,13}, however this proposed dose-response relationship is less clear in older adults. There is evidence via meta-analysis and meta-regression that in cohorts of older adults (mean age= 65.5±6.5 years), there exists a linear association between overall volume of training (e.g. sets performed per session) and gains in LBM\textsuperscript{6}. Notably, this relationship existed even when controlling for age, study duration, technique for assessing LBM, gender, training intensity, and training frequency. It remains a possibility that maximal training volume in the present study (15 sets/session) did not sufficiently progress, however another meta-analysis and meta-regression found that performing 2-3 sets of 7-9 repetitions of each exercise in a training session is sufficient to produce gains in lean mass\textsuperscript{31}. The relationship between overall training volume and lean mass gain in older adults is not fully elucidated, however it is plausible that this relationship played a role in the results herein.

Finally, the lack of observable difference in the body composition and strength outcomes between the GP and CP groups is well-supported by the literature. ten Haaf et al. report in a recent meta-analysis that the consumption of protein supplementation while undergoing concomitant RT produced no extra benefit to measures of DXA derived LBM\textsuperscript{134}. Indeed, several studies have reported that enhanced protein intake and/or protein supplementation do not lead to enhanced lean mass gain when undergoing a concomitant RT intervention\textsuperscript{22,23,27,28,33,111,135}. While there is evidence to suggest that differential protein intake can play a role in the augmentation of lean mass gain in older adults,
overall findings to this point remain equivocal, a notion supported by the results of this study. It is also possible that any theoretical differential group response would not be revealed in this analysis due to the limited sample size herein. Indeed, a post hoc power analysis conducted in G*power v3.1 showed that this study did not achieve the conventionally accepted adequate power level of 80%, owing particularly to sample size. While the required sample size for total lean/soft tissue mass was almost achieved (n=22; 11/group), the sample required to reveal significance in the interaction term of appendicular lean/soft tissue mass was much larger (n=132; 66/group). This is a consideration for future research attempting to provide a more adequately powered analysis of total and appendicular lean/soft tissue mass in response to a RT and protein intake paradigm in older adults.

**Strength Measures**

There were no significant findings for isokinetic dynamometry derived measures of peak torque in this study with the notable exception of a main effect of time for knee flexor peak torque at 60°/s. This is contrary to the majority of the current body of literature. Indeed, several meta-analyses as well as individual studies have found significant increases to varying strength measures after the initiation of a RT intervention. Importantly however, participants did not significantly decline in strength. While there was largely no improvement to peak torque within or between group, the lack of strength loss is perhaps indicative of an attenuation of strength loss and speaks to the importance of adopting RT in early adulthood. Indeed, given the strong evidence suggesting older individuals experience strength decline as a result of the aging process (reviewed in) and that older adults do not gain strength at the same rate as their
young counterparts\textsuperscript{25,37–40}, it is possible that this RT intervention served to maintain strength in these older adults as opposed to produce observable strength gains.

Another potential factor in the lack of significant strength gain associated with this intervention is the intensity of RT. In a meta-analysis examining the dose-response relationship of different factors in relation to strength and other functional outcomes, Steib et al. found that training intensities $>75\%$ 1 RM produced significantly greater strength gain than intensities of 55-75\% 1 RM (SMD=0.62; 95\% CI: 0.22-1.03) and intensities of $<55\%$ (SMD=0.88; 95\% CI: 0.21-1.55)\textsuperscript{137}. This finding is shared by a similar meta-analysis that found 70-79\% 1 RM had the largest effect on strength gain in a population of older adults (mean SMD\textsubscript{bs}=1.89)\textsuperscript{31}. Yet another meta-analysis found that when training was stratified into four subgroups of: low intensity ($<60\%$ 1 RM), low/moderate intensity (60-69\% 1 RM), moderate/high intensity (70-79\% 1 RM), and high intensity ($\geq80\%$ 1 RM) that moving into a higher intensity subgroup yielded a \textasciitilde5.5\% increase to strength measures in older adults ($n=1079$; mean age=67.4±6.3 years)\textsuperscript{7}. Importantly, the primary goal of the RT intervention in this study was to elicit maximal muscle hypertrophy, however it is possible that if training intensities were consistently at a higher range ($\geq75$-80\% 1 RM) that strength measures would have demonstrated more observable progress, as only 4 weeks of training in the present study were spent at intensities $\geq75\%$ 1 RM.

\textbf{Vastus Lateralis Thickness}

The body of literature assessing older adults ultrasound derived muscle tissue thickness response to RT is somewhat limited. Indeed, in a recent meta-analysis aiming at synthesizing measures of muscle quality, it was found that of the 12 studies assessing
morphological muscle change, only 5 included ultrasound derived measures, of which the majority exclusively examined ultrasound echo intensity\textsuperscript{112}. However, of the evidence that does exist, Mesquita et al. reported a significant increase to ultrasound derived vastus lateralis muscle thickness from $1.88\pm0.45$ cm to $2.02\pm0.37$ cm in a cohort of adults (n=16; mean age=59±4 years) undergoing 10 weeks of RT (2 sessions/week; 3 sets/exercise; 10-12 repetitions/set; RPE=7-9)\textsuperscript{52}. In another study examining 6 weeks of RT (2 sessions/week; 2-4 sets/exercise; 8-12 repetitions/set; 6-10 exercises/session; 70-85% 1RM) in healthy older adults (n=25; mean age≥71 years), Scanlon et al. reported no significant changes or interactions for vastus lateralis or rectus femoris muscle tissue thickness\textsuperscript{138}. In another study examining 6 weeks of RT in a cohort of older adults (n=23; age range=61-85 years), it was seen that ultrasound derived vastus lateralis cross-sectional area significantly increased from baseline in the training group, but not in the control group. Importantly, this study reported no significant changes in rectus femoris cross-sectional area, and there were no significant between group differences in vastus lateralis or rectus femoris cross-sectional area\textsuperscript{129}. Notably, Scanlon et al. reported a significant group*time interaction favoring the RT group over controls for measures of vastus lateralis cross-sectional area\textsuperscript{138}, perhaps suggesting that this measure is more sensitive to change in older adults and is not an adequate proxy for muscle tissue thickness despite both being derived by ultrasound. In yet another study assessing the effects of 10 weeks of 30 minutes of high intensity RT (3 sessions/week; 3 exercises/session; 3-4 sets/exercise; 6 repetitions/set; ≥80% 1 RM) combined with 34 g of a milk protein supplement each day, muscle tissue thickness of the vastus lateralis, rectus femoris, and the vastus intermedius demonstrated group*time interactions favoring
the RT and protein intake group\textsuperscript{139}. Given this information, it is possible that the present study did not find significant changes in muscle tissue thickness. The body of literature certainly provides evidence that muscle thickness can improve in older adults, however the relationship based on the current body of literature to this point remains to be fully elucidated.

Ultrasound derived muscle tissue thickness is relevant due to findings that indicate a correlation of muscle tissue thickness at the vastus lateralis ($r=0.756; P<0.001$), vastus intermedius ($r=0.815; P<0.001$), vastus medialis ($r=0.875; P<0.001$), and rectus femoris ($r=0.834; P<0.001$) with maximal voluntary contraction of the knee extensors in older adults\textsuperscript{140,141}. Ultrasonography derived vastus lateralis thickness has additionally been shown to be associated with the 6-minute walk test ($R^2=-0.45; P<0.01$), the timed up and go test ($R^2=-0.43; P<0.01$), stair climb power ($R^2=0.65; P<0.01$), stair descent power ($R^2=0.70; P<0.01$), and vertical jump height ($R^2=0.55; P<0.01$)\textsuperscript{142}. Such findings indicate that measures of ultrasound derived vastus lateralis thickness might hold some clinical relevancy in older adults.

**Experimental Considerations**

Similar to many studies involving intensive training interventions, the present study is limited to a small sample size. Thus, as shown by aforementioned power analysis, this study was underpowered to reveal meaningful training adaptations to muscle size. In this same vein, this study was underpowered to perform sub-analyses stratifying by either age or sex. Another limitation of the present study is the inability to collect meaningful biological markers from participants. Given the suggestion that concomitant protein intake and RT is beneficial for inducing anabolic cell signaling
events in older adults\textsuperscript{37,49}, the collection of relevant skeletal muscle signaling markers, such as phosphorylation status of mTOR, S6K1, and 4EBP1 derived via Western Blotting; and molecular tracer derived FSR; could have provided a broader picture about what was occurring at the molecular level in these participants. As previously discussed, the nature of self-report measures are an additional limitation of the present study. It is apparent that self-reports of dietary intake to not reflect actual intake with a high degree of certainty\textsuperscript{106–109}. This remains an unresolved limitation in the field of nutrition research. The duration of this study is another limitation. Owing to logistical constraints, the training period in this study could not be of desired duration (12-24 weeks) which has been shown to be effective at eliciting body composition changes in older adults. Another limitation lies in the inability to analyze measures of 3 RM on relevant exercises completed in this study. Owing to the nature of pin-loading exercise machines, a number of participants performed the maximum weight of the machine for both the leg press (n=2) and the leg extension (n=9). Given the inappropriate nature of excluding participants from analyses on the basis of maximizing the weight of the machine, analyses were forgone on participants 3 RM. Due to logistical considerations, the investigator performing ultrasonography measures was unable to be blinded from group assignment. Participants were not necessarily RT naïve, rather the inclusion criterion was that they had not formally adhered to a progressive RT program in recent months. This criterion was established owing to the logistics and feasibility of recruitment. Finally, the CP group was not provided with an isocaloric placebo supplement, a method that has been used previously\textsuperscript{133}, in order to equate overall caloric intake.
The main strength of this study was that in this randomized study, the graded protein structure of the intervention and the high adherence to protein intake protocols from participants in the GP group. To the author’s knowledge, this is the first study to examine changes in muscle morphology, body composition, and strength in response to chronic graded protein intake in older adults. Another strength of the current study is the relatively advanced age of participants herein. While RT based studies in adults are relatively commonplace, a mean age of 69.72±8.23 represents individuals entering their 8th decade of life, a point at which approximately 50% of muscle mass is lost (reviewed in143). A final strength of the present study is a multifactorial approach to assessing the aging muscle. A variety of measures were employed aimed at targeting not only muscle mass, but additionally muscle quality, muscle architecture and morphology, and muscle strength. This allowed for a more complete assessment of the individuals’ response to RT with differential protein intake.

Implications and Future Research

The implications of this study begin with the increase seen in muscle quality. This finding could indicate that, while overall lean/soft tissue mass was not improved, RT still serves some benefit to the force production capabilities per unit muscle in older adults. This cannot be overlooked, as strategies to enhance functionality in older adults are of the utmost importance. Another implication from the current study is the ubiquitous increase in appendicular lean/soft tissue mass that was seen among subjects. Given the key role of the lower extremity in ambulatory function and general locomotion, it is reasonable to assume that perhaps a gain in appendicular lean/soft tissue mass is beneficial to older adults who struggle in these areas. Finally, another implication from the current study is
found in the covariate analysis of strength measures. In all instances, baseline values of strength strongly and significantly predicted values at both MID and POST. This perhaps indicates the importance of adopting a RT program in early adulthood in order to maximize muscle mass prior to the course of aging becoming harsher in nature.

Potential avenues of future research lie primarily in the area of molecular signaling as it relates to graded protein intake strategies in response to RT. An investigation into whether or not the mTOR pathway and its downstream effectors were upregulated in both an acute and a chronic manner in response to this paradigm would elucidate many questions that still seem to be evasive. Furthermore, more expansive and otherwise larger study aiming to examine the paradigm presented herein is warranted given the small sample size of the present study. Additionally, a larger sample size would allow for effects of age and gender to be more thoroughly examined in response to graded protein intake with RT. Additionally, correlational analyses examining changes in lean/soft tissue mass, muscle thickness, and muscle quality in relation to strength measures would serve to demonstrate relationships among outcomes that might remain uncovered and are therefore warranted.

In summary, initial analyses indicate that the structure of the present intervention was feasible and well adhered to. Additionally, no significant group*time interactions were observed for any outcome variable measured. Appendicular lean/soft tissue mass and muscle quality scores at both 60°/s and 120°/s, and peak torque of the knee flexors at 60°/s demonstrated positive main effects of time, indicating improvement regardless of group. There were no other significant main effects of either group or time. This study
serves as an important first step in investigating the proposed strategy of grading protein intake to RT intensity/volume on skeletal muscle in older adults.
References


43. Churchward-Venne TA, Holwerda AM, Phillips SM, van Loon LJCh. What is the Optimal Amount of Protein to Support Post-Exercise Skeletal Muscle Reconditioning


68. Adamo ML, Farrar RP. Resistance training, and IGF involvement in the maintenance of muscle mass during the aging process. *Ageing Research Reviews*. 2006;5(3):310-331. doi:10.1016/j.arr.2006.05.001


130. Reid KF, Callahan DM, Carabello RJ, Phillips EM, Frontera WR, Fielding RA. Lower extremity power training in elderly subjects with mobility limitations: a


Appendix A: Tables and Figures Relating to Study Design

Figure 10: Resistance Training Timeline

<table>
<thead>
<tr>
<th>Initial Contact</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 7</th>
<th>Week 8</th>
<th>Week 9</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acclimation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesocycle 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesocycle 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M: Microcycle  
D: Deload

Table 8: Exercises Performed at Weekly Training Sessions

<table>
<thead>
<tr>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>Exercise</td>
<td>Exercise</td>
</tr>
<tr>
<td>Leg Press</td>
<td>Machine Chest Press</td>
<td>Leg Extension</td>
</tr>
<tr>
<td>Compound Machine Row</td>
<td>Calf Raises</td>
<td>Compound Machine Row</td>
</tr>
<tr>
<td>Machine Triceps Pressdown</td>
<td>Machine Bicep Curl</td>
<td>Machine Triceps Pressdown</td>
</tr>
<tr>
<td>Leg Extension</td>
<td>Leg Press</td>
<td>Leg Press</td>
</tr>
</tbody>
</table>
**Appendix B: Tables and Figures Relating to Measures and Outcomes**

**Table 9: Self-Reported Carbohydrate Intake Across Time**

<table>
<thead>
<tr>
<th>Carbohydrates (g/d or g/kg/d)</th>
<th>Week</th>
<th>Constant Protein</th>
<th>Graded Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>SD</td>
<td>Relative</td>
</tr>
<tr>
<td>1</td>
<td>194</td>
<td>67</td>
<td>2.54</td>
</tr>
<tr>
<td>2</td>
<td>177</td>
<td>37</td>
<td>2.35</td>
</tr>
<tr>
<td>3</td>
<td>154</td>
<td>38</td>
<td>2.06</td>
</tr>
<tr>
<td>4</td>
<td>163</td>
<td>43</td>
<td>2.22</td>
</tr>
<tr>
<td>5</td>
<td>162</td>
<td>39</td>
<td>2.17</td>
</tr>
<tr>
<td>6</td>
<td>171</td>
<td>44</td>
<td>2.32</td>
</tr>
<tr>
<td>7</td>
<td>171</td>
<td>43</td>
<td>2.30</td>
</tr>
<tr>
<td>8</td>
<td>169</td>
<td>34</td>
<td>2.25</td>
</tr>
<tr>
<td>9</td>
<td>149</td>
<td>40</td>
<td>2.00</td>
</tr>
<tr>
<td>10</td>
<td>171</td>
<td>43</td>
<td>2.25</td>
</tr>
</tbody>
</table>

#Significantly different from CP group for the same time period
**Table 10: Self-Reported Fat Intake Across Time**

<table>
<thead>
<tr>
<th>Week</th>
<th>Constant Protein</th>
<th>Graded Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>Relative</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>66</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>68</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>19</td>
</tr>
</tbody>
</table>

*Significantly different from Week 1
#Significantly different from CP group for the same time period
Table 11: Protein Supplement Adherence

<table>
<thead>
<tr>
<th>Week #</th>
<th>Bag Return (% supplement doses taken)</th>
<th>Self-Report (% supplement doses taken)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>Week 2</td>
<td>71</td>
<td>95</td>
</tr>
<tr>
<td>Week 3</td>
<td>76</td>
<td>100</td>
</tr>
<tr>
<td>Week 4</td>
<td>87</td>
<td>97</td>
</tr>
<tr>
<td>Week 5</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>Week 6</td>
<td>89</td>
<td>98</td>
</tr>
<tr>
<td>Week 7</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>Week 8</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Week 9</td>
<td>73</td>
<td>94</td>
</tr>
<tr>
<td>Week 10</td>
<td>63</td>
<td>100</td>
</tr>
<tr>
<td>Overall</td>
<td>81</td>
<td>98</td>
</tr>
</tbody>
</table>

Table 12: Training Session Attendance

<table>
<thead>
<tr>
<th>Week</th>
<th>GP (% SESSIONS ATTENDED)</th>
<th>CP (% SESSIONS ATTENDED)</th>
<th>TOTAL (% SESSIONS ATTENDED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>89</td>
<td>93</td>
<td>91</td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>7</td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>96</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td>9</td>
<td>89</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Overall</td>
<td>96</td>
<td>96</td>
<td>96</td>
</tr>
</tbody>
</table>
Figure 11: Training Total Volume Load vs. Time

Main Effect of Group: $P=0.657$
Main Effect of Time: $P<0.001$
$G^*T$ Interaction: $P=0.670$

![Box plot showing Training Total Volume Load vs. Time](image)

Figure 12: Lean/Soft Tissue Mass Index vs. Time

Main Effect of Group: $P=0.838$
Main Effect of Time: $P=0.281$
$G^*T$ Interaction: $P=0.207$

![Box plot showing Lean/Soft Tissue Mass Index vs. Time](image)
Figure 13: Ultrasound Reference Images at PRE and POST
J. Max Michel  
e: michjm20@wfu.edu  
p: (205) 907-7830

EDUCATION
Master of Science in Health and Exercise Science  
Wake Forest University  
(expected)  
Concentration: Health and Exercise Science

Bachelor of Science in Exercise Science  
Auburn University, School of Kinesiology  
Concentration: Exercise Science

PUBLISHED MANUSCRIPTS (NEWEST TO OLDEST)

PUBLISHED MANUSCRIPTS (NEWEST TO OLDEST)
1. Smith IL, Michel JM, Bailey EK, Costa JV, Madzima TA. Effects of a Breakfast Meal with a Ketogenic Supplement or Whey Protein on Metabolism, Appetite, Blood Biomarkers and Subsequent Energy Intake  
   a. In Review  
2. Michel JM, Norton SC, Costa JV, Alphin KH, Miller GD. The Effects of Graded Protein Intake in Conjunction with Progressive Resistance Training on Skeletal Muscle Outcomes in Older Adults  
   a. In Preparation

CONFERENCE PRESENTATIONS

APPLIED EXPERIENCE
Clinical Intern  
Healthy Exercise and Lifestyle Programs  
Clinical Research Center at Wake Forest University  
Winston-Salem, North Carolina  
2020-2021

Study Staff  
Weight Loss and Exercise for Communities with Arthritis in North Carolina (WE-CAN)
Clinical Research Center at Wake Forest University
Winston-Salem, North Carolina  2020-2021

Student Co-Principal Investigator
Effects of Graded Protein Intake on Skeletal Muscle Adaptation in Older Adults
Clinical Research Center at Wake Forest University
Winston-Salem, North Carolina  2021-2022

RESEARCH INTERESTS
1. Skeletal muscle adaptation to training and supplementation
2. Genetic and epigenetic regulation of skeletal muscle adaptation across a lifespan
3. Effects of aging on cell and molecular signaling in skeletal muscle

TEACHING EXPERIENCE AND GUEST LECTURES
HES 120: Weight Training               Fall 2020-
Spring 2022
Instructor
HES 350: Human Physiology               Summer 2021
Muscle Systems Physiology - Guest Lecture

LABORATORY TECHNIQUES
- Body Composition Testing (DXA, Bio-electrical impedance spectroscopy, skinfolds)
- Ultrasonography for muscle tissue thickness
- Isokinetic Dynamometry
- RNA Isolation (QIAGEN RNeasy and Invitrogen RNAqueuous)
- Real-Time Polymerase Chain Reaction (RT q-PCR)
- Tissue powdering (mortar, pestle, and liquid nitrogen)
- Spectrophotometry (Nanodrop Lite)

PROFESSIONAL MEMBERSHIPS
American College of Sports Medicine     2020-Present
National Strength and Conditioning Association               2022-Present