Impact of Muscle Material Properties on the Hypertrophic Response of Aged Women to Resistance Exercise

by

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Abstract

All of the reports that comprise this dissertation center around the overarching hypothesis that the attenuated response to resistance exercise observed in older adults is caused by an impaired ability of the muscle to sense mechanical stimuli as a result of increased muscle stiffness.

In the first study we investigated the difference in the response of young and aged women to a single bout of resistance exercise. We hypothesized that there would be a less robust hypertrophic response to the exercise bout in the older adults than the young adults. The results of this study showed that young and aged women have a different response to acute exercise. The aged women had an attenuated response when compared to their young counterparts. It was also found that p70S6k phosphorylation is a robust marker that can be used to quantify hypertrophic response to acute resistance exercise.

In the second study we investigated the impact of a long-term stretching intervention on the response of aged women to an acute bout of resistance exercise. The stretching was used as a means to modulate muscle stiffness to test they hypothesis that stiffness is related to hypertrophic response to exercise. The results of this study showed that stretching did improve response to an acute bout of resistance exercise. However, while there was a trend toward a reduction in muscle stiffness the change in muscle material properties was not significant.

In the third study we investigated the impact of a long-term stretching intervention on the hypertrophic response of aged women to a long-term resistance exercise intervention. We hypothesized that having older women participate in a stretching intervention prior to beginning a resistance training intervention would improve their response to the resistance training program. The results of this study showed that performing a stretching intervention prior to beginning a resistance exercise intervention improved the hypertrophic response to resistance exercise.

Taken together, these studies indicate that stretching does appear to be an effective means to improve response to both an acute bout of exercise and a long-term resistance exercise intervention.

Impact of Muscle Material Properties on the Hypertrophic Response of Aged Women to Resistance Exercise

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Chapter 1

Introduction

1.1 General Background

Sarcopenia is the loss of muscle mass and strength with advanced age (Haran, Rivas, & Fielding, 2012; Rosenberg, 1997). Sarcopenia not only contributes to decreased independence and quality of life for older adults, but is also a significant predictor of mortality (Janssen, Heymsfield, & Ross, 2002; Ozcan, Donat, Gelecek, Ozdirenc, & Karadibak, 2005; Rantanen, 2003).

In order to combat sarcopenia resistance exercise has been recommended for older adults. Significant strength and muscle size gains have been shown to be possible in even very old adults (Fiatarone et al., 1990). However, when young adults have participated in resistance programs at the same relative intensity as older adults, the results have consistently showed attenuation in the response of the older adults (Greig et al., 2011a; LaRoche, Roy, Knight, & Dickie, 2008; Raue, Slivka, Minchev, & Trappe, 2009).

One potential cause for the attenuated hypertrophic response to exercise observed between young and aged adults is decreased mechanotransduction in the skeletal muscle of the older group. This is a likely possibility because differences in response to exercise have been not only observed in long term training programs, but also following a single acute bout of resistance exercise (Drummond, McCarthy, Fry, Esser, & Rasmussen, 2008; Drummond et al., 2009; Fry et al., 2011; Haddad & Adams, 2006; Mayhew, Kim, Cross, Ferrando, & Bamman, 2009). Alteration in response within a short time following application of the stimulus indicates that the impairment may be in the signaling cascade that results in increased protein translation, rather than in the protein translation machinery itself.

It is known that the extracellular matrix of myocytes becomes stiffer with age and that this stiffness decreases the amount of strain registered by the mechanosensor of the myocyte for any applied stress (Engler et al., 2004). This is important because strain is the mechanical signal that leads to increased protein synthesis and muscle hypertrophy.

Stretching has been shown to increase range of motion in both young and older adults (Feland, Myrer, & Merrill, 2001; Ferber, Osternig, & Gravelle, 2002; Puentedura et al., 2011). Stretching has also been shown to be an effective means to decrease muscle stiffness in young men (Akagi & Takahashi, 2014; Akagi & Takahashi, 2013). This makes stretching an attractive intervention to potentially improve response to resistance exercise by decreasing muscle stiffness.

1.2 Purpose of the Study

The purpose of this study is to determine the impact altered muscle material properties have on the hypertrophic response of aged women to both acute and chronic resistance exercise.

1.3 Dissertation Overview

The remainder of this dissertation is comprised of four reports followed by an overall discussion. All of these chapters test the overall hypothesis is that the attenuated hypertrophic response to resistance exercise observed in older adults is caused by an impaired ability of skeletal muscle to sense mechanical stimuli as a results of increased muscle stiffness.

There is still disagreement surrounding the difference in the hypertrophic response of young and old adults. Therefore the first report is a comprehensive literature review on this topic.

The second report has the specific aim to determine typical protein phosphorylation response of young and aged adults within 30 minutes of an acute bout of resistance exercise. The primary hypothesis of this study is that the older adults would have a less robust hypertrophic response to a single bout of resistance exercise than the young adults. The secondary aim of this study is to establish a protein that can be used to measure differences in the hypertrophic response within 30 minutes of a single bout of resistance exercise.

The third report is an investigation of the change in the acute hypertrophic response to resistance exercise following a stretching intervention. The objective of this

study is to determine how a long term stretching intervention alters the impact of aging on acute response to resistance exercise in skeletal muscle of aged as compared to young adults. This intervention provides a means to test the hypothesis that muscle stiffness impacts the acute hypertrophic response to a single bout of resistance exercise.

The fourth report examines the potential of stretching to rescue the hypertrophic response to chronic resistance exercise in older adults. The purpose of this study is to determine whether the hypertrophic response to a long-term resistance training program is rescued in aged individuals by participation in a stretching intervention prior to beginning the training. The hypothesis of this study is that older adults who participate in a stretching intervention prior to participating in a resistance training program will exhibit a more robust hypertrophic response to the training than those who do not participate in a stretching intervention first.

The overall discussion provides a summary of all of the preceding reports as well as future directions for research that would expand on the findings of the studies included in this dissertation.

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Chapter 2

Literature Review

2.1 Introduction

Sarcopenia is defined as the gradual loss of muscle mass and strength with age (Baumgartner et al., 1998; Marcell, 2003). It is estimated that after the age of 50, strength decreases by as much as 15 percent per decade (Larsson, Grimby, & Karlsson, 1979). This strength loss is concerning because muscle strength is a significant predictor of mortality (Rantanen, 2003). Furthermore, sarcopenia is also associated with decreased independence and quality of life (Haran et al., 2012; Janssen et al., 2002; Ozcan et al., 2005; Rosenberg, 1997). This happens because as individuals experience strength loss the activities they perform daily become more difficult. It has also been shown that low levels of muscle mass and strength are associated with increased risk of falls and fractures (Short & Nair, 2001). According to the 2010 census, the population of older adults in America is growing at a faster rate than the overall population growth, meaning that older adults make up a larger percent of the total population now than they ever have before (Howden & Meyer, 2010), and it is estimated that the prevalence of sarcopenia in persons over the age of 80 is greater than 50 percent (Baumgartner et al., 1998). As a result, problems facing the aging population, such as sarcopenia, are increasingly important public health issues that must be addressed. One intervention that has been used extensively to combat sarcopenia in the aging population is resistance exercise (RE).

Many studies that have looked exclusively at the response of older adults to resistance exercise (RE) programs have found that following such programs there is a significant increase in muscle fiber cross-sectional area (Leenders et al., 2013a; Pyka, Lindenberger, Charette, & Marcus, 1994), whole muscle cross-sectional area (Chale et

al., 2013; Leenders et al., 2013a; Slivka, Raue, Hollon, Minchev, & Trappe, 2008), muscle strength (Chale et al., 2013; Pyka et al., 1994; Slivka et al., 2008), and physical function (Chale et al., 2013; Leenders et al., 2013a). While these results provide evidence that older adults do have the capacity to gain muscle mass, increase strength and improve physical function following RE programs, these studies did not examine whether older adults respond to RE as effectively as the young adults. These studies were typically longitudinal intervention studies with repeat measures comparisons. The absence of a young group does not provide any basis for comparison of the observed improvements to improvements that made by young adults involved in similar RE interventions. It is unclear if the improvements observed in the older adults following resistance training are comparable with those observed in young adults.

The purpose of this review is to compile findings of research conducted on the response to RE exhibited by aged adults, both human and animal, compared to the response exhibited by young adults. It will describe studies that examined both the acute and chronic responses of aged versus young populations to RE by providing evidence on gross functional and morphological changes, such as strength and muscle mass, as well as cellular and molecular changes, including muscle fiber cross sectional area, gene expression and cell signaling. A review of this kind has not previously been compiled, but is needed as the field of aging muscle research moves forward. It provides a lens through which the response of older adults to RE can be scrutinized. This will help future research to more specifically test specific types of programs and examine possible causes of sarcopenia that could eventually be eliminated. This could greatly improve quality of life for the growing population of elders in society.

2.2 Force Production/Strength

Strength measurements are often used to show the response to resistance training (RT) programs both in the research setting and in the general public. Strength measurements are usually presented as changes in the 1 repetition maximum (1RM) of a specific movement such as a squat or knee extension, or measurements are presented as changes in force production such as knee extensor torque measured using an isokinetic dynamometer.

2.2.1 Human Studies

In studies examining changes in strength in young and aged adults following long-term resistance training programs most studies show that both young and older adults make significant strength gains following such programs. However, these increases are attenuated in the older groups compared to the young in most of the studies. The differences between the gains in strength of the young and old group in each study vary from 1%- 13% with the average being an 8% greater increase observed in the young group (Bickel, Cross, & Bamman, 2011; Greig et al., 2011b; LaRoche et al., 2008; Lemmer et al., 2000; Mayhew et al., 2009; Raue et al., 2009; Roth et al., 2000).

In a study conduced by Kosek and colleagues the trend of greater response in the younger groups was not apparent. Following a 16-week RT program strength gains for three different movements (knee extension, squat and leg press) were 28-47% higher in the young group and 33-49% higher in the older group after, as compared to

before, training (Kosek, Kim, Petrella, Cross, & Bamman, 2006). This was also true for a study examining changes in maximal force production of the abductor digiti minimi (ADM) following 6 weeks of maximal RT. The young group improved force production by 25% and the older group increased force production 33% (Patten, Kamen, & Rowland, 2001). These studies did not show an attenuated response by the older participants. However, the ADM strengthening study also measured the maximal motor unit discharge rate (MUDR) in each group. Both groups showed a significant increase in maximal MUDR on the first day, but following the second day of training this rate decreased in the older group and remained elevated in the young (Patten et al., 2001). This indicates that the observed response may be a result of different adaptations to the stimuli in the young and old adults.

2.2.2 Animal Studies

Animal studies have resulted in similar findings with young animals producing greater force production following chronic stretch shortening cycle (SSC) testing. At baseline, isometric force production was similar between the young and old rats. Following 14 bouts of intense SSC over the course of 30 days the young had increased isometric force production by 25%. The old animals significantly decreased force production from baseline with a drop of 34% (Cutlip et al., 2006).

2.2.3 Summary of Force Production/Strength

Nine of the eleven studies presented here show a greater increase in strength in the young group, as compared to the old, following a resistance training program (Table 2.1). It is worth noting that the two studies that did not follow this trend were methodologically different than the other studies. One study included participants in the

aged who were younger than those included in the other studies (Kosek et al., 2006). The other study tested and trained of 5th finger abduction strength (Patten et al., 2001). This movement may not be generalizable to overall response to exercise.

Table 2.1 Studies comparing strength/force production gains between young and old adults

Author	Type of Intervention	Duration	Frequency	Age (yrs)	Gender	Results
Laroche, et al., 2008	Knee Extensor High Velocity/ High Resistance Training	8 weeks	3x/week	Y- 18-33	F	Y- ↑ peak torque by 16%
				O- 65-84		O- ↑ peak torque by 7%
Grieg, et al., 2011	Quadriceps RT	12 weeks	3x/week	Y- 19-30	F	Y- ↑ strength 27%
				O- 76-82		O- ↑ strength 16%
Raue, et al., 2009	Knee Extensor High Intensity PRT	12 weeks	3x/week	Y- 21 ± 2	F	Y- ↑ 1RM 36%
				O- 85 ± 1		O- ↑ 1RM 26%
Roth, et al., 2000	Knee Extensor High Resistance Unilateral RT	9 weeks	3x/week	Y- 20-30	F	Y- ↑ strength 38%
				O- 65-75		O- ↑ strength 25%
Mayhew, et al., 2009	Knee Extensor RT	16 weeks	3x/week	Y- 27.9 ± 1	Not specified	Y- ↑ strength 44%
				O- 64.4 ± 0.9		O- ↑ strength 38%
Kosek, et al., 2006	Knee Extensor RT	16 weeks	3x/week	Y- 20-35	M/F	Y- men ↑ knee extensor strength 34%, squat 37% and leg press 38%
						Y- women ↑ knee extensor strength 38%, squat 28% and leg press 47%
				O- 60-75		O- men ↑ knee extensor strength 44%, squat 49% and leg press 49%
						O- women ↑ knee extensor strength 37%, squat 36% and leg press 49%
Patten, et al., 2001	5th Finger Abduction	6 weeks	5x/week	Y- 23.2 ± 3.5	M	Y- ↑ force production 25%
				O- 75.8 ± 7.4		O- ↑ force production 33%
Lemmer, et al., 1999	Knee Extensor RT	9 weeks	3x/week	Ym- 25±1	M/F	Y men- ↑ 1RM 31%
				Yw- 26±1		Y women- ↑ 1RM 39%
				Om- 69±1		O men- ↑ 1RM 27%
				Ow- 68±1		O women- ↑ 1RM 29%
Bickel, et al., 2011	Knee Extensor RT	16 weeks	3x/week	Y- 20-35	Not specified	Y- ↑ 1RM 40.6
				O- 60-75	F	O- ↑ 1RM 39.6%
***Cutlip, et al., 2006**	Dorsiflexor SSC	4.5 weeks	3x/week	Y- 12 weeks	M	Y- ↑ 25% from baseline
				O- 30 months		O- ↓ 34% from baseline

* Indicates animal study

2.3 Hypertrophy

Hypertrophy, or increased muscle mass, is, also a commonly quantified factor to determine the effectiveness of RT programs. Hypertrophy is typically measured as the change in cross-sectional area (CSA) of either whole muscle, individual muscle fibers or the components of the muscle fibers themselves. Muscle weight and volume have also been used to quantify hypertrophy. Muscle volume has been shown to be a determinant of joint torque (Fukunaga et al., 2001).

2.3.1 Human Studies

It has been reported in studies that examined changes in whole muscle CSA following 12 week interventions that there was a greater increase in the young groups than the old when measuring thigh CSA (Raue et al., 2009), elbow flexor, knee flexor and knee extensor CSA. When measuring whole thigh CSA there was a 5% increase in the young group and no change in the old group. Another study measured changes in knee flexor, knee extensor and elbow extensor CSA. The young adults had 8% knee flexor and 22% elbow extensor CSA increases while the older adults had 1% and 9% CSA increases, respectively. Knee extensor CSA did not follow the same pattern as it increased by 4% in the young and 6% in the old group (Welle, Totterman, & Thornton, 1996).

In studies that measured change in muscle volume it was found that following a 16-week RT intervention young participants had a 6.2% increase in quadriceps muscle volume while older participants only increased by 2.5% (Greig et al., 2011a). Another study found that following 9 weeks of knee extensor training all groups had a significant

increase in muscle volume. The young men, old men and old women all increased by 12% and the young women increased by 6% (Ivey et al., 2000).

Among studies that measured changes in fiber size following a 16-week RT program one reported that both the young and older participants had a significant increase in the size of type II myofibers of 37% and 40%, respectively (Mayhew et al., 2009), but, another study showed a 25% increase in the young group and a 16% increase in the older group (Kosek et al., 2006). Similar results were found in a study examining changes in mean fiber area (MFA). It was reported that young men increased MFA 27% and the older men increased 17% (Petrella, Kim, Cross, Kosek, & Bamman, 2006). Another study showed type II fiber size increased 37% in the young and 28% in the older group following (Bickel et al., 2011). Even more drastic results were reported in another study that examined changes in young and older women following 12 weeks of high intensity progressive RT. There was a 28% increase in myofiber size in the young, but no change was reported in the older group (Raue et al., 2009).

2.3.2 Animal Studies

Animal models often quantify hypertrophy by isolated muscle mass following testing. The studies examined here used two different means of inducing hypertrophy to determine whether there is a difference in the hypertrophic response to RE between young and old rats. Both studies showed a more robust response in the young animals than the old (Blough & Linderman, 2000; Murlasits et al., 2006). The numerical data are given in Table 2.2.

2.3.3 Summary of Hypertrophy

Seven out of the ten studies presented in this section showed a greater hypertrophic response in the young group than the old group (Table 2.2). On average the young group increased 27% over baseline and the old group increased 9% over baseline in these studies. One study had mixed results among the muscle groups measured with one of three increasing CSA more in the old group than the young. However, when the data from this study was combined over all muscle groups measured the young increased CSA by and average of 11% and the old group only increased by an average of 5% (Welle et al., 1996). Another study reported no difference between the volume gains between the young and old groups (Ivey et al., 2000). The only study that showed a large difference between the young and old groups in which the old group had more hypertrophy than the young had the lowest average age among the old groups. This old group had an average age of 64±0.9 years. It is possible that this group was young enough to have response that was more robust than the other old groups (Mayhew et al., 2009). The overall pattern of response for hypertrophy is worse than it is for strength gains in older adults following resistance training programs. This likely indicates that older adults are still gaining neural adaptations from participation in resistance training programs, but building little muscle mass. This is particularly concerning because muscle hypertrophy, in addition, to increasing strength also has other health benefits.

Table 2.2 Studies comparing hypertrophy gains between young and old adults

Author	Type of Intervention	Intervention Length	Age (yrs)	Gender	Results
Mayhew et al., 2009	RT	16 weeks	Y- 27.9±1	M/F	Y- 37% ↑ in type II myofiber size
			O- 64.4±0.9		O- 40% ↑ in type II myofiber size
Kosek et al., 2006	Knee Extensor RT	16 weeks	Y- 20-35	M/F	Y- 25% ↑ in type IIa myofiber size
			O- 60-75		O- 16% ↑ in type IIa myofiber size
Petrella et al., 2006	Knee Extensor RT	16 weeks	Y- 27±1	M/F	Y-27% ↑ in mean fiber size
			O- 63.7±1		O- 17% ↑ in mean fiber size
Bickel et al., 2011	Knee Extensor RT	16 weeks	Y- 20-35	Not Specified	Y- 37% ↑ in type II myofiber size
			O- 60-75		O- 28% ↑ in type II myofiber size
Raue et al., 2009	High Intensity PRT	12 weeks	Y- 21±2	F	Y- 28% ↑ in myofiber size, 5% ↑ in thigh CSA
			O- 85±1		O- No change in myofiber size or thigh CSA
Welle et al., 1996	Knee Flex/Ext and Elbow Ext	12 Weeks	Y- 22-31		Y- 22% ↑ in elbow flexor CSA, 4% ↑ in knee extensor CSA, 8% ↑ in knee flexor CSA
			O- 62-72		O- 9% ↑ in elbow flexor CSA, 6% ↑ in knee extensor CSA, 1% ↑ in knee flexor CSA
Grieg, et al., 2011	Quadriceps RT	12 weeks	Y- 19-30	F	Y- 6.2% ↑ in volume
			O- 76-82		O- 2.5% ↑ in volume
Ivey, et al., 2000	Knee Extensor RT	9 weeks	Ym- 25±3	M/F	Y men- 12% ↑ in volume
			Yw- 26±2		Y women- 6% ↑ in volume
			Om- 69±3		O men- 12% ↑ in volume
			Ow- 68±2		O women- 12% ↑ in volume
***Murlasits et al., 2006**	Chronic SSC	4.5 weeks	Y- 3	M	Y- increased tibialis anterior wet weight by 15.6% from baseline
			O- 30		O- no change in tibialis anterior wet weight from baseline
***Blough & Linderman, 2000**	Ablation model of overload	8 weeks	Y- 6	M	Y- increased plantaris wet weight by 53%, increased plantaris CSA by 63%
			O- 36		O- no change in plantaris wet weight, increased plantaris CSA by 20% (non-significant increase)

* Indicates Animal Study

2.4 Growth Factors

There are many factors that have been identified as contributors to cell growth. It is known that at different points in the lifespan there are various concentrations of these factors (Owino, Yang, & Goldspink, 2001). The studies outlined in this section have looked for differences in these factors between young and old adults as a potential cause for altered hypertrophic response in that occurs with age.

Insulin-like growth factor (IGF-1), an important growth hormone that stimulates muscle mass, is produced by the liver and can be regulated by other cytokines including growth hormone (GH). There are multiple splice variants of IGF-1, including IGF-1Ea and IGF-1Ec (humans) /IGF-1Eb (rodents), that appear to be involved in the positive regulation of muscle hypertrophy and both of these are produced locally in active muscle (Goldspink, 1998; Owino et al., 2001; Yang, Alnaqeeb, Simpson, & Goldspink, 1996).

In studies that have examined the changes in IGF-1Ea mRNA expression in young and older adults following acute RE, it was found that there were no significant differences in expression following the bout of exercise (Greig et al., 2011a; Hameed, Orrell, Cobbold, Goldspink, & Harridge, 2003; Roberts, Dalbo, Hassell, & Kerksick, 2009). In a study that examined IGF-1Ea mRNA expression in response to a 16-week RT program, both the young and older adults showed a significantly increased rate of mRNA expression, but the magnitude of the expression change was very different between the groups with the young displaying greater expression (Petrella et al., 2006). IGF-1Ea mRNA expression in muscle was examined in animals using male Sprague-Dawley rats aged 3 months, 12 months and 24 months in a synergist ablation model of

overload. It was found that there was no change in IGF-1Ea mRNA expression with age or following overload (Owino et al., 2001).

IGF-1Ec/ IGF-1Eb, also called mechanogrowth factor (MGF), has been shown only to appear appreciably in muscle that has been exercised or damaged (Goldspink, 1998; Owino et al., 2001; Yang et al., 1996). In a study conducted in young and older men MGF mRNA expression was measured at baseline and 2.5 hours following an acute bout of heavy RE. It was found that there were no differences between expression in the groups at baseline, but following exercise there was a significant increase in the mRNA expression of MGF in only the young group (Hameed et al., 2003). In a training study, it was found that young adults increased MGF mRNA expression by 85% while the older adults increased expression by 40% at the end of 16 weeks of RT (Petrella et al., 2006).

In animal studies, MGF mRNA expression significantly increased in all groups following overload (Haddad & Adams, 2006; Owino et al., 2001). In one of the studies, the young group was significant at the level of $p<0.001$ while the level of significance for the mature and older groups was $p<0.05$ (Owino et al., 2001). In another there was no significant difference between the two groups (Haddad & Adams, 2006).

Not all studies use assays that differentiate between the various isoforms of IFG-1. One such study that examined the response of young and older adults to an acute bout of RE found that there was a significantly higher level of IGF-1 mRNA expression in young men as compared to older men both at baseline and following exercise (Dennis et al., 2008). In an animal study, the investigators measured the difference in mRNA levels of IGF-1 24 and 48 hours following an acute bout of heavy RE and found

that at both time points both groups had elevated levels of IGF-1 mRNA, but at 24 hours post-exercise the level in the young group was significantly greater than that of the older animals. At baseline, the older animals showed higher levels of IGF-1 mRNA expression (Haddad & Adams, 2006).

In addition to examining the levels of mRNA present for the various isoforms of IGF-1, there have also been studies that have monitored changes in the level of the IGF-1 receptor (IGF-1R). In one such study, it was found that although there was no significant difference in mRNA levels between the young and old men at baseline there was an increase in the level of IGF-1R mRNA in the young group 6 hours following RE and essential amino acid supplementation that was both significantly increased from baseline and significantly greater than the expression shown in the older group (Drummond et al., 2009). Another study found that there were no significant differences at baseline or 2.5 hours following RE (Greig et al., 2011a). The difference in response observed in these studies is likely due to the timing of the measurements.

2.4.1 Summary of Growth Factors

In studies that found differences in expression of any of the growth factors listed above, all found there to be greater expression in young adults when compared to old adults (Table 2.3). This is another example of decreased response to both acute and chronic resistance exercise exhibited by older adults.

Table 2.3 Studies comparing differences in growth factors between young and old adults

Author(s)	Intervention Type	Measurement Timing	Age (yrs)	Gender	Results
IGF-1Ea mRNA					
Roberts et al., 2009	Single bout of RE	Pre-RE, 5 minutes & 2.5 hr post-RE	Y- 21.3±0.6	M	Y-No difference in IGF-1Ea mRNA expression
			O- 67.6±1.3		O- No difference in IGF-1Ea mRNA expression
Hameed et al., 2003	Single bout of RE	Pre-RE & 2.5 hr post-RE	Y- 29.5±1.5	M	Y-No difference in IGF-1Ea mRNA expression
			O- 74.4±1.8		O-No difference in IGF-1Ea mRNA expression
Grieg et al., 2011	Single bout of RE	Pre-RE & 2.5 hrs post- RE	Y- 19-30	F	Y-No difference in IGF-1Ea mRNA expression
			O- 76-82		O- No difference in IGF-1Ea mRNA expression
Petrella et al., 2006	Both single bout of RE and 16 weeks of RT	Pre-RE, 24 hr post acute-RE & 24 hr post last training session	Y- 20-35	M/F	Y- No difference acutely, ↑ following 16 weeks of training
			O- 60-75		O- No difference acutely, ↑ following 16 weeks of training
***Owino et al., 2001**	Unilateral overload via tendon ablation	1, 2, 3, or 5 days following surgery	Y- 3 mos	M	Y- No difference in IGF-1Ea mRNA expression following overload
			Mature- 12 mos		Mature- No difference in IGF-1Ea mRNA expression following overload
			O- 24 mos		O- No difference in IGF-1Ea mRNA expression following overload
IGF-1 mRNA					
Dennis et al., 2008	Single bout of RE	Pre-RE & 72 hr post-RE	Y- 32±7	M	Y- No difference from baseline, but significantly greater level of IGF-1 expression than old at baseline and post-RE
			O- 72±5		O- No difference from baseline
***Haddad & Adams, 2006**	Single bout of heavy RE	Pre-RE, 24, & 48 hr post-RE	Young- 6 mos	M	Y- ↑ at both 24 and 48 hr following RE, significantly higher than the old group at 24 hr post-RE
			Old- 30 mos		O- ↑ at both 24 and 48 hr following RE
IGF-1R mRNA					
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Young- 29±2	M	Y- No difference at 3 hr post-RE, ↑ at 6 hr post-RE
			Old- 70±2		O- No difference at any of the measured time points
Grieg et al., 2011	Single bout of RE	Pre-RE & 2.5 hrs post- RE	Y- 19-30	F	Y- No difference in IGF-1R mRNA expression
			O- 76-82		O- No difference in IGF-1R mRNA expression
***Owino et al., 2001**	Unilateral overload via tendon ablation	1, 2, 3, or 5 days following surgery	Y- 6 mos	M	Y- ↑ in IGR-1R mRNA expression following overload
			Mature- 12 mos		Mature- No difference in IGF-1R mRNA expression following overload
			O- 30 mos		O- No difference in IGF-1R mRNA expression following overload
MGF mRNA					
Hameed et al., 2003	Single bout of RE	Pre-RE & 2.5 hr post-RE	Y- 29.5±1.5	M	Y- ↑ in MGF mRNA expression following RE
			O- 74.4±1.8		O- No difference in MGF mRNA expression following RE
Petrella et al., 2006	16 weeks of RT	Pre-RE & 24 hr post-RE	Y- 20-35	M/F	Y-↑ following acute RE, ↑ following 16 weeks of training

			O- 60-75		O- No significant change from baseline following acute RE or 16 weeks of training
***Owino et al., 2001**	Unilateral overload via tendon ablation	1, 2, 3, or 5 days following surgery	Y- 6 mos	M	Y- ↑ in MGF mRNA expression following overload ($p<0.001$)
			Mature- 12 mos		Mature- ↑ in MGF mRNA expression following overload ($p<0.05$)
			O- 30 mos		O- ↑ in MGF mRNA expression following overload ($p<0.05$)
***Haddad & Adams, 2006**	Single bout of heavy RE	Pre-RE, 24 & 48 hr post-RE	Y- 6 mos	M	Y- ↑ at both 24 and 48 hr post-RE
			O- 30 mos		O- ↑ at both 24 and 48 hr post-RE

* Indicates Animal Study

2.5 Gene and Protein Expression

Increase in muscle mass that occurs following RT is preceded by an increase in transcriptional or translational machinery that results in increased muscle protein content. As muscle mass or protein content is a product of the rate of protein synthesis and degradation, many studies have investigated how aging and RT effect each of these processes and the cellular machinery that regulate them. Below the cellular pathways that are involved in regulating muscle protein content with RE and/or sarcopenia are described.

2.5.1 Myogenic Regulatory Factors

Myogenic regulatory factors (MRFs) are a part of a superfamily of transcription factors that control the specification and differentiation of muscle cells. This family includes factors such as myogenin (Myf-1), myoD (Myf-3), Myf-5, and MRF4 (Myf-6/Herculin) (Buckingham, 1996; Sabourin & Rudnicki, 2000).

MyoD and myf-5 are “early” MRFs and their expression is responsible for stimulating myoblasts to enter differentiation and join muscle lineage (Bamman et al., 2004; Kosek et al., 2006). These MRFs are commonly measured as a means of determining whether the signal to produce myocytes is present. Studies have consistently shown that following an acute bout of RE myoD mRNA expression significantly increased in both the young and older participants (Drummond et al., 2009; Owino et al., 2001; Raue, Slivka, Jemiolo, Hollon, & Trappe, 2006). However, there is less agreement on the relative level of myoD mRNA at baseline. One study showed no difference between the groups at baseline (Drummond et al., 2009), another study

showed significantly higher expression in the young group at baseline (Owino et al., 2001), and the other showed significantly higher (58%) expression in the old group at baseline (Raue et al., 2006). There were no significant changes in myoD protein expression in either group following a short RE intervention, but baseline levels were significantly higher in the old group as compared to the young (Bamman et al., 2004). Following an acute bout of RE there was no significant difference in the change in myf-5 mRNA expression in either group (Raue et al., 2006).

When changes in the early MRFs have been examined following a long-term RT intervention it was found that there were significant increases in MyoD mRNA expression in both young and older adults of 90% and 54% respectively. However, there was no test performed for differences between these groups. Myf-5 expression also significantly increased (27%) in the in the young, but not the old group (Kosek et al., 2006).

The “late” MRFs, myogenin and MRF4/myf-6, terminally differentiate myoblasts toward the formation of multi-nucleated myotubes (Bamman et al., 2004; Buckingham, 1996; Drummond et al., 2009; Wright, Sassoon, & Lin, 1989). Studies that have looked at levels of myogenin present in old versus young adults have shown varying results. Many studies have shown significantly higher myogenin mRNA expression in the old group at baseline (Bamman et al., 2004; Haddad & Adams, 2006; Owino et al., 2001; Raue et al., 2006) with one showing a trend in the same direction (Drummond et al., 2009). This was also true for baseline myogenin protein levels (Bamman et al., 2004). However, another study showed no significant difference between mRNA expression of the groups at baseline (Roberts et al., 2009). Following an acute bout of RE, three

studies showed no significant changes in myogenin mRNA levels in either group (Bamman et al., 2004; Raue et al., 2006; Roberts et al., 2009). Three other studies found that there was a significant increase in myogenin mRNA expression in the young group following acute RE but not the old group (Drummond et al., 2009; Haddad & Adams, 2006; Kosek et al., 2006).

Myogenin mRNA levels significantly increased in both young and aged adults following a 16-week RT program (Kosek et al., 2006). Myf-6/MRF-4 mRNA expression was only reported in one study. Following RE both the young and older groups showed significantly increased levels of MRF4 mRNA expression (Raue et al., 2006). In one study, Myf-6 protein levels in older females tended to be higher than that of the young females ($p=0.059$) (Bamman et al., 2004), but another observed significantly increased levels of myf-6 protein in both groups post-RE. The levels measured in the young participants were higher than those of the older group at all time points (Kosek et al., 2006).

2.5.2 Summary of Myogenic Regulatory Factors

There is no difference between old and young adults in early MRF mRNA expression in response to acute RE. There may be difference in expression at baseline. However, with a long term RE program there is an attenuation in the expression of both myoD and myf-5 in the old group compared to the young. Most evidence suggests that there is greater myogenin mRNA expression in old adults at baseline, but only a significant increase in expression among young adults following an acute bout of RE (Table 2.4). More studies need to present data on changes in myf-6 expression before

conclusive statements can be made regarding differences between expression in young and old adults. These differences between young and old adults lack consistency and are not convincing evidence that the impairment causing a decreased hypertrophic response to exercise in older adults.

Table 2.4 Studies comparing differences in myogenic regulatory factors between young and old adults

Author(s)	Intervention Type	Muscle Biopsy Timing	Age (yrs)	Gender	Results
MyoD mRNA					
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	Significantly ↑ expression in both groups 6 hr post-RE
			O- 70±2		
Raue et al., 2006	Single bout of RE	Pre-RE & 4 hr post-RE	Y- 18-30	F	Significantly ↑ expression in older adults at baseline
			O- 80-89		Both old and young groups showed significant ↑ in expression post-RE
Kosek et al., 2006	16 weeks of RT	Pre-RT, 24 hr post first RE session & 24 hr post last RE session	Y- 20-35	M/F	Both old and young groups showed significant ↑ in expression post-RE
			O- 60-75		Young ↑ 90% and old ↑ 54%
***Owino et al., 2001**	Unilateral overload via tendon ablation	1, 2, 3, or 5 days following surgery	Y- 3 mos	M	Significantly ↑ expression in both young and old post overload
			Mature- 12 mos		Young showed significantly greater ↑ in expression
			O- 24 mos		
MyoD Protein					
Bamman et al., 2004	5 RT sessions	Pre-RE & 24 hr post last RE session	Y- 20-35	M/F	Significantly ↑ expression in young group both pre and post-RE
			O- 60-75		No significant changes in either group following RT
Myogenin mRNA					
Roberts et al., 2009	Single bout of RE	Pre-RE, 5 minutes & 2.5 hr post-RE	Y- 21.3±0.6	M	No significant changes in either group following RT
			O- 67.6±1.3		
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	Significant ↑ from baseline in young at 3 and 6 hr post-RE
			O- 70±2		Young showed significantly ↑ expression than old at 3 and 6 hr pos- RE
Kosek et al., 2006	16 weeks of RT	Pre-RT, 24 hr post first RE session & 24 hr post last RE session	Y- 20-35	M/F	Significant ↑ in young following first bout of RE
			O- 60-75		Significant ↑ in both groups following 16 weeks of RE
Bamman et al., 2004	5 RT sessions	Pre-RE & 24 hr post last RE session	Y- 20-35	M/F	Significantly ↑ expression in older adults at baseline
			O- 60-75		No significant changes in either group following RE
***Haddad & Adams, 2006**	Single bout of heavy RE	Pre-RE, 24, & 48 hr post-RE	Y- 6 mos	M	Significantly ↑ expression in old animals at baseline
			O- 30 mos		Significantly ↑ expression in old at 48 hr post-overload
Myogenin Protein					
Bamman et al., 2004	5 RT sessions	Pre-RE & 24 hr post last RE session	Y- 20-35	M/F	Significantly ↑ expression in young group both pre and post-RE
			O- 60-75		No significant changes in either group following RE
Kosek et al., 2006	16 weeks of RT	Pre-RT, 24 hr post first RE session & 24 hr post last RE session	Y- 20-35	M/F	No significant changes in either group following both acutely and following 16 weeks of RE
			O- 60-75		

Myf-6/MRF-4 mRNA					
Raue et al., 2006	Single bout of RE	Pre-RE & 4 hr post-RE	Y- 18-30	F	Significantly ↑ expression in old at baseline
			O- 80-89		Both old and young groups showed significant ↑ in expression post-RE with a trend (p=0.08) for greater expression in young than old
Myf-6 Protein					
Bamman et al., 2004	5 RT sessions	Pre-RE & 24 hr post last RT session	Y- 20-35	M/F	Expression tended to be higher in old females (p=0.059) than young females
			O- 60-75		

* Indicates Animal Study

2.5.3 Myostatin

Myostatin, also referred to as growth and differentiation factor 8 (GDF-8) is a member of the transforming growth factor-β family, which acts as a negative regulator of skeletal muscle growth by suppressing satellite cell proliferation and signaling satellite cell quiescence (Drummond et al., 2009; Lee & McPherron, 2001; McCroskery, Thomas, Maxwell, Sharma, & Kambadur, 2003). Myostatin mRNA expression in response to RE has been examined in several studies. One study reported no change from baseline expression in either old or young adults (Greig et al., 2011a). Others have reported decreases in both young and old groups following RE (Dennis et al., 2008; Raue et al., 2006). However, one of these studies reported that that decrease in the older group became non-significant with further statistical analyses (Dennis et al., 2008). Still another study reported a decrease in myostatin mRNA expression in young men, young women and old men, but not old women following RE (Kim, Cross, & Bamman, 2005). A decrease in only the older group was reported in another study (Dalbo et al., 2011).

Myostatin expression has also been monitored in animal models of RT in young and old rodents. At baseline young rats had significantly higher levels of myostatin mRNA expression, and although expression levels decreased in both groups it was only significant in the young group (Haddad & Adams, 2006).

2.5.4 Summary of Myostatin

The results presented in this section are variable. Half of the studies indicate that there is no difference in myostatin mRNA expression at baseline and that both young

and old groups show a decreased following acute exercise (Table 2.5). However, the variability in these data may be due to the vast differences in the time points between the studies. The only study that shows an increase in myostatin following exercise was the animal study and it is likely that the bout of exercise used in this study is much more intense than that which could be used in human exercise studies.

Table 2.5 Studies comparing differences in myostatin between young and old adults

Author(s)	Intervention Type	Measurement Timing	Age (yrs)	Gender	Results
Dalbo et al., 2011	Four bouts of RE	Pre- RE, 48 hr after the 1st and 2nd bouts	Y- 18-25	M	No difference in mRNA levels between young and old at baseline
		of RE, and 24 hr after the 3rd bout of RE	O- 60-75		↓ in mRNA expression between baseline and 3rd bout of RE in old group
Grieg et al., 2011	Single bout of RE	Pre-RE & 2.5 hrs post- RE	Y- 19-30	F	No difference in mRNA levels between young and old at baseline
			O- 76-82		No change from baseline in either group following RE
Raue et al., 2006	Single bout of RE	Pre-RE & 4 hr post-RE	Y- 18-30	F	Old expressed ↑ levels of mRNA at baseline compared to young
			O- 80-89		Both groups ↓ mRNA expression following RE
Dennis et al., 2008	Single bout of RE	Pre-RE & 72 hr post-RE	Y- 32±7	M	Young expressed significantly ↑ levels of mRNA at baseline
			O- 72±5		mRNA expression ↓ in young (52%) and old (42%) groups (*old group did not reach significance)
Kim et al., 2005	Single bout of RE	Pre-RE & 24 hr post-RE	Y- 20-35	M/F	No differences were reported between groups at baseline
			O- 60-75		Young men (56%), young women (48%) and old men (40%) all showed ↓
					expression following RE. No change was reported in old women.
***Haddad & Adams, 2006**	Single bout of heavy RE	Pre-RE, 24 & 48 hr post-RE	Y- 6 mos	M	Young expressed significantly ↑ levels of mRNA at baseline
			O- 30 mos		Young decreased expression at 24 and 48 hr post-RE. No change seen in old

* Indicates Animal Study

2.5.5 mTOR Signaling Pathway

Mammalian target of rapamycin, mTOR, plays a critical role in the signaling pathway that regulates cell growth and proliferation (Bodine et al., 2001; Funai, Parkington, Carambula, & Fielding, 2006; Yonezawa, 2004). The activation of mTOR the signaling pathway has been studied. Figure 2.1 is a simplistic representation of the proteins discussed in this section that are involved in mTOR singaling.

mTOR (Ser2448) phosphorylation in response to RE was found to increase in young adults in two studies, but the response of the older adults differed between the two (Drummond et al., 2008; Fry et al., 2011). In one study, there was no significant change in expression in the older group following RE (Fry et al., 2011), but in the other study there were significant increases in phosphorylation following RE and essential amino acid ingestion (Drummond et al., 2008).

2.5.5.1 Upstream modulators of mTOR signaling

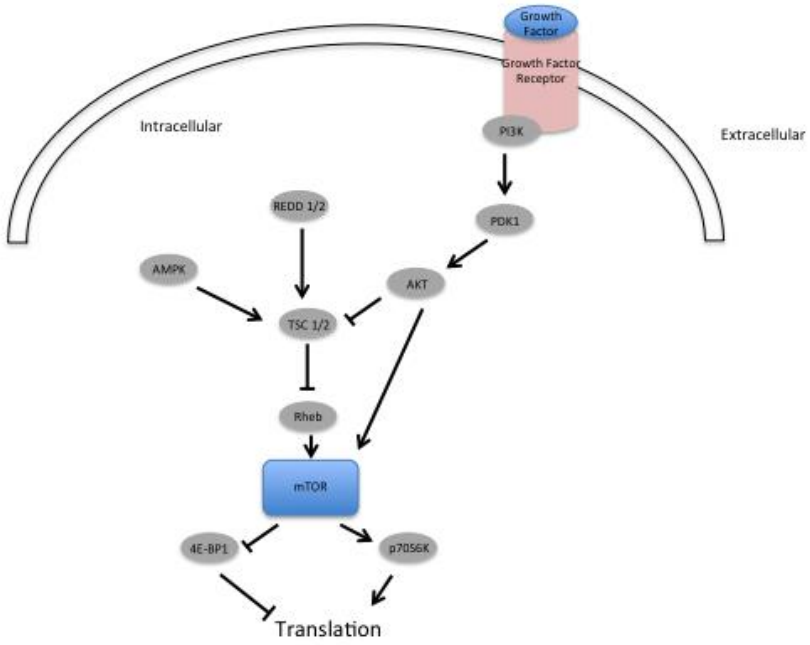

Figure 2.1 This is a simplistic representation of the mTOR signaling pathway discussed in this report.

Akt promotes cell growth through activation of mTOR complex 1 (mTORC1) (Manning & Cantley, 2007). Studies have consistently shown a significant increase in Akt phosphorylation (Ser473) in young adults following RE, with no change in the older group (Drummond et al., 2008; Fry et al., 2011; Haddad & Adams, 2006; Mayhew et al., 2009). One study reported a significant decrease in Akt phosphorylation in the older adults following RE (Rivas et al., 2012).

AMP-activated protein kinase (AMPK) has been shown to be an upstream negative regulator of mTOR signaling (Bolster, Crozier, Kimball, & Jefferson, 2002). One study showed no change in AMPK phosphorylation levels in either the young or older participants, but significantly higher levels of phosphorylated AMPK than the young at all time points (Fry et al., 2013), but another found that post RE levels of phosphorylated AMPKα were significantly higher in the older adults, but not the younger (Drummond et al., 2008). This may play a role in decreased levels of protein synthesis

in older adults that could potentially lead to the attenuated hypertrophic response observed in older adults.

Tuberous Sclerosis Complex (TSC1/TSC2) is a negative regulator activated by AMPK that acts upstream of mTOR (Drummond et al., 2009; Yonezawa, 2004). TSC1 mRNA expression tended to be lower ($p=0.05$) 3 hours post-exercise and was significantly lower at 6 hours post-exercise in the older participants compared to baseline. TSC2 mRNA expression levels were unchanged in the young group, but significantly decreased in the older participants post-exercise (Drummond et al., 2009).

REDD1 and REDD2 have been identified as negative regulators of mTOR signaling (Drummond et al., 2009). The behavior of REDD1 mRNA expression following RE has not been consistent in the literature. In one study expression was significantly decreased post-exercise in older men, but there was no significant changes in the young men (Drummond et al., 2009). However, in a study conducted with young and older women it was determined that REDD1 mRNA levels were significantly decreased in the young women, but not the older women(Greig et al., 2011a). REDD2 mRNA expression was significantly decreased in both young and old men post-exercise (Drummond et al., 2009).

Rheb is a GTPase that is a target of TSC2's GTPase-activating function. Rheb-GTP is a positive regulator of mTOR signaling (Drummond et al., 2009; Yonezawa, 2004). Drummond and colleagues showed Rheb mRNA levels in older adults did not change from baseline, but were significantly higher in the young group (Drummond et al., 2009).

2.5.5.2 Downstream effectors of mTOR

70 kDa ribosomal protein S6 kinase (p70S6k) has been identified as a downstream target of the mTOR pathway (Baar & Esser, 1999; Nader & Esser, 2001). In all studies that examined changes in p70S6k following RE there were no differences between the groups reported at baseline. Some studies have reported no changes in either young or old participants following RE (Drummond et al., 2009; Mayhew et al., 2009). Other studies have reported significantly elevated p-p70S6k following RE that eventually returns to baseline, but no change in the old group (Kumar et al., 2009; Rivas et al., 2012). An animal study showed that there was an increase in p-p70S6k in both the old and young groups 24 hours following 2 bouts of maximal isometric exercise, but both returned to baseline at 48 hours post-RE (Haddad & Adams, 2006).

2.5.6 Summary of age related differences in the mTOR signaling pathway

The variability in the reported differences between young and old adults in both positive and negative regulators of the mTOR signaling pathway make it difficult to clearly understand the alterations that exist between young and old adults (Table 2.6). There are several methodological factors that contribute to this including choice of time points and type of exercise selected. However, it appears that mTOR, Akt, and p70S6k all show an attenuation in the response of old adults, compared to young. This indicates that there are true differences in the response to resistance exercise between these two groups.

Table 2.6 Studies comparing differences in mTOR pathway signaling between young and old adults

Author(s)	Intervention Type	Biopsy Timing	Age (yrs)	Gender	Results
mTOR					
Drummond et al., 2008	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	Y- ↑ at 1, 3, and 6 hours following RE
			O- 70±2		O- ↑ at 1, 3, and 6 hours following RE
Fry et al., 2011	Single bout of RE and EAA	Pre-RE, 3, 6 & 24 hr post-RE	Y- 27±2	M	Y- ↑ at 3, 6 and 24 hours following RE
			O- 70±2		O- No change
Akt					
Mayhew et al., 2009	Single bout of RE	Pre-RE & 24 hrs post- RE	Y- 27.9±1	Not specified	Y- ↑ of 60%
			O- 64.4±0.9		O- Non-significant ↑ of 39%
Rivas et al., 2012	Single bout of RE	Pre-RE, immediately post-RE, and 6 hrs post-RE	Y- 22±0.6	M	Y- ↑ Phosphorylation following RE
			O- 74.1.5		O- ↓ Phosphorylation following RE
Fry et al., 2011	Single bout of RE	Pre-RE, 3 ,6 , & 24 hr post-RE	Y- 27±2	Both	Y- ↑ at 3 hours following RE
			O- 70±2		O- ↑ at 24 hours following RE
Drummond et al., 2008	Single bout of RE and EAA	Pre-RE, 1, 3 , & 6 hr post-RE	Y- 29.7±1.7	M	Y- ↑ in phosphorylation at 3 hours following RE
			O- 70±2.1		O- No change
***Haddad & Adams et al., 2006**	Single bout of heavy RE	Pre-RE, 24, & 48 hr post-RE	Y- 6 mos	M	Y- ↑ phosphorylation at 24 hours following RE
			O- 30 mos		O- No change
AMPK					
Fry et al., 2011	Single bout of RE	Pre-RE, 3 ,6 , & 24 hr post-RE	Y- 27±2	Both	No change in either group following RE
			O- 70±2		O- significantly ↑ phosphorylation at all time points
Drummond et al., 2008	Single bout of RE and EAA	Pre-RE, 1, 3 , & 6 hr post-RE	Y- 29.7±1.7	M	Y- no change following RE
			O- 70±2.1		O- phosphorylation ↑ at 1 and 3 hours following RE
TSC1/TSC2					
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	Y- no change in TSC1 or TSC2 following RE
			O- 70±2		O- TSC1 and TSC2 both ↓ following RE
REDD1/REDD2					
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	REDD1- ↓ in old, but not young at 6 hours following RE
			O- 70±2		REDD2- ↓ in both young and old at 3 and 6 hours following RE
Grieg et al., 2011	Single bout of RE	Pre-RE & 2.5 hrs post- RE	Y- 19-30	F	REDD1- ↓ in young but not old following RE
			O- 76-82		
Rheb					
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	Y- ↑ following RE
			O- 70±2		O- No change

p70S6k					
Mayhew et al., 2009	Single bout of RE	Pre-RE & 24 hrs post- RE	Y- 27.9±1	Not specified	No significant change was reported in either group
			O- 64.4±0.9		
Drummond et al., 2009	Single bout of RE and EAA	Pre-RE, 1, 3, & 6 hr post-RE	Y- 29±2	M	No difference between groups reported at baseline
			O- 70±2		No changes in mRNA levels reported at 3 or 6 hrs post RE
Kumar et al, 2004	Acute bout of RE	BL and every 30 min for 4 hr post-RE	Y- 24±6	M	No difference between groups reported at baseline
			O- 70±5		Y- ↑ Phosphorylation 1 hr post RE, returned to baseline at 2 and 4 hrs post RE O- No change at any time points
Rivas et al., 2012	Single bout of RE	Pre-RE, immediately post-RE, and 6 hrs post-RE	Y- 22±0.6	M	Y- ↑ Phosphorylation immediately following RE and 6 hr post RE
			O- 74.1.5		O- No change at any time points
***Haddad & Adams et al., 2006**	Single bout of heavy RE	Pre-RE, 24, & 48 hr post-RE	Y- 6 mos	M	Y- ↑ Phosphorylation 24 and 48 hours following RE Significantly ↑ than old at 48 hours
			O- 30 mos		O- ↑ Phosphorylation 24 hours following RE

* Indicates Animal Study

2.5.7 MAPK Signaling Pathways

The mitogen-activated protein kinase (MAPK) family of proteins responds to growth factors, environmental stressors and inflammatory cytokines (Wu, Fannin, Rice, Wang, & Blough, 2011). The MAPK family has four main signaling molecules in skeletal muscle (Kramer & Goodyear, 2007). The signaling pathways for each of the individual signaling molecules can be activated by resistance exercise (Kramer & Goodyear, 2007; Williamson, Gallagher, Harber, Hollon, & Trappe, 2003). MAPK signaling is involved in regulating gene expression, cellular metabolism, cell growth, and cell differentiation (Wu et al., 2011). The studies outlined below have examined factors that influence MAPK signaling for indicators that alterations exist between young and old adults that could have an effect of the hypertrophic response to RE.

2.5.7.1 Proinflammatory Cytokines

Cytokines are often involved in the regulation of immune responses and inflammation (Akira, Hirano, Taga, & Kishimoto, 1990). Several research groups have measured circulating levels of cytokines to identify differences in the response to RE between young and older adults.

Serum levels of Interlukin-6 (IL-6), Interlukin-8 (IL-8) and Tumor necrosis factor-α (TNF-α) have been shown to increase in both young and old adults following a single bout of heavy resistance exercise. No differences between groups were detected at baseline or following exercise (Thalacker-Mercer, Dell'Italia, Cui, Cross, & Bamman, 2010).

Ciliary neurotrophic factor (CNTF) is a cytokine in the IL-6 family that plays a myotrophic role (Vergara & Ramirez, 2004). In a study conducted in all men, baseline levels of CNTF mRNA were compared to the levels 72 hours following a single bout of RE. It was found that there was a significantly higher level of CNTF mRNA expression in young men as compared to older men both at baseline and following exercise (Dennis et al., 2008).

2.5.7.2 Heat Shock Proteins

Heat shock proteins are produced from highly conserved genes. They function predominantly to protect cell during times of stress. They have also been shown to increase with resistance exercise without the presence of additional stressors (Kilgore, Musch, & Ross, 1998; Locke & Noble, 1995). One study showed that there was a 41% greater HSP70 content in the older subjects than the young (Thalacker-Mercer et al., 2010). This may indicate that older muscle is more susceptible to damage and has adapted to be able to provide more protection during stress.

An animal study conducted by Murlasits and colleagues showed that following a chronic stretch-shortening protocol there was a significant increase in HSP72 protein in the trained limb of both the young and old rats of 969% and 409%, respectively. A similar increase in HSP25 protein was also observed with the young increasing 943% and old increasing 420% in the trained limb. The authors suggest that these data show a maladaptive response to repetitive RE in the old animals that is due in part to oxidative stress (Murlasits et al., 2006).

2.5.7.3 NF-κB

NF-κB is involved in the signaling pathways for many different types of cells and alters the production of many genes including those encoding cytokines (Kramer & Goodyear, 2007). Two separate studies have shown that both at baseline and following and acute bout of RE older adults express significantly higher levels of NF-κB protein (Rivas et al., 2012; Thalacker-Mercer et al., 2010).

2.5.7 Summary of MAPK Signaling Pathway

There is evidence to suggest that at baseline and following an acute bout of resistance exercise older adults older adults have more cellular stress than their young counterparts, but following a chronic exercise stimulus the adaptation to stress is less robust in the old than in the young (Table 2.7).

Table 2.7 Studies comparing differences in MAPK Signaling between young and old adults

Author(s)	Intervention Type	Biopsy Timing	Age (yrs)	Gender	Results
IL-6					
Thalacker-Mercer et al, 2010	Acute bout of RE	Pre-RE and 24 hr post-RE	Y- 37±1	M/F	Significant ↑ in both groups following RE, but no difference between groups
			O- 73±1		
IL-8					
Thalacker-Mercer et al, 2010	Acute bout of RE	Pre-RE and 24 hr post-RE	Y- 37±1	M/F	No significant difference between groups nor following RE
			O- 73±1		
TNF-α					
Thalacker-Mercer et al, 2010	Acute bout of RE	Pre-RE and 24 hr post-RE	Y- 37±1	M/F	No significant difference between groups nor following RE
			O- 73±1		
CNTF					
Dennis et al, 2008	Acute bout of RE		Y- 32±7	M	Significantly ↑ levels both at baseline and following RE in young compared to old
			O- 72±5		
HSPs					
Thalacker-Mercer et al, 2010	Acute bout of RE	Pre-RE and 24 hr post-RE	Y- 37±1	M/F	HSPA9, HSPE1, HSPB1 and HSPH1 mRNA were all upregulated in old following RE
			O- 73±1		41% greater ↑ in HSP70 protein expression in old than young following RE
***Murlasits et al, 2006**	Chronic stretch-shortening		Y- 3 mos	M	Significant ↑ (y-969%, o-409%) of HSP72 expression in trained leg in both groups following SSC protocol
			O- 30 mos		Significant ↑ (y-943%, o-420%) in HSP25 expression in trained leg in both groups following SSC protocol
NF-κB					
Rivas et al, 2012	Acute bout of RE	Pre-RE, immediately post and 6 hr post-RE	Y- 22±0.6	M	At all time points old expressed significantly ↑ levels of protein and phosphorylated NF-κB
			O- 74±1.5		
Thalacker-Mercer et al, 2010	Acute bout of RE	Pre-RE and 24 hr post-RE	Y- 37±1	M/F	At baseline, old expressed significantly higher protein levels- 26% higher
			O- 73±1		

* Indicates Animal Study

2.6 Ubiquitin/Proteasomal Pathway

The ubiquitin/proteasomal pathway (UPP) is involved in intracellular protein degradation (Glickman & Ciechanover, 2002). Protein degradation that occurs via this pathway is mediated by ubiquitin ligases and there are specific ligases for skeletal muscle (Reid, 2005). It has been observed that at baseline older women had significantly higher muscle-RING-finger protein 1 (MuRF-1) and forkhead box (FOXO3A) mRNA expression. Following an acute bout of RE older women had significantly higher mRNA levels of atrogin-1, but there was no change in young women. Both young and older women displayed increased MuRF-1 mRNA expression (Raue, Slivka, Jemiolo, Hollon, & Trappe, 2007). This difference in expression both at rest and following RE in the older adults may be involved in the muscle loss associated with sarcopenia.

2.7 MicroRNA

MicroRNAs (miRNAs) can regulate gene expression by posttranscriptional modification (Valencia-Sanchez, Liu, Hannon, & Parker, 2006). A study conducted by Drummond and colleagues showed that at baseline older males have significantly higher levels of primary miRNA expression. Following RE and essential amino acid supplementation both primary and mature miRNA expression was altered in the young men, but not old (Drummond et al., 2008). This different response between the two groups strengthens the hypothesis that young and old adults do not respond to RE in the same manner.

2.8 Serum Hormones

The hypertrophic response to RE has many contributing factors. The endocrine system is one of the better-known contributors to muscle hypertrophy because of the important role that hormones play in muscle protein synthesis and remodeling (Crewther, Keogh, Cronin, & Cook, 2006). Circulating levels of hormones such as growth hormone (GH) and testosterone have been studied in old and young adults to determine if there are differences in expression as a result of RE. It has been found that immediately following a single bout of RE both groups had a significant increase in GH. These studies also showed that the young group had significantly higher levels of growth hormone than the old following RE (Craig, Brown, & Everhart, 1989; Roberts et al., 2009; Smilios, Pilianidis, Karamouzis, Parlavantzas, & Tokmakidis, 2007).

Changes in GH were also examined in response to a 12-week progressive RT program and it was found that both groups showed a significant increase in GH following an acute bout of RE with the young group expressing significantly higher levels of GH than the older group (Craig et al., 1989).

Free testosterone has also been examined as a hormonal marker of response to RE. One study reported that young men had significantly higher levels of free testosterone before exercise, 5 minutes post-RE and 24 hours post-RE, but here were no changes in the levels of free testosterone in old men (Roberts et al., 2009), but another study found no differences at baseline and significant increases of serum testosterone immediately following RE in both groups (Smilios et al., 2007).

Petrella and colleagues examined differences in free testosterone levels following a 16-week progressive RT program. It was found that young men had 59%

more free testosterone than older men at baseline, but no changes were observed following the RT program (Petrella et al., 2006).

2.8.1 Summary of Serum Hormones

In studies that that examined both circulating levels of growth hormone and testosterone, young men had greater response to exercise stimulus than older men (Table 2.8). This finding provides further evidence that there is an attenuated serum growth hormone and testosterone response to resistance exercise in older adults.

Table 2.8 Studies that compare differences in serum hormones between young and old adults

Author(s)	Type of Intervention	Sample Collection Timing	Age (yrs)	Gender	Results
Growth Hormone					
Craig et al, 1989	Acute and 12 week RT Program	Pre-RE, immediately post and 15 minutes post- RE	Y- 23.2±1.5	M	Following acute RE both groups significantly ↑ expression
			O- 62.8±0.7		Following long term RT both groups had non-significant ↑ in baseline expression (y- 45%, o- 3%)
Roberts et al, 2009	Acute bout of RE	Pre-RE and 5 minutes post- RE	Y- 21.0±0.6	M	Both groups significantly ↑ from baseline
			O- 67.6±1.3		Young had significantly ↑ expression than the old group
Smilios et al, 2007	Acute bout of RE	Pre-RE, immediately post and 15 minutes post- RE	Y- 23±1	M	Both groups significantly ↑ from baseline at both time points
			O- 69±5		Young had significantly ↑ expression than the old group
Testosterone					
Craig et al, 1989	Acute and 12 week RT Program	Pre-RE, immediately post and 15 minutes post- RE	Y- 23.2±1.5	M	Basal levels of testosterone ↓ in both groups following long term RT (not significant)
			O- 62.8±0.7		Testosterone ↑ (not significant) in both groups following acute RE
Petrella et al, 2006	16 week RT Program	Before start of training and after 16 weeks of RT	Y- 20-35	M/W	No differences in total testosterone between young and old men
			O- 60-75		Free testosterone was significantly↑ in young men (59%)

2.9 Muscle Contractile Proteins

It has been hypothesized that some of the decrease in muscle mass and strength in the older population is a result of decreased amounts of muscle contractile proteins, specifically myosin heavy chain (Balagopal, Schimke, Ades, Adey, & Nair, 2001).

One study that examined MHC I and MHC IIa gene expression found that of the genes examined following an acute bout of RE 463 genes changed expression in young women compared to a change in 63 genes observed in the older women (Raue et al., 2012). Following a 12 week RT program, it was found that only 12 genes were differently expressed in young women, while 144 genes changed expression in the older women (Raue et al., 2012). In another study that also examined MHC isoforms following high RE, it was shown that older men expressed a lower percentage of MHC II isoforms than young men (Hameed et al., 2003). A study by Roberts and colleagues examined expression of embryonic MHC expression and found no significant differences between the young and older groups at baseline or 24 hours following RE. However, there was a trend toward decreased expression in the young group that failed to reach statistical significance ($p=0.06$) (Roberts et al., 2009). These findings support the hypothesis that gene transcription in response to RE is attenuated in old adults compared to.

2.10 Myofibrillar Protein Synthesis/ Fractional Synthesis Rate

There have been studies that have examined muscle protein synthesis rate (Brock Symons, Sheffield-Moore, Mamerow, Wolfe, & Paddon-Jones, 2011; Kumar et al., 2009; Mayhew et al., 2009; Schulte & Yarasheski, 2001; Welle, Thornton, & Statt,

1995; Yarasheski, Zachwieja, & Bier, 1993) and studies that have looked at muscle protein breakdown (Fry et al., 2013) to determine if there are differences in the rate of protein synthesis and degradation that may contribute to the attenuated hypertrophic response observed in older adults compared to their young counterparts.

There is some disagreement among these studies regarding the difference in FSR at baseline with some showing the older group significantly lower than the young (Schulte & Yarasheski, 2001; Welle et al., 1995; Yarasheski et al., 1993) and others showing no difference between the two groups (Brock Symons et al., 2011; Drummond et al., 2008; Kumar et al., 2009; Mayhew et al., 2009). The studies that examined the difference in FSR following an acute bout of RE also had varying findings. Two studies showed a significant increase in the FSR of the young group, but not the old (Kumar et al., 2009; Mayhew et al., 2009), another showed a significant increase in both groups, but the response of the older group was delayed (Drummond et al., 2008), and the last showed a significant increase in both groups (Brock Symons et al., 2011). The authors of the last study did speculate that this result might have been a result of their screening procedures that excluded older individuals who may have been more representative of the general population.

Two studies examined changes in the FSR following a 2 week RT program and both showed that at the end of the program the FRS for both groups was the same (Schulte & Yarasheski, 2001; Yarasheski et al., 1993).

Another group examined differences in FSR in young and older adults over the course of a 3-month RT program. It was found that at baseline the FSR of the older

group was 30% lower than that of the young group, and following training it was still 27% lower than the young group (Welle et al., 1995).

Muscle protein breakdown (MPB) was examined in young and aged adults following a single bout of resistance activity in order to determine if there were differences in this response that may influence muscle protein synthesis. No differences in the MPB response between young and older adults were found, but the authors noted that it is possible that they missed peak increases due to the biopsy time course used (Fry et al., 2013).

2.10.1 Summary of Myofibrillar Protein Synthesis/ Fractional Synthesis Rate

There is not overwhelming agreement in the data regarding age-related changes in the balance of protein synthesis and breakdown following resistance exercise. Much of the disagreement in the data is likely a result of different time points at which the changes were examined (Table 2.9).

Table 2.9 Studies that compare differences in MPS/FSR between young and old adults

Author(s)	Type of Intervention	Sample Collection Timing	Age (yrs)	Gender	Results
MPS/FSR					
Yarasheski et al, 1993	2 week RT Program	BL and post- 2 week RT	Y-(F) 24±1 (M) 24/24	M/F	FSR of MPS ↓ in old at baseline
			O- (F) 60/73 (M) 63±1		FSR of the old group ↑ significantly more than the young- both ↑ significantly over BL
Schulte & Yarasheski, 2001	2 week RT Program	BL and post- 2 week RT	Y- 23-32	M/F	FSR for MHC and mixed muscle protein ↓ in old at baseline
			O- 78-84		FSR for MHC and mixed muscle protein ↑ in both groups following RT
Welle et al, 1995	3 month RT Program	BL and post- 3 month RT	Y- 22-31	M/F	FSR of the old group significantly ↓ than the young at BL
			O- 62-72		Old still 27% ↓ than young post-RT
Mayhew et al, 2009	Acute bout of RE	BL and 24 hrs post-RE	Y- 27.9±1	Not Specified	FSR significantly ↑ in young 24 hr post-RE, no change in old
			O- 64.4±0.9		
Kumar et al, 2004	Acute bout of RE	BL and every 30 min for 4 hr post-RE	Y- 24±6	M	FSR significantly ↑ in young 1-2 hr post-RE, no change in old
			O- 70±5		
Brock Symons et al, 2011	Acute bout of RE	BL and 40 min post-RE	Y- 29±3	M/F	Both groups significantly ↑ FSR following RE
			O- 67±2		
Drummond et al, 2008	Acute bout of RE	BL, 1, 3, and 6 hrs post-RE	Y- 29.7±1.7	M	Both groups significantly ↑ FSR following RE, but response in old group was delayed
			O- 70±2.1		
MPB					
Fry et al, 2013	Acute bout of RE	BL, 3, 6, and 24 hrs post-RE	Y- 27±2	M/F	No differences in MPB were observed between the young and old groups
			O- 70±2		

2.11 Satellite Cells

Satellite cells are mononucleated cells that function as a source of nuclei for myocytes (Moss & Leblond, 1971). The activity of these cells following RE has been examined as a potential point of divergence in response of between young and aged adults to such activity. In a study conducted by Walker and colleagues, the number of satellite cells present in each of the groups was not different at baseline, but 24-hours following a single bout of RE young men showed a significant increase in Pax7+ cells as compared to young women and both older men and women (Walker et al., 2012). Another study found no difference between the groups at baseline, but following 9 weeks of RT all groups significantly increased the proportion of satellite cells present (Roth et al., 2001).

2.12 Conclusions

Based on the available data, evidence suggests that young and old adults do not respond in the same manner to either acute RE or chronic RT. In many areas of response outlined in this review it appears that older adults do respond to the resistance stimuli however, the robustness of the outcome is attenuated compared to the response of young adults. This appears to be the case when examining both performance-based outcomes (ie. 1RM, muscle or fiber size) and gene and protein expression outcomes. These discrepancies in the response to exercise indicate that young adults receive more benefit from both acute and chronic resistance exercise.

Some of the studies presented in this review do show a more robust response in the older group following exercise. However, when these studies are examined more

closely it appears that often the age range or mean age of the participants in the old group is in the low 60s. It is possible that the impairment observed in the other studies with older “old” participants is not present until individuals reach their seventh decade.

It was also observed that the muscles being exercised in some of the groups that did not follow the trend of attenuation in the older group were not muscles that are used often or would be expected to experience a large degree of atrophy, such as the abductor digiti minimi.

One possible explanation for the differences in hypertrophic response could be varying levels of effort during resistance training interventions. Similar results in both animal and human studies suggest that this is not the cause of the diminished hypertrophic response in old adults. Therefore, the difference in hypertrophic response between young and old adults outlined in this review likely indicates that there may be some internal factor influencing the hypertrophic response. Few studies have proposed a mechanism for the age-related differences in response to exercise. More research is needed to determine what this may be and whether it can be manipulated to restore the hypertrophic response of older adults to that similar to young adults.

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Chapter 3

Study 1: Old and Young Women Respond Differently to an Acute Bout of Resistance Exercise

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3.1 Abstract

Sarcopenia, the loss of muscle mass and strength with age, is highly prevalent in the older adult population. However, the mechanism for how aging leads to the loss of muscle mass and strength is not fully understood. One potential mechanism is reduced ability of skeletal muscle to detect mechanical stimuli induced by contractile activity ,or mechanosensation. If less mechanical stimuli is being sensed by the muscle for a given activity, the hypertrophic response to this activity will be diminished compared to the response in those without impaired mechanosensation. The primary purpose of this study was to examine the hypertrophic response of old and young women to a single bout of lower extremity resistance exercise. The secondary purpose of this study was to establish a protein that can be used to measure differences in the hypertrophic response within 30 minutes of a single bout of resistance exercise. We hypothesized that there would be a less robust response to the exercise bout near the time of the stimulus in the older adults than the young adults.

The participants for this study were 8 healthy young women (24±3) and 8 healthy aged women (74±6). Participants completed a single unilateral bout of resistance exercise to maximally engage the plantarflexors. Muscle biopsies were immediately collected from the lateral gastrocnemius, bilaterally. Western blot analyses were conducted to examine changes in p70S6k, Akt, and Focal Adhesion Kinase (FAK) phosphorylation levels both between the exercised and control limbs and between groups. No difference was found between groups at in the control limb, but the young had a significant increase following exercise in both p70S6k and Akt phosphorylation following exercise, but no change in FAK. The old group showed no significant change

in any proteins examined. The young showed a significantly greater p70S6k response than the old group. This study shows that there is a differential response in young and old women immediately following an acute bout of resistance exercise within 30 minutes of an acute bout of resistance exercise. The timing of this response lends more evidence that the cause of sarcopenia may be associated with decreased mechanosensation by skeletal muscle.

3.2 Introduction

Sarcopenia, the age-related loss of muscle mass and strength, is a commonly observed phenomenon in older adults (Baumgartner, Stauber, McHugh, Koehler, & Garry, 1995; Baumgartner et al., 1998; Rosenberg, 1997). Studies have shown that there are myriad consequences from the loss of muscle mass and strength that adversely impact older adults. Decreased muscle mass has been associated with increased incidence of functional impairment and disability in both men and women (Janssen et al., 2002). Individuals with a decline in muscle mass and strength are at greater risk of adverse consequences. The ability to live independently is impaired and quality of life has been shown to decrease significantly (Ozcan et al., 2005). Specifically, the loss of strength due to sarcopenia is associated with slower walking speeds, decreased grip strength, balance impairment, increased instance of falls and fractures, and higher mortality risk (Fujita et al., 1995; Laukkanen, Heikkinen, & Kauppinen, 1995; Rantanen, 2003; Short & Nair, 2001).

It is estimated that the prevalence of sarcopenia is greater than 50% in adults over the age of 80 (Baumgartner et al., 1998). According to the 2010 census, the population of adults over the age of 65 is growing at a faster rate than the population under the age of 45 (Howden & Meyer, 2010). This means that the population at risk of developing sarcopenia is growing and presents as one of the health concerns that must be addressed with urgency.

Resistance training is an effective intervention for individuals seeking means to combat age-related muscle mass loss and improve function. Some studies have shown remarkable success with improving function in older adults (Fiatarone et al., 1990).

However, studies have consistently shown that older adults have a diminished response to chronic exercise training when compared to their younger counterparts (Bickel et al., 2011; Cutlip et al., 2006; Greig et al., 2011b; LaRoche et al., 2008; Lemmer et al., 2000; Mayhew et al., 2009; Raue et al., 2009; Roth et al., 2000). One example is a study that followed young and old women participating in a 12 week high-intensity resistance training program. The older women showed improvement in 1-repetition maximum strength (1RM), but the increase was less than that seen in the young women tested. The young women saw a 36% increase in 1RM from baseline while the older women only improved their 1RM 26% over baseline. Additionally, there was no increase in muscle cross-sectional area or type I or IIa muscle single-fiber function in the older women (Raue et al., 2009). Another study with both men and women found that after completion of a 16-week resistance training program there was less hypertrophy in the older compared to younger subjects. It was also found that there was an increase in myogenic regulatory factors in the young group that were absent in the older group (Kosek et al., 2006).

In studies that have examined differences in the acute response to resistance exercise a diminished response has been observed in the older adults at different points in the signaling cascade. Both positive and negative regulators of muscle protein synthesis have been studied at various time points following acute resistance exercise. The responses observed in these studies have been inconsistent. Much uncertainty remains as to the exact response, but results have consistently shown an altered response to acute response between young and old adults with the older adults typically responding less robustly (Dalbo et al., 2011; Dennis et al., 2008; Drummond et al.,

2008; Drummond et al., 2009; Greig et al., 2011b; Kosek et al., 2006; Raue et al., 2006; Rivas et al., 2012).

This variable, yet attenuated response to exercise observed in the aged population may indicate that the hypertrophic response is impaired during long-term resistance exercise training. However, it is unclear at what level the impairment occurs. It is likely that all signaling past the point of impairment will be attenuated. We reasoned that reduction in attenuated response to resistance exercise might be a result of reduced ability of muscles to detect mechanical stimuli (mechanosensation) (Figure 3.1). The exact identity of mechanosensor that responds to contraction is elusive, but evidence to suggest that Focal Adhesion Kinase (FAK) is phosphorylated very quickly following mechanical stimulation (Klossner, Durieux, Freyssenet, & Flueck, 2009; Rice et al., 2007).

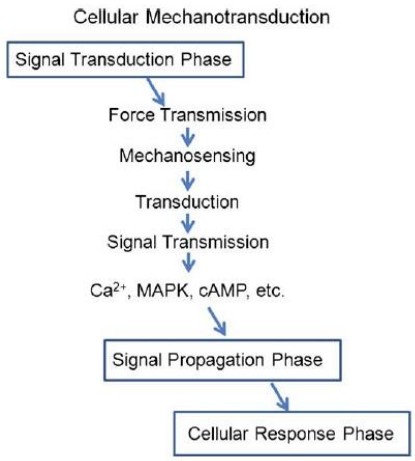


Figure 3.1 Basic schematic of the mechanotransduction cascade in skeletal muscle (Wu et al., 2011)

One of the potential downstream effectors of FAK are molecules that are implicated in protein translation during muscle hypertrophy with RE. p70S6k has been identified as a critical player in the regulation of protein synthesis and hypertrophy (Baar & Esser, 1999; Bodine et al., 2001; Nader & Esser, 2001; Terzis et al., 2008). However, it appears that p70S6k is differentially activated by different types of stimulation (Burry, Hawkins, & Spangenburg, 2007; Nader & Esser, 2001). It has previously been established that p70S6k is a downstream target for the Akt/mTOR pathway for muscle protein synthesis and hypertrophy (Bodine et al., 2001; Hornberger

et al., 2003; Pallafacchina, Calabria, Serrano, Kalhovde, & Schiaffino, 2002). However, a more recent study demonstrated that p70S6k is activated following mechanical stimulation in a manner that is mediated by Focal Adhesion Kinase (FAK) and appears to be Akt-independent (Klossner et al., 2009). The Klossner study suggests that there may be an alternative pathway involved in muscle protein synthesis regulation that is more responsive to acute mechanical stimuli.

The purpose of this study was to examine whether FAK, Akt, and p70S6k phosphorylation induced by a single bout of lower extremity resistance exercise is attenuated in old compared to young women within 30 minutes of the exercise. The secondary purpose of this study was to establish a protein that can be used to measure differences in the hypertrophic response within 30 minutes of a single bout of resistance exercise. We hypothesized that there would be a less robust response to the exercise bout in the older adults than the young adults.

3.3 Methods

3.3.1 Participants

Sixteen healthy, female participants were recruited to participate in this study. The participants were either in the young group (24±3, *n*=8) or the aged group (74±6, *n*=8) (Table 3.1). All participants completed a health questionnaire prior to being accepted for participation to ensure that all inclusion criteria were met and that they were free of all exclusion criteria including neurological disorders, lower extremity osteoarthritis, peripheral artery disease, cancer, diabetes, and cardiac disease. Only females were recruited to participate in this study because there was to be a small

sample size and it was unclear whether gender may play a role in the response to resistance exercise. At this time, women are living to older ages than men, thus this research has greater applicability to the female population. All participants read and signed an informed consent form prior to beginning any of the experimental protocol. The East Carolina University Institutional Review Board approved all experimental procedures.

Table 3.1: Demographic information for study participants. All values are given as mean ± standard deviation.

	n	Age	Height (in)	Weight (lbs)
Young	8	24±3	66.4±3.6	144.5±20.9
Old	8	74±6	61.6±2.5	150.8±29.7

3.3.2 Experimental Protocol

All study participants reported to the East Carolina University Biomechanics Lab to complete a resistance exercise protocol with their right leg using the isokinetic dynamometer (HUMAC NORM Testing & Rehabilitation System, CSMI Medical Solutions, Stoughton, MA). The gastrocnemius muscle was selected as the muscle in which response to exercise would be examined. The exercise bout consisted of 3 sets of 10 repetitions of both isometric and isokinetic maximal ankle plantarflexion. Isokinetic repetitions were performed at 60 degrees per second. Participants were positioned supine with knees and hips in anatomical position for these exercise sets. Participants also completed 3 sets of 10 repetitions of maximal isometric and isokinetic knee flexion. Participants were positioned supine with knee in full extension for ankle repetitions. Isometric repetitions were conducted with the ankle in neutral position (ankle angle of 0 degrees of flexion). For all knee flexion trials, participants were in a seated position with both hips and knees flexed to 90 degrees. All isometric repetitions were performed at a knee angle of 90 degrees of flexion. Both ankle plantarflexion and knee flexion were included in this exercise protocol to target both actions of the gastrocnemius muscle group. Participants were allowed to rest between each set of all exercises. When all exercise sets were complete participants were transported to the East Carolina Heart Institute for gastrocnemius muscle biopsies.

Two muscle biopsies were collected from each participant. The procedure involved the administration of local anesthesia (1% lidocaine), a small incision, and sample extraction from the mid portion of both the right and left lateral gastrocnemius using a 4-mm Bergstrom biopsy needle. All muscle biopsies were collected within 30

minutes of the resistance exercise bout. Muscle samples were immediately frozen in liquid nitrogen for later analysis and stored at -80° C.

3.3.3 Western Blotting

Muscle samples were homogenized using a ground glass homogenizer (Glas-Col, Terre Haute, IN) in a buffer of 10 mM HEPES (pH 7.4), 125 mM sucrose, 1 mM EDTA, 10 mM $Na_4P_2O_7 \bullet 10H_2O$, 10 mM β-glycerophosphate, 2 mM NaF, 1 mM Na_3VO_4, and commercially prepared protease inhibitor cocktail (Sigma, St. Louis, MO). Homogenization was done on ice. Total protein content of each sample was determined using a BCA protein assay kit (Pierce, Rockford, IL) (Ebben, 2009). Protein homogenates were diluted to in a loading buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 100 mM DTT, 0.02% bromophenol blue). Homogenates in loading buffer were heated at 100° C for 3 minutes.

Proteins were separated by electrophoresis. 50 µg of protein from each homogenate and a protein homogenate used as a standard were loaded into a 10% sodium doecyl sulfate-polyacrylamide gel. Proteins were transferred to a PVDF membrane for 4 hours at a temperature of 4° C in standard transfer buffer. PVDF membranes were used because of their durability to withstand reprobing (Yeung & Stanley, 2009).

To probe for phosphorylated p70S6k, membranes were blocked in 5% nonfat dry milk in TBS-T (10 mM Tris-HCl (pH 7.3), 150 mM NaCl, 0.05% Tween 20) at room temperature for 1 hour. After blocking, membranes were incubated in phospho-p70S6k (Thr 389) (1:1000) (Cell Signaling) diluted in 5% milk in TBS-T at 4° C overnight. After washing in TBS-T, membranes were incubated 1-hour in anti-rabbit secondary antibody (1:2500) conjugated with horseradish peroxidase (HRP) at room temperature. HRP activity was visualized using enhanced chemiluminescence solution (GE Healthcare,

Piscataway, NJ) and exposure to autoradiographic film (Blue Devil Film, Genesee Scientific, San Diego, CA).

The membrane was stripped in 62.5 mM Tris-HCl (pH 6.7), 2% SDS and 100 mM 2-mercaptoethanol (reducing agent) at 60° C for 30 minutes to remove phospho-p70S6k antibody. Membranes were once again blocked in 5% nonfat dry milk in TBS-T at room temperature for 1 hour. After blocking, membranes were incubated in total p70S6k antibody (1:1000) (Cell Signaling) diluted in 5% milk in TBS-T at 4° C overnight. After washing in TBS-T, membranes were incubated 1-hour in anti-rabbit secondary antibody (1:2500) conjugated with HRP at room temperature. HRP activity was visualized using enhanced chemiluminescence solution and exposure to autoradiographic film. Subsequent probing with additional antibodies for total Akt and p-Akt (Cell Signaling) was conducted and imaged in a similar manner. Methods for total FAK (Millipore) and p-FAK (Cell Signaling) probing were the same except that 10% polyacrylamide gels were used. All films were imaged via transmissive scanning with a HP ScanJet G4050 (Hewlett-Packard, Palo Alto, CA). All films were analyzed with SigmaScan Pro 5.0 (Jandel Scientific, Systat, Point Richmond, CA). All of the films were analyzed by averaging three analyses of each film. A standard protein was loaded on each gel and the intensity values for each film were normalized to the intensity of the standard protein. In order to compare the arbitrary units across all participants the numerical values presented are the ratio of phosphorylated protein to total protein in the control limb, ratio of phosphorylated protein to total protein in the exercised limb, and the overall ratio, which is the ratio from the exercised limb over the ratio from the control limb.

3.3.4 Statistical Analysis

Statistical analyses were conducted using MatLab (MathWorks, Natick, MA.). Differences between the young and old adults groups at baseline were determined using a two sample, two-tailed t-test. Due to the fact that response to the exercise stimuli was hypothesized to be reduced in older adults the remainder of the statistical analyses were one-tailed tests. One-tailed, paired sample t-tests were conducted to quantify differences present between the exercised and control limbs. This was used to determine whether the old adults exhibited an attenuated response to the acute resistance exercise stimuli as compared to the young adults. One-tailed chi-squared analyses were also conducted to determine whether there is a difference in the proportion of responders in the young and old groups for each protein. Responders were defined as participants who exhibited positive changes in phosphorylation following the bout of resistance exercise. An a priori level of $p<0.05$ was used to determine statistical significance for all comparisons. In accordance with the recommendations made by Curran-Everett and Benos, p-values that are around 0.10 will be discussed as trends (Curran-Everett & Benos, 2004).

3.4 Results

3.4.1 FAK

There were no differences between the young and old adults in the control limb regarding the ratio of phosphorylated to total FAK ($p=0.86$) (Table 3.4), nor were there any statistical changes observed following exercise in either the young ($p=0.34$) or old groups ($p=0.07$) (Figure 3.3). The response of the older adults trended toward

significance, but the change was small. There was also no significant difference between the proportion of responders and non-responders between the two groups with 4 of 8 responding in the young group and 6 of 8 responding in the old group (chi-squared= 0.291, p=0.29).

Table 3.2: This table shows the ratio of p-FAK to total FAK in both the unexercised (control) limb and the exercised limb as well as the ratio comparing the two. All values are given as mean ± standard deviation.

	Control Limb (p-FAK/total)	Exercised Limb (p-FAK/total)	Overall Ratio (Exercised/Control)
Young	0.334±0.079	0.345±0.110	1.033±0.208
Old	0.382±0.087	0.417±0.088	1.111±0.168

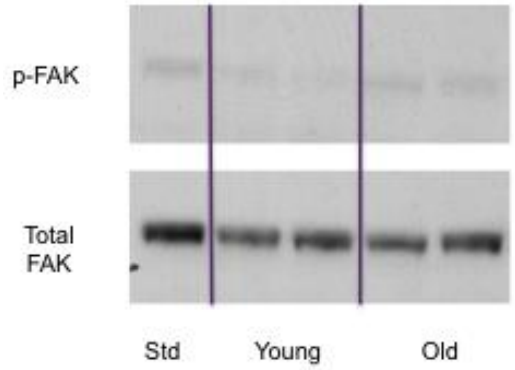


Figure 3.2 Representative blot for both p-FAK and total FAK

3.4.2 Akt

There were no differences between the young and old adults in the control limb regarding the ratio of phosphorylated to total Akt (p=0.41) (Table 3.3). Following exercise, the young adults showed a significant increase in the proportion of phosphorylated to total Akt (p=0.01) over the control limb, but there was no change in the old group (p= 0.56) (Figure 3.2). There was also no significant difference between the proportion of responders and non-responders between the two groups, however there was a trend toward more responders in the young group than the old with 8 of 8 young responding and 6 of 8 old responding (chi-squared= 2.286, p=0.07).

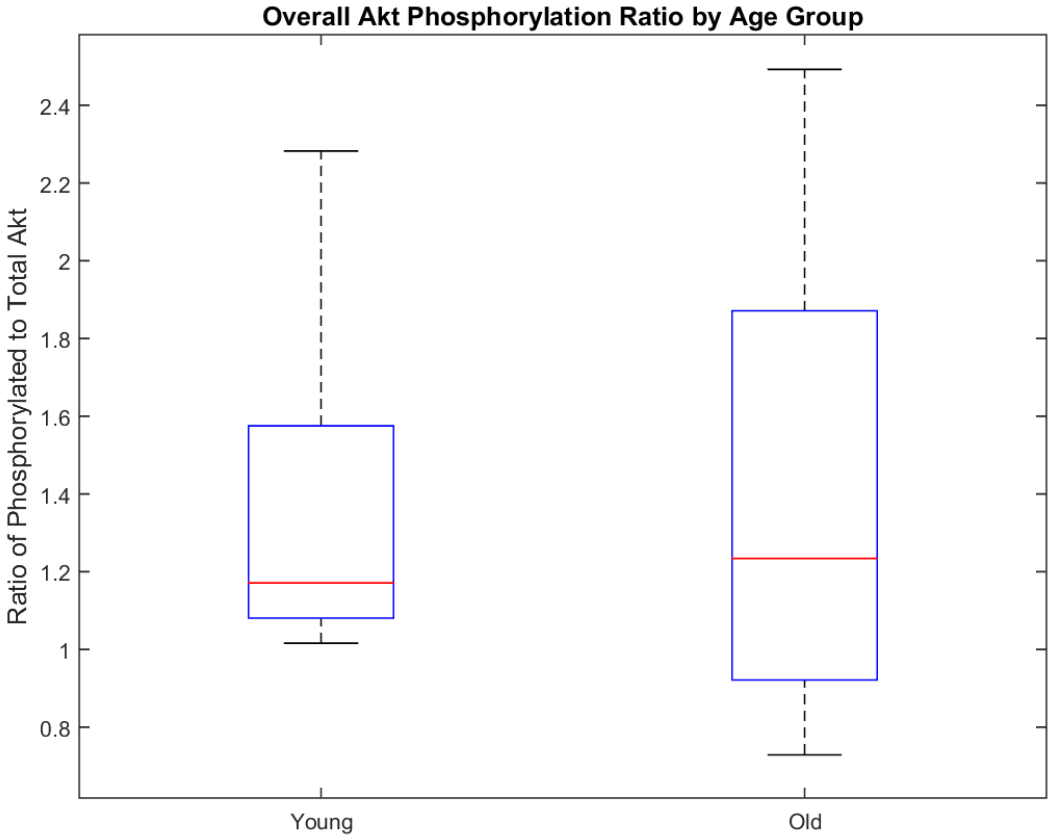


Figure 3.3: This represents the overall ratio of p-Akt/total Akt in the control limb to p-Akt/total Akt in the exercised limb. The line in the box represents the median value for the group represented. The top line of the box represents the 1st quartile and the bottom line of the box represents the 3rd quartile. The value represented by the line extending from the top of the box represents the maximum value for the group while the line extending from the bottom represents the minimum value for the group. The median and quartile values for the old group were calculate without the outlier represented by the cross at the top of the plot.

Table 3.3: This table shows the ratio of p-Akt to total Akt in both the unexercised (control) limb and the exercised limb as well as the ratio comparing the two. All values are given as mean ± standard deviation.

	Control Limb (p-Akt/total)	Exercised Limb (p-Akt/total)	Overall Ratio (Exercised/Control)
Young	1.179±0.743	1.489±0.735	1.369±0.455
Old	1.103±0.743	1.403±0.704	1.409±0.638

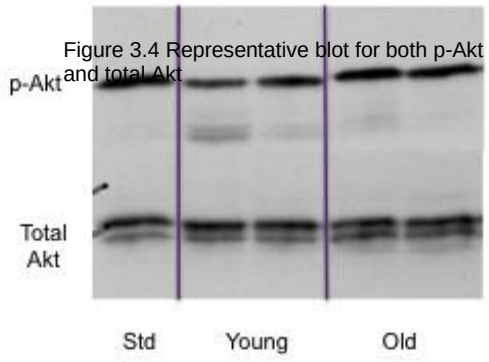


Figure 3.4 Representative blot for both p-Akt and total Akt

3.4.3 p70S6k

It was determined that there were no differences between the young and old adults in the control limb regarding the ratio of phosphorylated to total p70S6k (p=0.22). Following exercise, the young adults showed a significant increase in the proportion of phosphorylated to total p70S6k (p=0.03) over the control limb. The old adults showed no significant increase in the proportion of phosphorylated to total p70S6k over the control limb (p=0.13). There was also no significant difference between the young and old groups following exercise (p=0.16). However, upon further analysis one of the older adults was determined to be an outlier based on the Grubbs test for outliers. When the statistical analysis was recalculated excluding the outlier in the old adult data set the difference in response between groups was significant (p=0.02) (Figure 3.1) with the young group exhibiting a more robust response than the old group. There were 8 of 8 responders in the young group and 5 of 8 in the old group. The chi-squared statistic to determine whether there was a difference in the proportion of responders between the two groups was 3.692, which is significant (p=0.03) (Table 3.2).

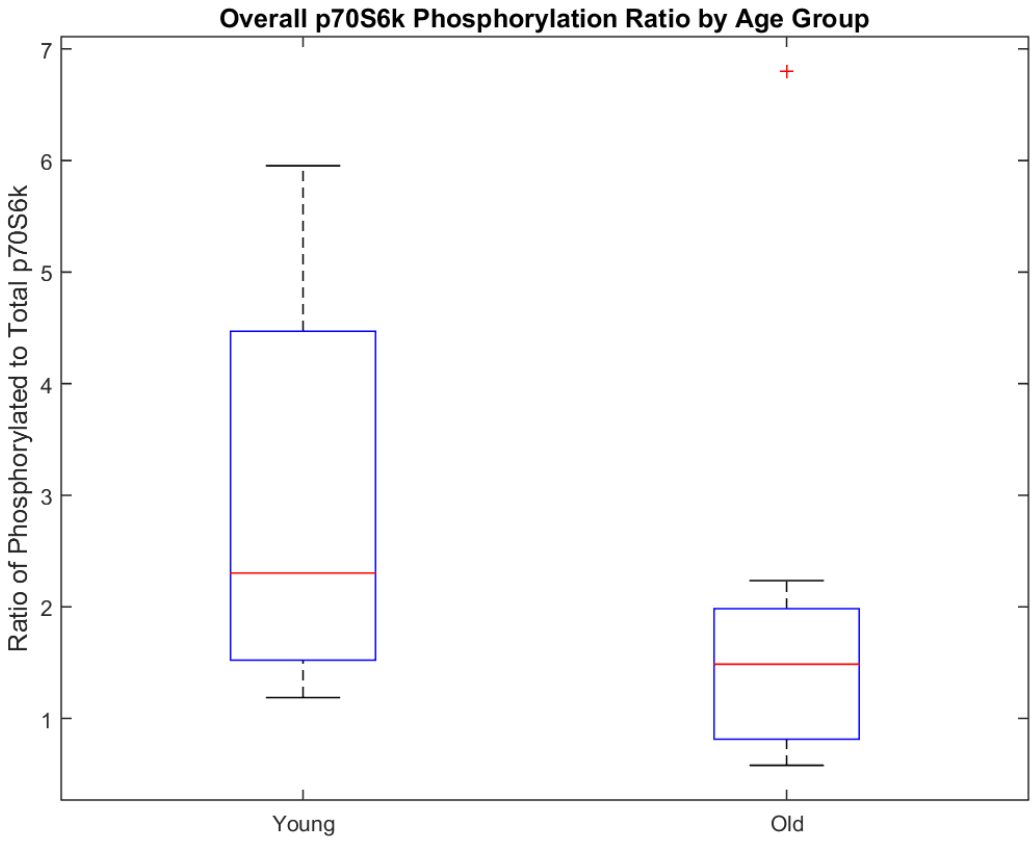
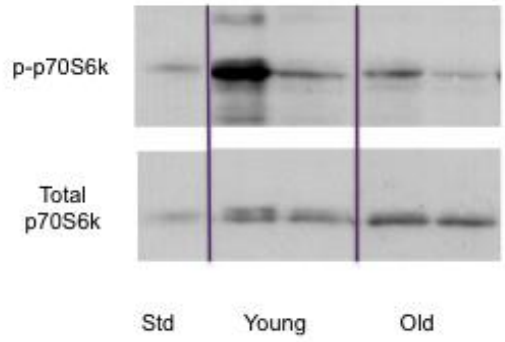


Figure 3.5: This represents the overall ratio of p-p70S6k/total p70Sk6 in the exercised limb to p-p70S6k/total p70Sk6 in the control limb. The line in the box represents the median value for the group represented. The top line of the box represents the 1st quartile and the bottom line of the box represents the 3rd quartile. The value represented by the line extending from the top of the box represents the maximum value for the group while the line extending from the bottom represents the minimum value for the group. The median and quartile values for the old group were calculate without the outlier represented by the cross at the top of the plot.

Table 3.4: This table shows the ratio of p-p70S6k to total p70S6k in both the unexercised (control) limb and the exercised limb as well as the ratio comparing the two. All values are given as mean ± standard deviation. The mean given for the old group included the outlier.

	Control Limb (p-p70S6k/total)	Exercised Limb (p-p70S6k/total)	Ratio (Exercised/Control)
Young	0.347±0.194	1.123±1.059	2.966±1.680
Old	0.247±0.068	0.507±0.589	1.993±1.889

Figure 3.6 Representative blot for both p-p70S6k and total p70S6k



3.5 Discussion

We hypothesized that there would be a less robust response to the single bout of resistance exercise in the older adults than in the young adults. Based on the phosphorylation results of the p70S6k, this hypothesis is supported.RE increased in Akt phosphorylation in the young over the control limb, but no changes were seen in phosphorylation with the old group. No changes in FAK were observed in either group. p70S6k phosphorylation was selected as a marker of response to exercise because it has been previously associated with increased muscle mass following a long-term intervention in animals (Baar & Esser, 1999). As previously stated, it is well established that p70S6k is a downstream target of the Akt/mTOR pathway that is involved in muscle protein synthesis and hypertrophy (Bodine et al., 2001; Hornberger et al., 2003; Pallafacchina et al., 2002). However, in studies that have examined p70S6k phosphorylation in the context of the Akt/mTOR pathway it has been shown that this phosphorylation event is observed 3 hours following the stimuli and remains elevated from baseline for up to 36 hours (Baar & Esser, 1999). A study by Klossner and colleagues showed that in a rodent unloading-reloading model there was p70S6k phosphorylation without preceding Akt phosphorylation indicating that there may be a mechanotransduction pathway that stimulates muscle protein synthesis and hypertrophy that is outside of the Akt modulated mTOR pathway (Klossner et al., 2009). This same study also showed that there was FAK phosphorylation that preceded the observed p70S6k phosphorylation. Another recent study also found p70S6k phosphorylation to be elevated in young adults 30 minutes following a bout of resistance exercise. This study also a significant increase in the phosphorylation of p70S6k in older

adults at this time point, if they had ingested a protein-rich beverage (Francaux et al., 2016). This indicates that p70S6k phosphorylation may be triggered by two different mechanisms.

Similar to the findings of Klossner and colleagues, we observed that there was increased p70S6k phosphorylation in the young group following exercise, but unlike their findings there was no significant increase in phosphorylated FAK in either group following exercise. The time between the introduction of the mechanical stimuli was shorter in our model, but it is possible that because they employed a rodent hind limb unloading-reloading model the stimuli was more intense than a single bout of resistance exercise. There was an increase in Akt phosphorylation in the young groups following the bout of exercise, which appears to indicate that the observed p70S6k phosphorylation response may be Akt modulated. It is possible that, although the muscle biopsies were harvested within 30 minutes of the exercise, we missed the time point with the most robust FAK phosphorylation response that results from mechanostimulation.

We are able to see an altered response between the young and old groups within 30 minutes of the resistance exercise stimuli. This indicates that the impairment is likely upstream of the muscle protein synthesis cascade. Decreased mechanosensation is a probable cause for the attenuated p70S6k phosphorylation observed in the old group. It is possible that increased muscle stiffness causes the muscle to register less strain for any stimuli, thereby attenuating the hypertrophic response.

One limitation regarding the muscle biopsies is that only a single biopsy was taken. It is possible that had repeat biopsies been collected the older adults would have

exhibited a more robust response to the exercise stimuli. It is possible that there is an alteration in the time course for the response to resistance exercise in older adults. This study enrolled only female participants because there is evidence to suggest that men and women respond differently to long-term resistance exercise and we did not want to confound the data by introducing a possible gender effect (Bamman et al., 2003a). Another potential limitation of this study is that the control muscle biopsy and exercised muscle biopsy were taken from different limbs. This assumes that both limbs are in the same basal state and that stiffness is similar bilaterally. This design was still selected rather than taking repeat biopsies from the same limb because we wanted to ensure that all participants were able to give a true maximal effort during the bout of resistance exercise and not be limited by pain or concern of injuring themselves.

Overall, the results of this study indicate that there is an attenuated response to resistance exercise in older women than younger women following a single bout of resistance exercise. Based on the results of this study, it was also determined that it is reasonable to use p70S6k phosphorylation as a marker for response to an acute bout of resistance exercise in human study participants. Future work in this area should work to determine the mechanoreceptor in skeletal muscle. Additional studies that examine how changes in the material environment of skeletal muscle, possibly increased muscle stiffness, impact the robustness of the hypertrophic response.

3.6 Acknowledgements

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Chapter 4

Study 2: Impact of a Long-term Stretching Intervention on Acute Response to Resistance Exercise in Aged Women

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4.1 Abstract

Sarcopenia is a growing public health concern. One means to combat the loss of skeletal muscle and strength associated with sarcopenia is by having older adults engage in resistance exercise. However, it has been shown in a number of studies that older adults have a less robust hypertrophic response to resistance exercise than young adults. One factor that may be impacting this altered response in older adults is increased levels of muscle stiffness. The purpose of this study was to determine how decreased muscle stiffness impacts the acute response to resistance exercise. We chose to implement a stretching program to modulate muscle stiffness to test the hypothesis that muscle stiffness impacts the response to an acute bout of resistance exercise. The participants for this study were 8 healthy, young women (24±3) and 8 healthy older women (74±6). Participants completed a single unilateral bout of resistance exercise to maximally engage the plantarflexors. Muscle biopsies were immediately collected from the lateral gastrocnemius, bilaterally. The older group took part in an 8-week stretching intervention. Following the intervention, they performed the same bout of unilateral resistance exercise and, again, bilateral muscle biopsies were collected. Western blot analyses were conducted to examine changes in p70S6k phosphorylation between the exercised and control limbs and differences in the older group following the stretching intervention. Prior to the stretching intervention there was a significant difference between the response of the young and old adults. Following the intervention there was no significant difference in the response of the two groups to a single bout of resistance exercise. However, the change in response of the old group from pre- to post-intervention failed to reach significance. There was no significant

change in muscle stiffness following the stretching intervention. This study was not able to confirm that decreased muscle modulus is the factor responsible for improved response to acute resistance exercise, it did confirm that stretching improves hypertrophic response to acute resistance exercise in older women.

4.2 Introduction

Sarcopenia is defined as age-related muscle wasting and the accompanying loss of function (Haran et al., 2012; Rosenberg, 1997). Sarcopenia is associated with decreased independence for older adults. As individuals become weaker activities of daily living become more difficult to perform without assistance. Simple tasks such as rising from a chair, ambulating, and self-administered hygiene require more effort when an individual has low relative muscle mass. The increased effort required to perform these or other similar functional skills may eventually lead to disability (Janssen et al., 2002). Decreased strength and muscle size are associated with increased instance of falls and fractures (Short & Nair, 2001). Based on this information, it is easy to understand how muscle mass has been strongly correlated with quality of life for older adults (Ozcan et al., 2005). Furthermore, muscle strength has been shown to be a significant predictor of mortality (Rantanen, 2003). This means that older adults who have lower levels of strength are at a higher risk of dying than their stronger peers. As the older portion of the population in the United States continues to increase at a faster rate than the overall population, it is imperative that interventions to combat and prevent sarcopenia are developed (Howden & Meyer, 2010).

Resistance exercise is a common prescription for persons of any age who are interested in increasing strength and muscle size. Resistance exercise has been suggested as a potential treatment for older adults with sarcopenia as a means to improve function and increase muscle mass and strength, thereby lowering the risk of both disability and mortality. Studies have shown that older adults respond positively to resistance exercise training programs (Fiatarone et al., 1990). However, when studies

had old and young adults engage in similar resistance training programs the older group consistently displayed an attenuated response compared to that of the young group. This pattern has been shown with many different outcome variables. Studies have shown that both young and old adults are capable of improving their strength as measured by increased 1-repetition maximum (1RM) or increased peak torque production, but the young participants had a significantly greater improvement than the old participants (Greig et al., 2011a; LaRoche et al., 2008; Raue et al., 2009; Roth et al., 2000). Studies have also shown a difference in the response to resistance training in relation to change in myofiber size. Five different studies reported an increase in myofiber size in young participants following a resistance training intervention and four out of these five studies showed a greater response in the young group than the old group following training (Bickel et al., 2011; Kosek et al., 2006; Petrella et al., 2006; Raue et al., 2009). The other study showed a 40% increase in myofiber size in the old adults and a 37% increase in the young adults (Mayhew et al., 2009). The study that did not show attenuation in the older group had a mean age of 64.4±0.9 for the older group. This is relatively young for an old cohort and these participants may have had a response to the exercise stimulus that was more similar to young adults than more senior adults.

There have been several studies conducted that have sought to determine what, if any, differences exist between young and old adults following an acute bout of resistance exercise. The outcome variables examined to determine response to exercise in these studies have typically been relative phosphorylation levels of proteins involved in the Akt/mTOR muscle protein synthesis pathway or known inhibitors of this

pathway's activity such as myostatin. Studies have consistently found significantly increased mTOR phosphorylation at all time points ranging from 1-24 hours post-exercise in the young adults. However, the response of the older adults is less consistent, increasing in one study (Drummond et al., 2008), and remaining unchanged in another (Fry et al., 2011).

70 kDa ribosomal S6 kinase (p70S6k) is a downstream target of the mTOR pathway (Baar & Esser, 1999; Nader & Esser, 2001) that regulates protein synthesis in response to RE. However, a more recent study suggested that p70S6k phosphorylation may be activated by another mechanically stimulated, faster-acting pathway that is Akt-independent (Klossner et al., 2009). Work in our lab has also shown p70S6k phosphorylation to be increased in young adults within 30 minutes of a single bout of exercise (Hibbert, Jones & Domire, in preparation). Observation of increased phosphorylation in only the young group so soon after the mechanical stimulus indicates that impairment in the hypertrophic response occurs very soon after application of the stimulus. It is probable that the impairment is in mechanosensation, or the amount of strain registered by the muscle cell for an applied force.

It is known that there are differences in the material properties of muscle between young and old adults. Domire and colleagues showed this using Magnetic Resonance (MR) elastography to take muscle stiffness measurements in vivo. It was found that tissue homogeneity decreased with age (Domire, McCullough, Chen, & An, 2009). Similarly, it has been shown that the stiffness of rat muscle epimysium increases with age (Gao, Kostrominova, Faulkner, & Wineman, 2008).

Thus, it is conceivable that the decreased hypertrophic response to resistance exercise observed in older adults may be due to increased muscle stiffness. In vitro experiments have shown cell growth and differentiation are impacted by the stiffness of the substrate on which it is grown (Engler et al., 2004). The substrate stiffness may influence the mechanotransduction signal cascade in the cell. This has been shown in studies that have found the muscle modulus to be highly inversely correlated with muscle cross-sectional area (Pauwels, Dowling, Okafor, Breighner, & Domire, 2012). If increased stiffness alters mechanotransduction, older adults who have greater muscle stiffness may be receiving less of the hypertrophic signal from exercise than the less stiff muscles of their young counterparts would from the same stimulus. This means that as the muscle stiffens the muscle is receiving less signal to hypertrophy than it did when it was less stiff. Thus, exercise in the form of everyday activity or workout routines will provide less benefit for those with stiff muscles.

The purpose of this study was to determine how decreased muscle stiffness induced by a stretching intervention impacts the acute response to resistance exercise. We chose to implement a stretching program to modulate muscle stiffness to test the hypothesis that muscle stiffness impacts the response to an acute bout of resistance exercise. Both muscle stiffness and signaling proteins were measured using ultrasound elastography and in muscle biopsies that were collected immediately after a single bout of resistance exercise, respectively.

4.3 Methods

4.3.1 Participants

The participants in this study were also participants in another study conducted by our lab group examining differences between the immediate response of young and old women to an acute bout of lower extremity resistance exercise. The data presented for the young and old women as pre-test data were the results of the aforementioned study. 16 healthy, female participants were recruited to participate in this study. 8 participants were young (24±3) and 8 participants were older (74±6) (Table 4.1). All participants completed a health questionnaire prior to being accepted for participation to ensure that all inclusion criteria were met and that they were free of all exclusion criteria. Only females were recruited to participate in this study because there was to be a small sample size and it was unclear whether gender may play a role in the response to resistance exercise. At this time, women are living to older ages than men, thus this research has greater applicability to the female population. All participants read and signed an informed consent form prior to beginning any of the experimental protocol. The East Carolina University Institutional Review Board approved all experimental procedures.

Table 4.1: Demographic information for study participants. All values are given as mean ± standard deviation.

	n	Age	Height (in)	Weight (lbs)
Young	8	24±3	66.4±3.6	144.5±20.9
Old	8	74±6	61.6±2.5	150.8±29.7

4.3.2 Experimental Protocol

All study participants reported to the biomechanics laboratory at East Carolina University for ultrasound imaging of the medial and lateral gastrocnemius of their right leg, including muscle stiffness measurement using shearwave elastography, and to complete a resistance exercise protocol with their right leg using the isokinetic dynamometer (HUMAC NORM Testing & Rehabilitation System, CSMI Medical Solutions, Stoughton, MA). The plantarflexors were selected as the target for these interventions because they are a bi-articular muscle group that is easily stretched and is a good muscle for muscle biopsy collection. The exercise bout consisted of 3 sets of 10 repetitions of both isometric and isokinetic maximal ankle plantarflexion. Isokinetic repetitions were performed at 60 degrees per second. Participants were positioned supine with knees and hips in anatomical position for these exercise sets. Participants also completed 3 sets of 10 repetitions of maximal isometric and isokinetic knee flexion. Participants were positioned supine with knee in full extension for ankle repetitions. Isometric repetitions were conducted with the ankle in neutral position (ankle angle of 0 degrees of flexion). For all knee flexion trials, participants were in a seated position with both hips and knees flexed to 90 degrees. All isometric repetitions were performed at a knee angle of 90 degrees of flexion. Both ankle plantarflexion and knee flexion were included in this exercise protocol to target both actions of the gastrocnemius muscle group. Participants were allowed to rest between each set of all exercises. When all exercise sets were complete participants were transported to the East Carolina Heart Institute for gastrocnemius muscle biopsies.

Two muscle biopsies were collected from each participant. The procedure involved the administration of local anesthesia (1% lidocaine), a small incision, and sample extraction from the mid portion of both the right and left lateral gastrocnemius using a 4-mm Bergstrom biopsy needle. All muscle biopsies were collected within 30 minutes of the resistance exercise bout. Muscle samples were immediately frozen in liquid nitrogen for later analysis and stored at -80° C.

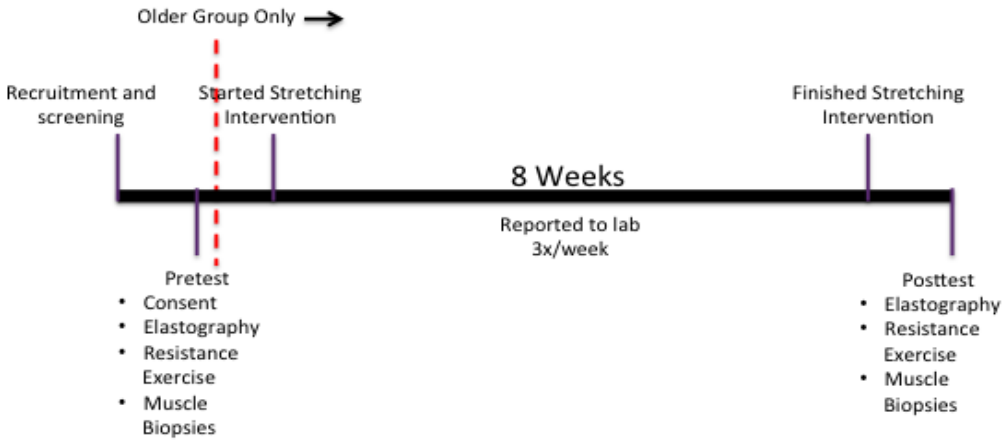

Figure 4.1 Timeline of the methodology followed in this study. Only the older adults performed the stretching intervention.

4.3.3 Elastography

Ultrasound shearwave elastography was used to measure muscle stiffness for the purposes of this study. Elastography was developed in 1991 to detect non-uniform areas in tissue (Ophir, Cespedes, Ponnekanti, Yazdi, & Li, 1991). Elastography uses an imaging technique to measure wave motion through a tissue and calculate tissue material properties based on the mechanics of wave propagation. Elastography has been applied to study muscle in a variety of different conditions (Ringleb et al., 2007). Because of the proximity of skeletal muscle to the surface of the skin, ultrasound elastography is a viable option to measure the stiffness of the gastrocnemius muscles

(Figure 4.1). Additionally, elastography has been shown to be a reliable and repeatable method for determining muscle stiffness (Eby et al., 2013).

The muscle stiffness value for each collection was determined by calculating the average modulus values from the middle frame of three 10-second video clips. The modulus is determined using a 2.0 mm circular region of interest positioned in the center of the muscle of interest. The mean modulus for all the pixels in the circle is recorded (Figure 4.2).

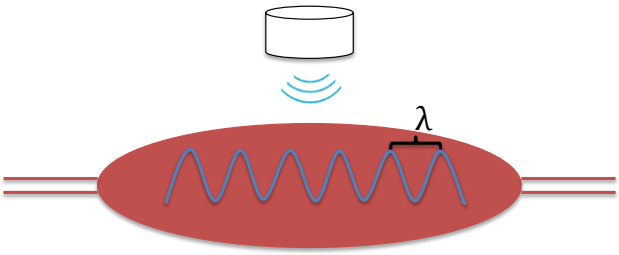

Figure 4.2: Ultrasound elastography uses a focused ultrasound pulse to induce tissue deformation and measure the resulting wave using standard B-mode imaging. This figure shows the propagation of ultrasound pulse from the transducer head into the muscle, and the propagation of the resulting shear wave. The shear modulus of the muscle is calculated as $\mu = f^2.\lambda^2.\rho$ where f- frequency, λ- wave velocity, and ρ- tissue density.

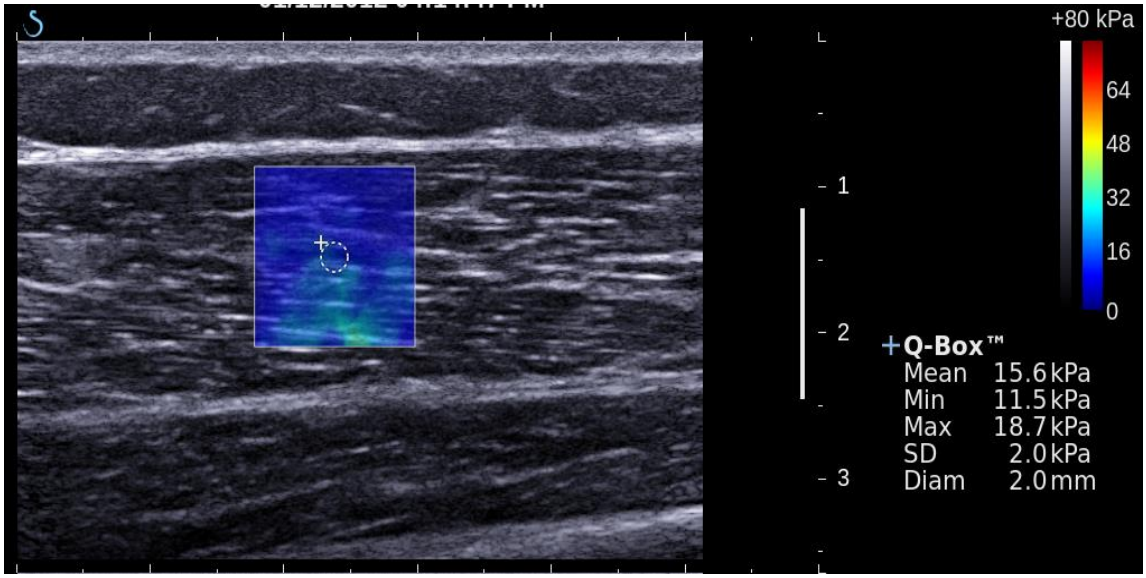

Figure 4.3: The colored box above is the area receiving the pulsed ultrasound. The color variation within the box indicates areas of differing stiffness. The circle in the middle of the box is the area from which the stiffness measurement will be taken. On the right side of the screen the mean stiffness is indicated, as well as the min and max stiffness and the standard deviation within the circular region of interest.

4.3.4 Stretching Intervention

In order to experimentally test the hypothesis that increased muscle stiffness contributes to the attenuated response to resistance exercise observed in older adults, it was necessary to find a means to manipulate muscle stiffness in vivo. Stretching has been shown to be an effective intervention to increase range of motion. Two separate studies have shown that stretching, specifically Proprioceptive Neuromuscular Facilitation (PNF) stretching, is a successful intervention for increasing range of motion in adults aged of 50-79 (Feland et al., 2001; Ferber et al., 2002). This increase in range of motion may indicate that stretching can influence a change in the material properties of muscle over time. A recent study conducted in young adults showed that following an acute bout of static stretching there was a significant increase in range of motion and a significant decrease in stiffness of the gastrocnemius musculotendinous unit (Akagi & Takahashi, 2013). The same research group conducted a longer study that examined changes in muscle stiffness following 5 weeks of gastrocnemius static stretching. They showed a significant decrease in muscle stiffness following 5 weeks of static stretching in the gastrocnemius, again, in young adults (Akagi & Takahashi, 2014).

Participants were given a week to heal following the muscle biopsies prior to reporting to the biomechanics laboratory to begin their PNF stretching intervention. Participants were taken through a balanced, bilateral lower body hold-relax PNF stretching program that targeted the hamstrings, quadriceps, gluteals, piriformis, and plantarflexors (gastrocnemius/soleus). Participants reported to the lab 3 times each week for 8 weeks. The same experienced practitioner worked with all of the study participants to ensure continuity of program implementation across all sessions.

A post testing session was scheduled to take place within a week of the successful completion of all 8 weeks of the stretching intervention. This testing session was exactly the same as the pretesting session including ultrasound imaging the plantarflexors, a unilateral bout of acute maximal lower extremity exercise, and bilateral gastrocnemius muscle biopsies.

4.3.5 Western Blotting

Muscle samples were homogenized using a ground glass homogenizer (Glas-Col, Terre Haute, IN) in a buffer of 10 mM HEPES (pH 7.4), 125 mM sucrose, 1 mM EDTA, 10 mM $Na_4P_2O_7 \bullet 10H_2O$, 10 mM β-glycerophosphate, 2 mM NaF, 1 mM Na_3VO_4, and commercially prepared protease inhibitor cocktail (Sigma, St. Louis, MO). Homogenization was done on ice. Total protein content of each sample was determined using a BCA protein assay kit (Pierce, Rockford, IL) (Ebben, 2009). Protein homogenates were diluted to in a loading buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 100 mM DTT, 0.02% bromophenol blue). Homogenates in loading buffer were heated at 100° C for 3 minutes.

Proteins were separated by electrophoresis. 50 µg of protein from each homogenate and a protein homogenate used as a standard were loaded into a 10% sodium doecyl sulfate-polyacrylamide gel. Proteins were transferred to a PVDF membrane for 4 hours at a temperature of 4° C in standard transfer buffer. PVDF membranes were used because of their durability to withstand reprobing (Yeung & Stanley, 2009).

To probe for phosphorylated p70S6k, membranes were blocked in 5% nonfat dry milk in TBS-T (10 mM Tris-HCl (pH 7.3), 150 mM NaCl, 0.05% Tween 20) at room temperature for 1 hour. After blocking, membranes were incubated in phospho-p70S6k (Thr 389) (1:1000) (Cell Signaling) diluted in 5% milk in TBS-T at 4° C overnight. After washing in TBS-T, membranes were incubated 1-hour in anti-rabbit secondary antibody (1:2500) conjugated with horseradish peroxidase (HRP) at room temperature. HRP activity was visualized using enhanced chemiluminescence solution (GE Healthcare, Piscataway, NJ) and exposure to autoradiographic film (Blue Devil Film, Genesee Scientific, San Diego, CA).

The membrane was stripped in 62.5 mM Tris-HCl (pH 6.7), 2% SDS and 100 mM 2-mercaptoethanol (reducing agent) at 60° C for 30 minutes. Membranes were again blocked in 5% nonfat dry milk in TBS-T at room temperature for 1 hour. After blocking, membranes were incubated in total p70S6k antibody (1:1000) (Cell Signaling) diluted in 5% milk in TBS-T at 4° C overnight. After washing in TBS-T, membranes were incubated 1-hour in anti-rabbit secondary antibody (1:2500) conjugated with HRP at room temperature. HRP activity was visualized using enhanced chemiluminescence solution and exposure to autoradiographic film. All films were imaged via transmissive scanning with a HP ScanJet G4050 (Hewlett-Packard, Palo Alto, CA). All films were analyzed with SigmaScan Pro 5.0 (Jandel Scientific, Systat, Point Richmond, CA). All of the films were analyzed by averaging three analyses of each film. A standard protein was loaded on each gel and the intensity values for each film were normalized to the intensity of the standard protein. In order to compare the arbitrary units across all participants the numerical values presented are the ratio of phosphorylated protein to

total protein in the control limb, the ratio of phosphorylated protein to total protein in the exercised limb, and the overall ratio, which is the ratio from the exercised limb over the ratio from the control limb.

4.3.6 Statistical Analysis

All statistical analyses were calculated in MatLab (MathWorks, Natick, MA). An a priori significance level of $p>0.05$ was used. According to the recommendation of Curran-Everett and Benos, p-values that are around 0.10 will be discussed as trends (Curran-Everett & Benos, 2004). Differences between the control limb of the young and old adult groups were determined using a two sample, two-tailed t-test. Due to the fact that response to the exercise stimuli was hypothesized to be reduced in the older adults the remainder of the statistical analyses were one-tailed tests. One tailed paired sample t-tests were conducted to determine whether the old adults exhibited an attenuated response to the acute resistance exercise stimuli as compared to the young adults. One-tailed chi-squared analyses were also conducted to test the uniformity of response. This allowed us to see if the number of responders following the intervention was different than the number of responders at baseline. Participants were classified as responders for the purpose of this calculation if they showed a positive increase in phosphorylation following resistance exercise.

4.4 Results

4.4.1 p70S6k

p70S6k was used as the marker of response for this study. Based on a study previously conducted by our lab we found p70S6k to be a robust indicator for response

to an acute bout of resistance exercise in both young and old adults (Hibbert, Jones, & Domire, in preparation). The baseline data for the older adults and the data for the young adults were previously presented in the aforementioned study. It was determined that there were no differences between the young and old adults at baseline regarding the ratio of phosphorylated to total p70S6k ($p=0.22$). Following exercise, the young adults showed a significant increase in the proportion of phosphorylated to total p70S6k ($p=0.03$) over baseline. The old adults showed no significant increase in the proportion of phosphorylated to total p70S6k from the control limb ($p=0.46$). However, it was determined that one of the older adults was an outlier based on the Grubbs test for outliers. When the statistical analysis was recalculated excluding the outlier in the old adult data set the difference in response between groups was significant ($p=0.02$). There were 8 of 8 participants in the young group who responded to the exercise, but only 5 of 8 responded in the old group. The chi-squared statistic to determine whether there was a difference in the proportion of responders between groups was 3.692 for the young and old adults before the stretching intervention, which is significant ($p=0.03$). Following the stretching intervention, there were no outliers among the older adults and there was no significant difference between the level of p70S6k phosphorylation between the young and old groups ($p=0.46$). There was no significant difference between the response of the old group pre and post stretching intervention ($p=0.10$). However, this p-value does fall in the range that indicates that there is a trend toward a difference between the two groups. The chi-squared value for the proportion of responders became non-significant following the stretching intervention with a value of 3.692 ($p=0.65$). This means that more of the older adults responded to the exercise

stimulus. The chi-squared value for the proportion of responders and non-responders in the old group from pre to post intervention was non-significant at 0.291 (p=0.30) as there were now 6 of 8 participants who showed as positive response to the resistance exercise.

Table 4.2: This table shows the ratio of p-p70S6k to total p70S6k in both the unexercised (control) limb and the exercised limb as well as the ratio comparing the two. The * represents data that was previously presented. All values are given as mean ± standard deviation.

	Control Limb (p-p70S6k/total)	Exercised Limb (p-p70S6k/total)	Overall Ratio (Exercised/Control)
*Young	0.347±0.194	1.123±1.059	2.966±1.680
*Old Pre	0.247±0.068	0.507±0.589	1.993±1.889
Old Post	0.317±0.301	0.580±0.627	2.831±3.267

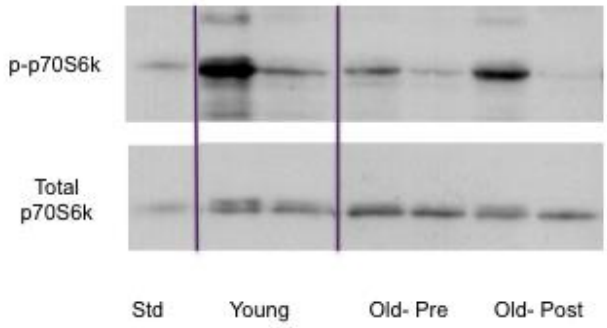

Figure 4.4 Representative blot for both p-p70S6k and total p70S6k

4.4.2 Muscle Stiffness

There was no significant difference in muscle stiffness between the medial or lateral gastrocnemius of young and old groups at baseline ($p=0.5868$ and $p=0.5800$). There was no significant change in the medial or lateral gastrocnemius muscle stiffness of the older adults overall following the stretching intervention ($p=0.14$ and $p=0.11$). While not reaching statistical significance, these p-values indicate that there is a weak trend toward a change in muscle stiffness following the stretching intervention. The fact that there was a decrease in both muscles measured makes the argument for a true change, rather than a change caused by chance more compelling. There was a large amount of variability in the change in muscle stiffness following the stretching intervention. This large variability paired with a relatively small sample size may complicate the visibility of a true change. The standard deviation of the change in the medial gastrocnemius muscle stiffness was 5.29 kPa and the standard deviation for the change in the lateral gastrocnemius was 6.77 kPa.

Table 4.3: This table shows the muscle stiffness values for each group. All values are given as mean ± standard deviation.

	Medial Gastrocnemius Stiffness (kPa)	Lateral Gastrocnemius Stiffness (kPa)
Young	16.78±4.13	16.21±2.37
Old Pre	17.24±3.54	16.62±4.38
Old Post	14.74±5.29	14.03±3.50

4.5 Discussion

Based on these data, the response measured by p70S6k phosphorylation observed in the older adults following the stretching intervention became more similar to the response observed in the young adults. While there was not a significant difference between the phosphorylation ratio of the older adults from before the intervention to after the intervention, the difference between the response of the young and old groups following exercise changed from being significantly different to statistically insignificant. This may indicate that PNF stretching influenced a positive change in the response to a single bout of lower extremity exercise, but was not successful in causing a significant decrease in muscle stiffness in the older adults.

The results of this study are different from those shown in the stretching intervention presented by Akagi and colleagues (Akagi & Takahashi, 2014). This may be due to the fact that all of the participants in that study were young men. It is also possible that the failure to see a significant change in muscle stiffness in our study was due, in part to the fact that none of the older participants who were enrolled in this study started with very stiff muscles. The participants with the greatest muscle stiffness values at baseline had stiffness values that were just over one standard deviation from the mean. In contrast, a study conducted by Domire and colleagues, had 3 of 20 participants who had stiffness 3 standard deviations above the mean (Domire et al., 2009). Although this study was conducted in a different muscle group (tibialis anterior), it is possible that by not having any participants who began the intervention with very stiff muscle, there was a floor-effect for the intervention. The participants may have had a limited response due to a smaller available range for decreased stiffness. It is possible

that the participants recruited for this study may not be the individuals expected to have high levels of muscle stiffness. All of the older participants were at least 70 years old, but they were all general healthy, ambulatory and living independently.

Another facet of the material properties of muscle that may have been affected by the stretching intervention is the homogeneity of stiffness in the muscle. Domire and colleagues noted that there was decreased homogeneity of stiffness in the older participants in their study (Domire et al., 2009). It is possible that the stretching intervention caused changes in the muscle that made the stiffness more uniform throughout the tissue. These changes may not have been large enough to be detectible with the elastography measurements used in this study, but were enough to make a difference in the response of the muscle to an acute bout of resistance exercise.

It is also possible that stiffness is modulated by activity level. The gastrocnemius muscles are heavily involved in ankle plantarflexion (Murray, Guten, Baldwin, & Gardner, 1976). Ankle plantarflexion is one of the major components of the gait cycle. This involvement could affect the state of the muscle at each visit. Different types of footwear may also contribute to changes observed in the muscle that are not caused by the stretching intervention. It is possible that the variability of activity level among the participants obscured our ability to detect true changes in the material properties of the muscle. Participants were asked to maintain the same activity level throughout the intervention, but some changes due to the change of seasons or activity involvement may be expected.

Given that there was an observable change in the p70S6k phosphorylation response to the stretching intervention, but the change in stiffness did not reach

significance, it appears that modulus may not be the rescuing factor for improved response to acute resistance exercise. Changes did occur at some level in the mechanotransduction signaling cascade that improved response to acute resistance exercise in the older women. It is possible that the contraction phase of the PNF stretching intervention was enough of a stimulus to serve as exercise for the participants and the improved response was a training effect.

Due to the fact that there are weak trends present in the muscle stiffness measurements for both gastrocnemius muscles coupled with high variability in the measurement, sample size is a limitation of this study. This study was designed to have each participant act as their own control by collecting biopsies from one exercised limb and one unexercised limb in order to diminish the effects cause by a small sample size. One of the other limitations regarding the muscle biopsies is that only a single biopsy was taken. It is possible that had repeat biopsies been collected the older adults may have exhibited a more robust response to the exercise stimuli. It is possible that there is an alteration in the time course for the response to resistance exercise in older adults. This study enrolled only female participants because there is evidence to suggest that men and women respond differently to long-term resistance exercise and we did not want to confound the data by introducing a possible gender effect (Bamman et al., 2003b).

Future studies that screen participants initially and enroll participants who are very stiff (stiffness greater than 25 kPa) may provide a clearer picture as to the effects of stretching on the material properties of muscle. Other means for measuring muscle stiffness would also be useful. Using atomic force microscopy to measure stiffness on a

microscale may be helpful in elucidating changes that occur on the fiber level of muscle with stretching or other interventions aimed at altering material properties. Another measurement that may be useful to measure changes in stiffness would be changes in passive joint stiffness measured using the isokinetic dynamometer. This may provide information regarding changes in the stiffness of connective tissues as well.

It would also be worthwhile to measure other outcome variables to determine what other benefits stretching may provide older adults even if it proves not to be an effective mechanism for decreasing muscle stiffness over time. Future studies should also explore other mechanisms that may alter muscle stiffness. If a significant decrease in muscle stiffness is observed there is a possibility that a more robust response to resistance exercise would also be observed.

In summary, it appears that participation in a PNF stretching intervention improved the hypertrophic response to a single bout of acute resistance exercise in older women. The mechanism still remains to be determined, but implementation of a stretching intervention may be helpful in improving the hypertrophic signal of exercise.

4.6 Acknowledgements

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Chapter 5

Study 3: A Long-Term Stretching Intervention Positively Impacts Hypertrophic Response to Resistance Training in Aged Women

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5.1 Abstract

It has been shown that while older adults are capable of exhibiting a positive hypertrophic response to resistance exercise, this response is attenuated compared to young adults. Older adults have increased muscle stiffness and it is possible that this leads to an impairment the mechanical signal from exercise. In turn, the reduced ability of muscles to sense mechanical signal might be reflected in their ability to promote muscle hypertrophy. The purpose of this study was to test the hypothesis that a prior stretching intervention would increase the ability of skeletal muscle to hypertrophy in response to a resistance training (RT) intervention. The participants for this study were 8 healthy young women in the RT only group (20.4±1.2), 8 healthy old women in the stretch + RT group (74.1±6.2) and 8 healthy old women in the RT only group (73.8±4.5). Participants in the old stretch + RT group participated in a stretching intervention during which they reported 3 days each week for 8 weeks for assisted lower extremity stretching. Following the completion of this stretching intervention they participated in a RT intervention that was also 3 days each week for 8 weeks of lower extremity RT. The other two groups participated in only the RT. Response to the resistance exercise was observed based on changes in the plantarflexors. This muscle group was selected because it is bi-articular, easy to stretch, and easily targeted for strength training. There was a significantly greater response in both hypertrophy and strength in the old stretch +RT than the old RT only group following the RT intervention. Both the young RT only and old stretch + RT groups showed significantly greater plantarflexor torque production, but there was no significant increase in the old RT only group. The old stretch + RT group also showed a significant increase in gastrocnemius volume

following RT. The young group trended toward a significant increase in volume, but there was no change in muscle volume in the old RT only group. These data indicate that stretching is an effective way to rescue the hypertrophic effect of RT in older women.

5.2 Introduction

There are numerous reasons that contribute to sarcopenia being one of the greatest public health concerns facing society today. Muscle strength is a significant predictor of mortality (Rantanen, 2003). This means that older adults who have low levels of muscle strength are more likely to die than their stronger peers. The age-related loss of muscle mass and strength, or sarcopenia, is also associated with decreased independence (Haran et al., 2012; Rosenberg, 1997). This occurs because as individuals become weaker activities of daily living become more difficult to complete. Activities that were once simple and effortless, such as rising from a chair, ambulating and completing simple household tasks become difficult. These difficulties, often termed functional impairments are significantly more likely to occur in individuals with low relative muscle mass (Janssen et al., 2002). It is not surprising that it has also been shown that muscle mass is strongly correlated with quality of life in older adults (Ozcan et al., 2005). In addition to requiring more effort or assistance to perform habitual activities, persons with decreased muscle size and strength are at increased risk for falls and fractures (Short & Nair, 2001). It is estimated that strength losses associated with sarcopenia are 15 percent per decade beyond the age of 50 (Larsson et al., 1979). The prevalence of sarcopenia in persons over the age of 80 is estimated to be greater than 50 percent (Baumgartner et al., 1998). These facts become more alarming after examining population growth patterns that show the older portion of the population in the United States is growing more quickly than the overall population of the country (Howden & Meyer, 2010). This means that the health concerns effecting older adults are increasingly prevalent and must be addressed.

Resistance training in older adults can significantly improve function and strength (Fiatarone et al., 1990; Leenders et al., 2013b; Pyka et al., 1994; Slivka et al., 2008). However, aged appear to respond less robustly when compared to young adults. These studies have shown attenuated strength gains in the older groups (Greig et al., 2011a; LaRoche et al., 2008; Raue et al., 2009; Roth et al., 2000), diminished or absent increases in muscle cross-sectional area (Kosek et al., 2006; Petrella et al., 2006; Raue et al., 2009), and decreased expression in genes associated with hypertrophy (Dennis et al., 2008; Owino et al., 2001).

There are many levels of the hypertrophic response to chronic exercise interventions that may be impaired resulting in the observed attenuation. However, studies have shown that there is also a decreased response to an acute bout of exercise (Drummond et al., 2008; Drummond et al., 2009; Fry et al., 2011; Raue et al., 2006; Rivas et al., 2012; Roberts et al., 2009). Furthermore, impairment has been observed as soon as 30 minutes following a bout of resistance exercise (Hibbert, Jones, & Domire, in preparation). This attenuation of hypertrophic markers so soon after stimulus application indicates that the impairment in the mechanotransduction cascade is very early, potentially as early as sensation of the mechanical stimuli.

One possible mechanism for this decreased response to resistance exercise observed in older adults is that increased extracellular matrix (ECM) stiffness is altering mechanotransduction. It is known that there is greater ECM stiffness in old muscle (Gao et al., 2008). It has also been shown that the stiffness of a cell's environment influences the way the cell interacts with its environment (Engler et al., 2004). Greater muscle stiffness could alter mechanotransduction because less strain will be registered by the

mechanosensor for any given input. This means that a person with stiff muscles will have to perform more exercise than a person with more compliant muscles to have the same amount of signal propagated through the hypertrophic pathway.

Stretching has been shown to be an effective intervention to increase range of motion in older adults (Feland et al., 2001). In a study by Akagi and colleagues, it was shown that there was a decrease in muscle stiffness following 5 weeks of a stretching intervention in young men (Akagi & Takahashi, 2014). Which indicates that stretching can be used to modulate muscle stiffness. The purpose of this study was to test the hypothesis that having older adults participate in a stretching intervention prior to beginning a resistance training intervention will improve their response to the resistance training program. The dependent variables being used to determine response to the resistance training program for the purpose of this study are change in isokinetic plantarflexor torque and change in the combined volume of the medial and lateral gastrocnemius.

5.3 Methods

5.3.1 Participants

The participants recruited for this study were 16 healthy, aged women and 8 healthy, young women (Table 5.1). Participants were excluded from participation if they had been previously diagnosed with Parkinson's disease, stroke, peripheral artery disease, cancer, diabetes, or osteoarthritis in the lower extremities. Participants were also excluded if they had high blood pressure or were significantly overweight (BMI > 32). The aged women were divided into two groups. The first group was assigned to

complete both stretching and resistance exercise (stretch + RT). The second group was assigned to resistance exercise only (RT only). The young women also performed only the resistance exercise intervention.

Table 5.1: Demographic Information for study participants. All values are given as mean ± standard deviation.

	n	Age	Height (in)	Weight (lbs)
Young- RT Only	8	20.4 ±1.2	66.7±2.4	148.9±24.4
Old- Stretch+RT	8	74.1±6.2	61.6±2.5	150.8±29.7
Old- RT Only	8	73.8±4.5	64.0±2.9	149.3±28.9

5.3.2 Procedures

All participants reported to the biomechanics laboratory for pre-intervention testing that included ultrasound imaging of the plantarflexors and both isometric and isokinetic strength testing. The plantarflexors were selected as the target for these interventions because they are a bi-articular muscle group that is easily stretched and can be targeted for isolated strengthening. The young participants (young RT only) and the older exercise only group (old RT only) were asked to report three days per week for 8 weeks to complete a resistance training program. The stretching and resistance exercise group (old stretch + RT) were asked to report three days per week for 8 weeks to complete a PNF stretching intervention. They were then given the option to complete the same 8-week resistance exercise program that the other groups completed. Following the completion of both the stretching and resistance exercise interventions participants reported to the biomechanics laboratory for post-intervention testing that was the same as the pre-intervention test.

5.3.2.1 Ultrasound Imaging

A series of longitudinal and cross sectional images of both the medial and lateral gastrocnemius were collected using b-mode ultrasound (Aixplorer; SuperSonic Imagine, Aix-en-Provence, France). These images were used to calculate the volume of these muscles by using the cross-sectional area calculated using image processing software (OsiriX Imaging Software, Pixmeo, Bern, Switzerland; ImageJ, U. S. National Institutes of Health, Bethesda, Maryland) at 6 different points along the length of the muscle and the distance between the images. Muscle volume was calculated from these images by averaging the cross-sectional area of each end of the sections and multiplying this by

the width of the slice. The first and last images were taken 1cm from the end of each muscle. The volume of the remaining muscle was calculated as a cone with a base of the same circumference as the CSA of the corresponding image.

$$volume = \left(\left(\frac{1}{3} CSA\ S0\right) * 1cm\right) + \left(\frac{CSA\ S0 + CSA\ S1}{2} * sw\right) + \left(\frac{CSA\ S1 + CSA\ S2}{2} * sw\right) + \left(\frac{CSA\ S2 + CSA\ S3}{2} * sw\right)$$
$$+ \left(\frac{CSA\ S3 + CSA\ S4}{2} * sw\right) + \left(\frac{CSA\ S4 + CSA\ S5}{2} * sw\right) + \left(\left(\frac{1}{3} CSA\ S5\right) * 1cm\right)$$

Formula 5.1 Muscle volume calculation- CSA is the cross-sectional area of the corresponding slice (S*n*), sw is the slice width calculated as 1/5 the length of the muscle after subtracting the most proximal and distal centimeters

The use of ultrasound imaging to calculate muscle volume has been validated to MRI (Ahtiainen et al., 2010) and direct cadaveric measurements (Infantolino, Gales, Winter, & Challis, 2007).

5.3.2.2 Strength Testing

Participants performed 1 set of 5 repetitions of maximal, isokinetic plantarflexion at 60 degrees per second using an isokinetic dynamometer (HUMAC NORM Testing & Rehabilitation System, CSMI Medical Solutions, Stoughton, MA). Prior to beginning the test the subject will be allowed two practice repetitions at 50% effort to familiarize them with the dynamometer.

5.3.2.3 Stretching Intervention

PNF stretching was chosen as the intervention rather than static stretching because studies have shown that while there is a significant increase in ROM following static stretching, the increase in ROM following PNF stretching is significantly greater than the increase seen following static stretching (O'Hora, Cartwright, Wade, Hough, & Shum, 2011; Sharman, Cresswell, & Riek, 2006).

When performing the hold-relax method of PNF stretching the participant was passively moved to the end ROM for the muscle being stretched held for 10 seconds, and then asked to push against the stretch, activating the muscle being stretched, for 7 seconds. They then relaxed and the stretch was be taken to a new end point. This was performed 3 times, bilaterally, on the hamstrings, quadriceps, piriformis, gastrocnemius, and hip extensors. The intervention was 24 sessions that took place over the course of 8 weeks.

5.3.2.4 Resistance Training Intervention

The resistance training intervention will be a general lower extremity strength training intervention. Although the plantarflexors are the muscle group that will be tested and measured to evaluate response to the exercise program. A general lower extremity program was formulated for the benefit of the participants. It is possible that asking participants to take part in an unbalanced exercise program could result in discomfort or even injury.

Prior to beginning the prescribed exercises for each session, participants completed a 5-minute warm-up on a cycle ergometer. Participants performed 3 sets of

10 repetitions of each of the following exercises, resisted knee flexion, knee extensions, leg press, calf press, band-resisted plantarflexion, and band-resisted dorsiflexion. In order to find an appropriate resistance to use for each exercise maximal testing was performed on the first day participants reported for training. Participants were familiarized with each exercise and performed a warm-up set with low resistance. The resistance was then increased until a resistance was identified at which only one repetition could be completed. This was determined to be the 1 repetition maximum (1RM) for that participant for that exercise. Participants were allowed as much rest as needed between testing sets. The training load for the subsequent sessions was determined as a percentage of the 1RM. During the first two weeks, participant performed the exercises at 50, 60, and 70% of their 1RM. The next two weeks increased to 60, 70, and 80% of their 1RM. Maximal testing was repeated on the first training day of the fifth week. If the participant was able to increase their maximal resistance, these new numbers were used to calculate the appropriate training load. 50, 60, and 70% of their new 1RMs were used for weeks 5 and 6, and the training load increased to 60, 70, and 80% for the final two weeks. All training sessions were supervised to ensure both safety and compliance. 24 training sessions were completed in no more than 9 weeks, with no more than 3 training sessions taking place in any week.

5.3.3 Statistical Analysis

All statistical analyses were calculated in MatLab (MathWorks, Natick, MA). An a priori significance level of $p>0.05$ was set to determine statistical significance. A 2x2

mixed model analysis of variance (ANOVA) was used to determine differences between the groups and changes following the strength training intervention. One tailed t-tests were used as post-hoc analyses to measure the specific difference between the groups that were shown to be different by the repeat measures ANOVA. One tailed tests were used to test the hypothesis that the old stretch + RT group will have a more robust response to exercise than the old RT only group. These post-hoc analyses were conducted on the changes scores, which are defined as the percent change from before to after the resistance training intervention, for the purpose of this study.

5.4 Results

5.4.1 Isokinetic Plantarflexor Torque

There was a significant group by time interaction for isokinetic plantarflexor torque ($p=0.01$). When post-hoc analysis were conducted on the change scores for plantarflexor torque it was found that there was no significant difference between the percent change in torque between the young RT only and stretch + RT group ($p=0.17$). There was a significant difference in the percent change in torque between the young RT only and old RT only groups ($p=0.02$). There was also a significant difference between the percent change in the old RT only and the stretch + RT groups ($p=0.05$). The young RT only and old stretch + RT groups showed a significant increase in torque from pretest to posttest ($p=0.002$ and $p=0.005$). There was no significant difference in torque between the pre- and posttests of the old RT only group ($p=0.41$).

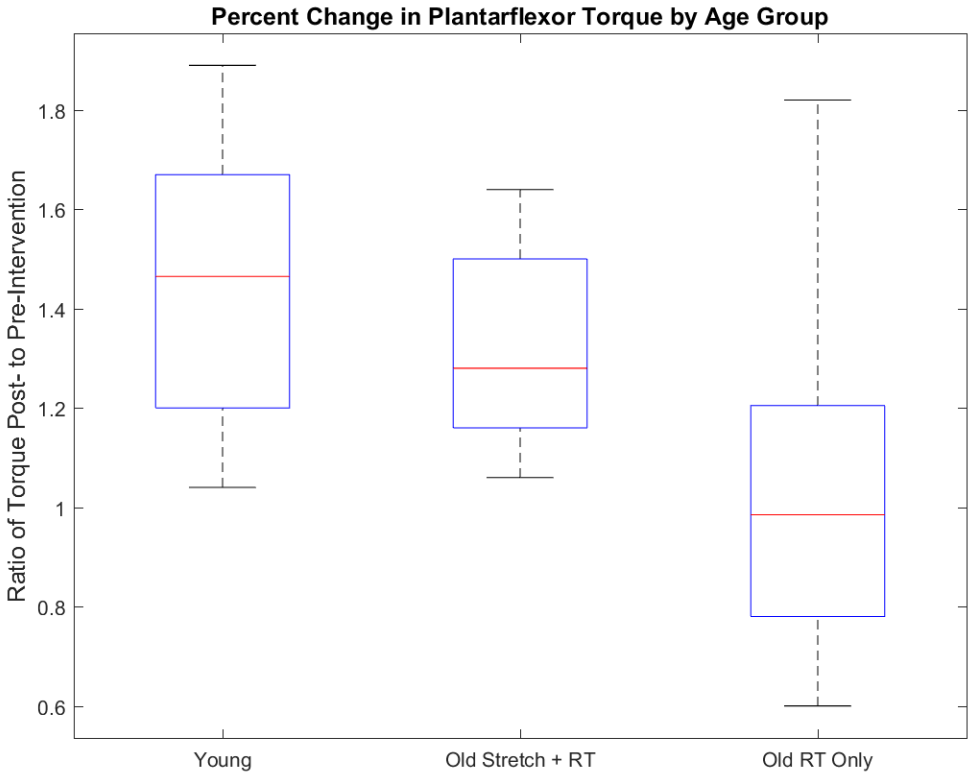

Figure 5.1: This represents the ratio of post-intervention torque/ pre-intervention torque. The line in the box represents the median value for the group represented. The top line of the box represents the 1st quartile and the bottom line of the box represents the 3rd quartile. The value represented by the line extending from the top of the box represents the maximum value for the group while the line extending from the bottom represents the minimum value for the group.

Table 5.2: This table shows the ankle plantarflexor torque values for both the pre- and posttest sessions. The last column shows the ratio of change over the course of the RT intervention. All values are reported as the mean ± standard deviation.

	Pretest Torque (Nm)	Posttest Torque (Nm)	Ratio of Change (posttest/pretest)
Young- RT Only	46.47±11.99	65.96±17.47	1.45±0.29*
Old- Stretch + RT	32.15±10.27	42.07±14.07	1.32±0.21*
Old- RT Only	37.61±12.93	38.67±18.12	1.05±0.39

5.4.2 Gastrocnemius Muscle Volume

There was a significant time by group interaction for muscle volume (p=0.02). When post-hoc analysis were conducted on the change scores for muscle volume it was found that there was no significant difference between the young RT only and stretch + RT group (p=0.96). There was also no significant difference between the young RT only and old RT only groups (p=0.19). There was a significant difference between the two old groups (p=0.03). There was a significant increase in the muscle volume of the old stretch + RT group following the resistance exercise intervention (p=0.02). The young RT only group showed a trend for increased muscle volume following the training intervention (p=0.09), but no significant change was detected in the old RT only group (p=0.45).

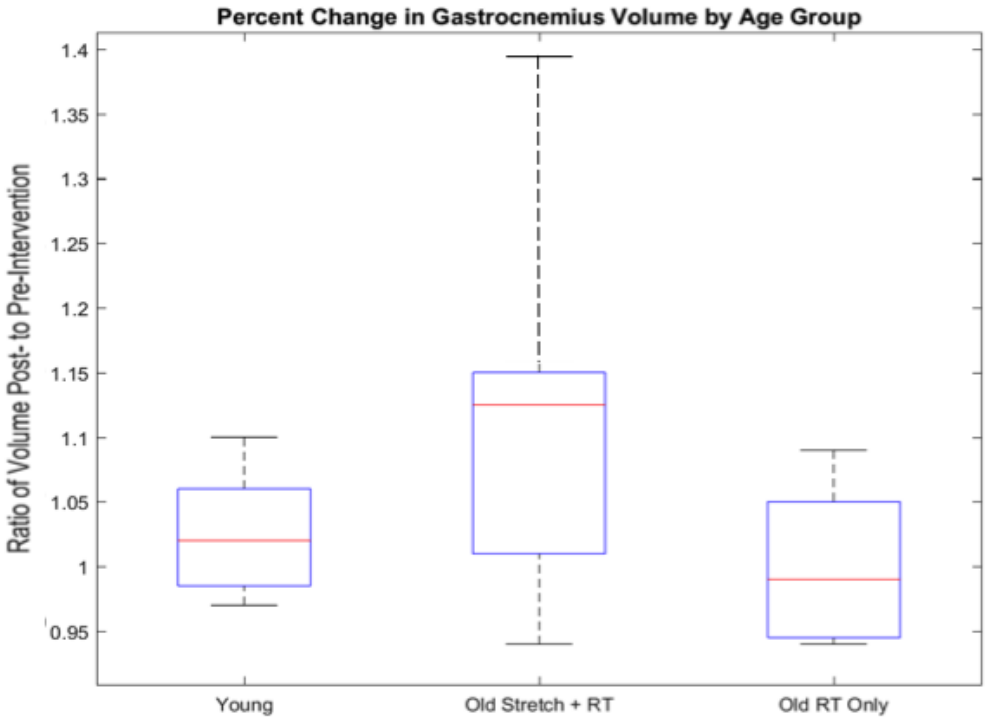

Figure 5.2: This represents the ratio of post-intervention volume/ pre-intervention volume. The line in the box represents the median value for the group represented. The top line of the box represents the 1st quartile and the bottom line of the box represents the 3rd quartile. The value represented by the line extending from the top of the box represents the maximum value for the group while the line extending from the bottom represents the minimum value for the group. The median and quartile values for the old stretch + RT group were calculated without the outlier represented by the cross at the top of the plot.

Table 5.3: This table shows the gastrocnemius muscle volume values for both the pre- and posttest sessions. The last column shows the ratio of change over the course of the RT intervention. All values are reported as the mean ± standard deviation.

	Pretest Volume (cm^3)	Posttest Volume (cm^3)	Ratio of Change (posttest/pretest)
Young- RT Only	298.66±66.86	306.99±73.30	1.03±0.05
Old- Stretch + RT	205.93±36.04	225.81±28.11	1.11±0.14*
Old- RT Only	223.86±44.09	223.19±46.72	1.00±0.06

5.5 Discussion

Based on these data, it appears that having older adults participate in a long term stretching intervention prior to beginning a resistance training intervention significantly increases their response to the resistance training. The old stretch + RT group showed significant increases in both ankle plantarflexor torque production and gastrocnemius muscle volume. This group was also significantly different from the other group of older adults who did not participate in a stretching intervention prior to beginning the resistance training intervention and exhibited no change in torque. It is also important to note that there was not a significant difference between the changes in the young RT only and old stretch +RT group.

When the percent changes are examined for each group it appears that the young adults still have a more robust increase in plantarflexor torque production than either of the other groups. The young RT only group increased plantarflexor by 45% while the old stretch + RT group increased by 32% and the old RT only group increased by a non-significant 5%. However, the story changes slightly when examining the percent changes in muscle volume follow the intervention. The old stretch + RT group increase muscle volume by 11% while the young RT only group only increase by 3%. No change was seen in the old RT only group. This finding is very different than the findings of previous studies that have reported large strength gains in both the young and old adults groups with strength training programs. For example, one study reported knee extensor strength gains of 36% in the young group and 26% in the old group, but when whole thigh cross-sectional area changes were evaluated there was a 5% increase in the young group and no change in the old group (Raue et al., 2009).

Increases in muscle mass are a very striking finding because increased muscle mass has been shown to decrease mortality risk (Landi et al., 2012).

The study we conducted focused on changes in ankle plantarflexor torque and gastrocnemius volume. To our knowledge, there have not been any long-term resistance training studies with old and young adults that have focused on these variable as the main outcome variables. Previous studies that have compared the response of old and young adults by measuring change in muscle volume have measured changes in quadriceps muscle volume. Both studies showed increased muscle volume following a resistance training intervention. In one study, there was a difference in the volume increase between the old and young groups, 2.5% and 6.2% respectively (Greig et al., 2011a). In the other study there was a 12% increase in the young men, old men and old women, but only a 6% increase in the young women (Ivey et al., 2000). This difference in response was not reported to be statistically significant.

In studies examining changes in strength/torque production in young and aged adults following long-term resistance training programs most studies show that both young and older adults make significant strength gains following such programs. However, these increases are typically attenuated in the older groups compared to the young. The difference in the percent gains between the young and age groups ranges between 1 and 13% with an average difference of 8% (Greig et al., 2011a; LaRoche et al., 2008; Mayhew et al., 2009; Raue et al., 2009; Roth et al., 2000).

Our results between the young RT only group and RT only group were consistent with these findings. However, the stretch + RT group had significantly greater gains of

both muscle volume and torque than the RT only group thereby narrowing the gap between the hypertrophic response with the young RT only group.

It is currently unclear what factor facilitated the increase in the hypertrophic response to the resistance training program. It is possible that changes in muscle stiffness facilitate the observed response. However, in a previous study only trends were found for changes in muscle stiffness following a long term stretching intervention. Overall, the correlation between change in volume and muscle stiffness had a r-squared value of 0.16. It is possible that changes were obscured by the variability in stiffness values across the group, but further investigation is needed to determine what impact changes in stiffness have on response to exercise.

There are several other possible explanations for how a stretching intervention may improve the hypertrophic response to a chronic resistance training intervention. One hypothesis is that there was increased neural drive present in the group that underwent the stretching intervention prior to beginning the resistance training. The rationale behind this idea is that there was neural inhibition present prior to beginning the stretching intervention and by stretching this inhibition was diminished. Another hypothesis was that this type of stretching intervention was enough of a stimulus that it was a stretching intervention in itself. However if these alternate hypotheses were true it would be expected that the group that had previously stretched would be stronger at baseline. There were no statistical differences in ankle plantarflexor torque at baseline between the old stretch + RT and the old RT only groups. It is possible that there is another unknown mechanism by which stretching is facilitating a more robust hypertrophic response to resistance training.

One of the limitations of any study with human participants that uses maximum voluntary contraction as a means to elicit torque values has limitations that come from participant motivation and the possibility of muscle inhibition that comes from decreased use of the muscle group being tested. There have been studies that have shown that older adults have lower levels of muscle activation during maximal contractions than younger adults, which may contribute to an exaggeration in the difference in maximal strength between young and old adults (Stevens, Stackhouse, Binder-Macleod, & Snyder-Mackler, 2003). This is why we chose to measure muscle volume as well as torque values before and after the resistance training intervention. Muscle volume has been shown to be highly correlated with joint torque (Fukunaga et al., 2001). We wanted to be sure that we were able to capture the changes occurring in the muscle following the resistance training intervention, despite any inhibition or participant motivation changes.

One limitation of the study presented here are that all of the participants included were women. We chose to do this to limit the potential hormone effect that could be introduced by including males. It is known that males have higher levels of testosterone, which is influential in muscle hypertrophy (Griggs et al., 1989). An inherent limitation of any strength training intervention is the dependence on participant effort and compliance. Study personnel supervised all training sessions to reinforce proper exercise form and protocol completion.

These data suggest that participation in a PNF stretching intervention prior to beginning a resistance training program is helpful in helping older adults to increase both plantarflexor torque production and muscle size. However, it is still unclear what is causing this change. The lack of change in muscle size in the older adults who

participated in only the resistance training program provides more evidence that there is an impairment in the hypertrophic signaling pathway present in older adults. It is possible that stretching is positively impacting factor involved in rescuing the hypertrophic response to exercise. Future studies should look for other changes in the muscle protein synthesis pathway following a stretching intervention to attempt to better understand what may be causing increased torque production and muscle volume. However, based on the findings of this study it is strongly recommended that anyone who is conducting a resistance training program with older adults include a stretching component.

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Chapter 6

Overall Discussion

6.1 Summary

The purpose of this dissertation was to determine the impact that altered muscle material properties have on the response of aged women to both acute and chronic resistance exercise. Each of the three reports included in this dissertation were designed to provide information necessary to answer a different part of this question. The first report provides information that contributes to baseline understanding of differences in the response of exercise between young and aged adults. The second and third reports deal directly with the two parts of the hypothesis, the impact of altered material properties on response to acute resistance exercise and the impact of altered material properties on response to chronic resistance exercise.

The purpose of the first study was to examine the response of young and aged women in response to a single bout of lower extremity resistance exercise. In this study, it was important to establish that there was a detectable difference in response between the two groups as soon as thirty minutes following resistance exercise. Neither group showed differential activation of FAK following an acute bout of resistance exercise. It was found that there was a difference in p70S6k phosphorylation following exercise with the young adults exhibiting a more robust response. A difference in response that is present soon after application of the mechanical stimulus indicates that the impairment in response is occurring early in the mechanotransduction cascade. The results of this study provided baseline differences between the two age groups that were used to determine the effectiveness of subsequent interventions.

The purpose of the second study was to determine how decreased muscle stiffness impacts the acute response to resistance exercise. It was found that following

an 8 week stretching intervention the response to acute resistance exercise observed in older adults became similar to the response of young adults. Prior to beginning the intervention the response of the two groups was significantly different and following the intervention there was not statistical difference between the two. This indicates that the response of the older adults was more similar to that of the young following the stretching intervention. The change in muscle stiffness following the stretching intervention trended in the direction of indicating a true change as a result of the stretching intervention, but it did not reach statistical significance.

The purpose of the third study was to test the hypothesis that having older women participate in a stretching intervention prior to beginning a resistance training intervention would improve their response to the training program. Following the resistance training intervention the older adults who participated in the stretching intervention prior to strength training had significant increases in both plantarflexor torque and gastrocnemius muscle volume. These increases were significantly greater than those observed in the group of older adults who only participated in the resistance training program and were similar to those measured in the young group.

6.2 Future Research Directions

The results of the study outlined in chapter 3 show that the impairment in the mechanotransduction cascade is likely upstream of most of the hypertrophic signaling, but it is still unclear what is causing the impairment. The hypothesis of this work has been that increased muscle stiffness is contributing to diminished response to exercise in older adults. The targets chosen in chapter 3 were selected because they have previously been shown to be phosphorylated following mechanical stimulation. However, one piece of information that is still needed to complete the understanding of cellular mechanotransduction is identifying the mechanosensor in skeletal muscle. Studies to determine what the mechanosensor is and its immediate targets will help to further describe the pathways involved in hypertrophy as well as reveal points at which dysfunction occurs. Once the mechanosensor for skeletal muscle is known it would be possible to examine differences in the abundance of the mechanosensor in young and old adults. It would also be possible to design knockout studies that could directly link changes in mechanosensation and hypertrophy.

Stretching was used as an intervention to alter material properties of skeletal muscle. Stretching was chosen because it is non-invasive, non-pharmacological, easy to perform independently, and poses low risk to the person stretching. This intervention showed a modest decrease in muscle stiffness. Stretching may prove to be more effective if the study participants selected to participate in the study all have higher muscle stiffness at baseline. This would be advantageous for several reasons. One reason is that there may be a more uniform lack of hypertrophic response in these participants prior to the stretching intervention. Thus, changes in the muscle that follow

the stretching intervention that are related to hypertrophic response may result in a much more robust difference in response between the control and exercised limbs than were observed in the study in chapter 4. Another reason that recruiting participants who are stiffer may be beneficial is that any changes in muscle stiffness caused by the stretching intervention may be more easily measured using ultrasound elastography. It was noted that there was a great deal of variability in the muscle stiffness measurements. While it would not be expected that the variability would be less in a group that has stiffer muscles, if there were greater changes in stiffness over the course of the intervention the variability would not be as much of a factor in obscuring the evidence of a true change in muscle stiffness.

Previous studies have shown that muscle stiffness in older adults is more heterogeneous than that of younger adults (Domire et al., 2009). Therefore, it is possible that the measured protein phosphorylation response reported in chapters 3 and 4 could have been impacted by the specific sample of muscle tissue selected. If a particularly stiff or compliant portion of tissue was extracted during the muscle biopsy procedure the results showing hypertrophic response may have been skewed. Another means of evaluating changes induced by the stretching intervention would be evaluating changes in muscle homogeneity and response to exercise on a cellular level using atomic force microscopy and immunohistochemistry. Examining changes in the stiffness of the tissue and the response to exercise of the response to mechanical stimuli of that specific area of tissue may be more sensitive to the changes that are being cause by the stretching intervention. This would effectively negate variation observed in response cause by the sampling area. It is possible that stretching is inducing changes in the

stiffness of the muscle on a cellular level and this is not captured with whole muscle stiffness measurements. Examining both stiffness and response to mechanical stimuli on a cellular level and in the same portion of tissue the results may be more clear in showing the changes that occur following a stretching intervention.

It is important that other means of decreasing muscle stiffness are also explored because it is not possible to effectively stretch every muscle in the body thus making systemic muscle stiffness decreases difficult, if not impossible. Alternative means to decrease stiffness are also necessary in the case that a population is not capable of stretching. It is important to be aware that while robust hypertrophic and strength changes were observed following the stretching intervention, there was only a moderate effect of stretching on muscle stiffness. One treatment that may provide a more robust decrease in skeletal muscle stiffness is the use of advanced glycation end-product (AGE) crosslink breakers. AGEs are a likely cause for the age-related increase in skeletal muscle stiffness. It has been shown that tissue with higher AGE accumulation is stiffer than tissue with less accumulation (Reddy, 2004). It has also been shown that there is increased AGE accumulation in aged skeletal muscle (Snow, Fugere, & Thompson, 2007). AGE cross-link breakers have been pursued as a treatment for myocardial and vascular stiffening associated with both age and diabetes. Studies have shown these cross-link breakers to be an effective treatment for decreasing both myocardial and vascular stiffness and improving cardiovascular function (Asif et al., 2000; Kass et al., 2001). While it is true that the muscle in vasculature and the heart is not skeletal muscle, if the cross-link breakers were effective in decreasing the stiffness caused by AGE accumulation in these tissues it follows that it may be an effective

means which could decrease muscle stiffness in skeletal muscle. The prescription of cross-link breakers could be followed by mechanical stimuli or resistance exercise and the hypertrophic response could be measured to determine if pharmacologically induced decreased muscle stiffness improved the hypertrophic response in a similar way that stretching did, as shown in chapter 5. This study would have to take place in an animal model due to the fact that there are not currently any AGE cross-link breakers approved for use in humans.

One area of this research that needs to be explored further is to elucidate what the components of the stretching program are necessary to elicit the improved response to exercise observed in the study outlined in chapter 5. Further work should seek to determine the optimal length of the stretching program as well as the optimal type of stretching. It would be much easier for individuals to engage in a static stretching program without assistance than a PNF stretching program. It should also be determined what, if any, maintenance is required to maintain the state of the muscle that enables a more robust response to resistance exercise. Performing these studies with larger participant groups would help increase the generalizability of the results, thus making it more likely that stretching would be adopted by clinicians working with older adults.

6.3 Conclusions

Study 1 Hypothesis

It was hypothesized that there would be a less robust hypertrophic response to the acute exercise bout in the older adults than the young adults.

Study 1 Conclusions

The results of this study showed significantly less p70S6k and Akt phosphorylation in the exercised limb of the older adults than the young adults. There was also a significant difference in the number of responders between the young and old groups with 8 of 8 participants responding in a positive manner to the resistance exercise stimulus, but only 5 of the 8 older participants responding in a positive manner. This study provides additional evidence that there is an impairment in the mechanotransduction cascade in the skeletal muscle of aged adults.

Study 2 Hypothesis

It was hypothesized that muscle stiffness impacts the response to an acute bout of resistance exercise. A stretching intervention was used to decrease muscle stiffness in order to improve response to resistance exercise in older adults.

Study 2 Conclusions

There was a trend present for improvement in the older adults following the stretching intervention. The old adults went from statistically different from the young

adults at baseline to showing no statistical difference from the young group after the stretching intervention. The change in muscle material properties was highly variable. Muscle stiffness changes trended toward, but did not reach, significance. This study provides additional evidence that change in the material properties may contribute to increased response to exercise. This change may be more important in the context of response to long term resistance training.

Study 3 Hypothesis

It was hypothesized that having older women participate in a stretching intervention prior to beginning a resistance training intervention would improve their response to the training program

Study 3 Conclusions

This hypothesis was confirmed. The participants who performed 8 weeks of PNF stretching prior to beginning a resistance training program gained significantly more ankle plantarflexor torque and muscle volume as a result of a resistance training program than their peers who did not complete the stretching intervention. The large changes observed in the older adults who participated in a stretching intervention prior to strength training provide compelling evidence to suggest that all older adults should participate in a stretching program prior to beginning resistance training.

Overall Conclusions

The research presented in these studies supports the overall hypothesis that the attenuated hypertrophic response to resistance exercise observed in older adults is

caused by an impaired ability of skeletal muscle to sense mechanical stimuli as a results of increased muscle stiffness. Stretching was designed to be an experimental manipulation of muscle stiffness and following implementation of a stretching intervention the metrics for both mechanosensation and hypertrophic response increased. The evidence that stretching changed skeletal muscle stiffness was inconclusive, however there was a trend that suggested that it decreased stiffness. Independent of whether the mechanism of improvement was increased mechanosensation, stretching was effective and has substantial potential to improve quality of life for aged adults.

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Appendix A: Institutional Review Board Approval Letters

EAST CAROLINA UNIVERSITY
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4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · **www.ecu.edu/irb**

Notification of Initial Approval (Committee)

From:	Biomedical IRB
To:	Jamie Hibbert
CC:	Zachary Domire Patrick Rider
Date:	5/28/2014
Re:	UMCIRB 14-000505 Effects of a Long-Term Stretching Intervention on the Mechanical Properties of Muscle Tissue in Young and Older Adults

I am pleased to inform you that at the convened meeting on 5/28/2014 at 12:15 PM of the Biomedical IRB, the committee voted to approve the above study. Approval of the study and the consent form(s) is for the period of 5/28/2014 to 5/27/2015.

The Biomedical IRB deemed this study Greater than Minimal Risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Document	Description
Informed Consent(0.04)	Consent Forms
Recruitment(0.01)	Recruitment Documents/Scripts
Study Protocol(0.08)	Study Protocol or Grant Application

The following UMCIRB members were recused for reasons of potential for Conflict of Interest on this research study:

None

The following UMCIRB members with a potential Conflict of Interest did not attend this IRB meeting: R. Hickner - COI

IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418

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Notification of Initial Approval: Expedited

From: Biomedical IRB
To: Jamie Hibbert
CC: Zachary Domire
Patrick Rider
Date: 2/16/2015
Re: UMCIRB 15-000099
Effects of a Long-Term Stretching Intervention on the Response to Resistance Training in Young and Older Adults

I am pleased to inform you that your Expedited Application was approved. Approval of the study and any consent form(s) is for the period of 2/14/2015 to 2/13/2016. The research study is eligible for review under expedited category # 4. The Chairperson (or designee) deemed this study no more than minimal risk.

Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The Investigator must adhere to all reporting requirements for this study.

Approved consent documents with the IRB approval date stamped on the document should be used to consent participants (consent documents with the IRB approval date stamp are found under the Documents tab in the study workspace).

The approval includes the following items:

Name	Description
Older Adult- Informed Consent	Consent Forms
Protocol	Study Protocol or Grant Application
Recruitment Wording	Recruitment Documents/Scripts
Young Adult- Informed Consent	Consent Forms

The Chairperson (or designee) does not have a potential for conflict of interest on this study.