# Can stretching as pre-intervention improve aged muscle's response to a resistance training intervention?

by

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Sarcopenia represents an important problem in older women affecting their physical function. Resistance training interventions in older adults have been widely investigated, and the effect they have in this population have shown to be positive but blunted compared to young adults. Muscle stiffness in older adults is greater than in younger populations and this could be aiding to cause a blunted response to resistance training, and as it has been shown that stretching can decrease muscle stiffness, it may benefit older adults.

The purpose of the study was to determine if a pre intervention of stretching followed by a resistance training intervention, would produce a larger effect in older adults, than a pre intervention of resistance training followed by a resistance training intervention. Isokinetic plantarfexor torque and combined volume of the medial and lateral gastrocnemius were used to determine this effect.

Sixteen healthy women (average age, 75.44 years) were randomly divided in 2 groups: resistance training + resistance training (RT + RT), and stretching + resistance training (S + RT). The RT + RT group performed 16 weeks of resistance training, and the S + RT performed 8 weeks of stretching followed by 8 weeks of resistance training. Isokinetic plantarflexor torque was measured with 1 set of 5 repetitions in a dynamometer at 60 degrees per second. Muscle volume was measured with b-mode ultrasound of the medial and lateral gastrocnemius.

There were no statistical significant differences found in torque and volume when comparing both 16 week treatments. The only significant time by group interaction found was when comparing torque values of the pre-intervention of RT + RT group with the resistance training portion of the S + RT group, however the results are driven by a drop in mid-test values. In conclusion based on our findings performing a stretching intervention prior to a resistance training program doesn’t enhance the response to resistance training more than resistance training alone in older adults. However these findings may have been influenced by a low effort when performing the strength assessments, and other limitations identified, which is something that need to be considered with future research.

# Stretching as pre-intervention can improve aged muscle's response to a resistance training intervention

A Thesis

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In Partial Fulfillment of the Requirements for the Master of Science in Kinesiology

Biomechanics Concentration

by

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# Chapter 1. Introduction

## 1.1-General background

Sarcopenia is an age-related loss of skeletal muscle mass and function (Fielding et al., 2011). In the year 2000 sarcopenia was associated with a healthcare cost of $18.5 billion, representing 1.5% of the total healthcare expenditures in the United States (Janssen, Shepard, Katzmarzyk, & Roubenoff, 2004). As the population of older adults continues to increase, these expenditures will also continue to increase if ways to treat and prevent this condition aren’t found. In order to do that, there needs to be a better understanding of what sarcopenia is, and what the causes and implications of this condition are on the aging population.

The direct consequences of sarcopenia are loss of skeletal muscle mass and function. In older adults with class II sarcopenia there is greater likelihood of functional impairment and physical disability compared to older adults without sarcopenia, the likelihood is twice as great for men and three times as great in women (Janssen, Heymsfield, & Ross, 2002). Participants were considered to have class II sarcopenia when their skeletal muscle index was below 2 standard deviations of young adult values. Functional impairment and physical disability are measured by activities like climbing 10 stairs, lifting/carrying 10 pounds, standing from a chair, stooping/crouching/kneeling, and performing household chores; the inability to successfully perform these activities decreases quality of life and independence in older adults (Janssen et al., 2002).

There are many risk factors that may be involved in the onset and progression of sarcopenia. These risk factors can be grouped in different categories: age-related (sex hormones, apoptosis, mitochondrial dysfunction), disuse (immobility, physical inactivity), inadequate nutrition or malabsorption, neuro-degenerative diseases (motor neuron loss), and endocrine (insulin resistance, abnormal thyroid function, GH, IGF-1) (Cruz-Jentoft et al., 2010).

Resistance training is a commonly accepted treatment for older adults with sarcopenia. However, previous studies have shown that the response to this training is not as beneficial compared to younger adults. LaRoche, Roy, Knight, & Dickie (2008) in their study found that with 8 weeks (24 sessions) of isokinetic resistance training comparing old participants versus young, experimental versus control, and pre training versus post training, there was a trend ($p = 0.06$) in the three-way interaction for greater improvement in the young training (+16%) compared with the old training (+7%), young control (-4%), and old control groups (+6%), when calculating the pre-post % difference of peak knee extensor torque. Supporting this study, there are other studies that show young adults have around a 10% more increase in strength compared to old adults after a resistance training intervention, showing the blunted response of older adults to resistance training (Greig et al., 2011; Raue, Slivka, Minchev, & Trappe, 2009).

This diminished response is also seen in the increase in muscle mass following training. After resistance training interventions young participants show significant increases in muscle's cross sectional area (CSA), and older adults show little to no increases (Greig et al., 2011; Raue et al., 2009). In addition there is a difference in the growth in fiber type between young and older participants. After a resistance training protocol young participants had more growth in type II myofibers than their older counterparts, and were also the only ones who experienced type I myofiber growth (Kosek, Kim, Petrella, Cross, & Bamman, 2006; Kosek & Bamman, 2008).

The blunted hypertrophic response to resistance training is also seen acutely. Testing gene expressions by muscle biopsies hours after a resistance training intervention, show differences between old and young adults. Some mRNA genes have shown to significantly increase (TIMP, ACTC1) and some to decrease (REDD1, GDF8) as an acute response to resistance training in young adults, however these gene expressions show no significant difference before and after resistance training in older adults (Dennis et al., 2008; Greig et al., 2011).

As a possible explanation to the impaired response of aged muscle to resistance training, we propose a change in a mechanical property: muscle stiffness. Muscle stiffness is the muscle's ability to resist an external force that modifies its shape, and it is known to be greater in older populations when compared to young (Agyapong-Badu, Warner, Samuel, & Stokes, 2016; Ditroilo, Cully, Boreham, & De Vito, 2012; Palmer & Thompson, 2017).

Extracellular matrix (ECM) stiffness is greater in old muscle (Gao, Kostrominova, Faulkner, & Wineman, 2008). Engler et al. (2004) showed that increased stiffness in the ECM of muscle cells affects the interaction with the environment, causing cells to have a reduced striation. This effect of stiffness on cells can affect mechanotransduction causing stimuli to have less effect on muscle fibers. Therefore, for the same force stiffer cells will transmit weaker signals and have less effect on muscle fibers.

Stretching seems like a logical intervention for older adults with stiffer muscles, as it has shown to significantly decrease muscle stiffness (Akagi & Takahashi, 2014; Hirata, Kanehisa, & Miyamoto, 2017; Nakamura et al., 2014; Taniguchi, Shinohara, Nozaki, & Katayose, 2015). Having a pre intervention of stretching before a resistance training protocol, has shown to significantly increase muscle volume and strength in older women, compared to only having the resistance training protocol without any pre intervention (Hibbert, 2016). Because of the design and results (no pre-intervention at all in the resistance training control group and lack of statistically significant change in muscle stiffness in the stretching group) of Hibbert's study (2016), there are still uncertainties whether if stretching prior to resistance training helps the blunted response of resistance training in older adults. This is because the group that performed stretching before the resistance training protocol, overall had 8 more weeks of treatment. This leads us to question if this effect is seen because one group had a pre intervention and the other did not (regardless of the type of pre intervention), or if stretching should be considered as a pre intervention in older adults because it makes them respond better to resistance training interventions. Also the stretching intervention showed a trend for a decrease in muscle stiffness but it was not a statistically significant difference.

## 1.2- Hypothesis

The primary hypothesis of this study is that performing a stretching intervention prior to a resistance training program enhances the response to resistance training more than resistance training alone in older adults.

## 1.3- Purpose

The purpose of this study is to determine if a pre intervention of stretching followed by a resistance training intervention, produces a larger effect in older adults, than a pre intervention of resistance training followed by a resistance training intervention. The dependent variables that are going to determine this effect will be isokinetic plantarflexor torque and combined volume of the medial and lateral gastrocnemius.

## 1.4- Delimitations

1.) All participants will be healthy, indicating that they have not been previously diagnosed with Parkinson’s disease, stroke, peripheral artery disease, cancer, diabetes or osteoarthritis in lower extremities.

2.) All participants will be older adult women. Bioavailable testosterone has shown to predict skeletal muscle mass in male older adults (Iannuzzi-Sucich, Prestwood, & Kenny, 2002). Because of the known hormone differences between men and women, recruitment was delimited to women. Also women with class II sarcopenia have a greater likelihood of functional impairment and disability, being three times greater in women with class II sarcopenia than women with a normal skeletal muscle mass index (Janssen et al., 2002).

3.) Participants will have a Body Mass Index of less than 32 kg/m$^2$, as ultrasound images can be difficult to obtain in participants with higher BMI's

4.) The pre interventions will be 3 times a week for 8 weeks, either PNF stretching or resistance training.

5.) The intervention will be 3 times a week for 8 weeks of resistance training.

6.) Biomechanical analysis will focus on isokinetic plantarflexor torque and combined volume of the medial and lateral gastrocnemius.

## 1.5- Operational Definitions

Older adults: age 65 or higher.

Response to resistance training program: change in isokinetic plantarflexor torque and change in combined volume of the medial and lateral gastrocnemius.

Isokinetic plantarflexor torque: torque produced in a plantarflexion at 60 degrees per second.

Muscle volume: sum of the average of the cross-sectional area of each end of 6 sections (at 6 equidistant points along the length of the muscle) multiplied by the width of the CSA slice.

Combined volume of the medial and lateral gastrocnemius: sum of the muscle volume of the medial gastrocnemius and the lateral gastrocnemius.

## Chapter 2. Literature Review

It is our intention to determine if stretching as a pre intervention makes older adults respond better to a resistance training program than a pre intervention of resistance training. The following chapter will include information on 2.1-sarcopenia's background; 2.2- older adult's response to resistance training, 2.3-discussion on muscle stiffness: a possible cause for the blunted response to resistance training; and then 2.4- stretching: potential intervention to enhance the response to resistance training.

### 2.1-Sarcopenia's background

Sarcopenia is an age-related loss of skeletal muscle mass and function. It is a complex syndrome associated with muscle mass loss alone or in conjunction with an increased fat mass (Fielding et al., 2011). It is also associated with reduced endurance, physical inactivity, slow gait speed, and decreased mobility (Landi et al., 2012). To decrease the risk of disability, it is recommended to maintain a skeletal muscle mass relative to body height above 10.75 $kg/m^2$ and 6.75 $kg/m^2$ for older men and women respectively (Janssen et al., 2004).

According to the International Working Group on Sarcopenia, the diagnosis of sarcopenia is based on two factors: low whole body or appendicular fat-free mass, and poor physical functioning (Fielding et al., 2011). The European Working Group on Sarcopenia recommends using the presence of both low muscle mass and one criterion for low muscle function (strength or performance (e.g. usual gait speed)) for the diagnosis (Cruz-Jentoft et al., 2010).

The prevalence of sarcopenia is different for men and women, it varies in older adults that are institutionalized, and depending on the physical activity level. When classifying sarcopenia based on skeletal muscle mass index and utilizing a national representative sample, 52% and 69% of the older (≥60 years) men and women respectively had sarcopenia (Janssen et al., 2002). For institutionalized older adults, the prevalence was 68% and 21% among male and female residents respectively, showing the patients with sarcopenia were at the highest risk of death, regardless the age, gender and other confounding factors (Landi et al., 2012). In the Third National Health and Nutrition Examination Survey the prevalence of class II sarcopenia was about twice as great in the older adults who were inactive compared to those who were at least moderately active (≥3 times/week) (Janssen et al., 2002).

The high prevalence of sarcopenia among older adults and its association with functional impairment and disability, confirms that it is a significant public health problem (Fielding et al., 2011; Janssen et al., 2002). In 2000 a healthcare cost of $18.5 billion towards sarcopenia related conditions represented the 1.5% of the total healthcare expenditures in the United States (Janssen et al., 2004) and has been increasing as life expectancy has increased steadily with medical progression.

The risk factors of sarcopenia are multifactorial and can include: disuse, changing endocrine function, chronic diseases, inflammation, insulin resistance, and nutritional deficiencies (Fielding et al., 2011). According to Morley (2016) a decrease in motor unit number and lack of muscle usage, are the two most important risk factors of sarcopenia. Reduced muscle activation has shown to be responsible for decreased torque production in older adults compared to the younger population (Morse et al., 2004). Physical activity levels are associated with muscle activation capacity (Cook, Kanaley, & Ploutz-Snyder, 2014), and physical activity levels are low in older adults. In the Third National Health and Nutrition Examination Survey fewer than 2% of the older subjects reported to perform resistance exercise on a regular basis (≥1 per week) (Janssen et al., 2002).

As physical activity levels can be associated with muscle activation capacity, and with decreased torque, the decrease in torque can be attributed to a low physical activity level. However, this can only explain the decrease in torque to an extent, because as we will demonstrate later, even by increasing their physical activity levels with exercise interventions, older adults do not have the same benefits increasing torque as younger adults. Starting at 40 years of age, muscle strength decreases even in master level athletes (Faulkner, Davis, Mendias, & Brooks, 2008).

One of the consequences of sarcopenia is muscle mass loss. The loss in skeletal muscle mass is reflected in the lower muscle CSA shown in older adults compared to young adults. This difference in the lower limb CSA's between old and young adults can vary from 14-23% (Narici, Maganaris, Reeves, & Capodaglio, 2003; Nilwik et al., 2013; Palmer & Thompson, 2017; Raue et al., 2009). At a smaller level, findings suggest that sarcopenia involves a loss of sarcomeres in series as well as in parallel, which can be seen in shorter fascicle lengths and smaller pennation angles when comparing older adults with younger adults (Morse, Thom, Birch, & Narici, 2005; Narici et al., 2003; Stenroth, Peltonen, Cronin, Sipila, & Finni, 2012).

The loss in skeletal muscle function is also a consequence of sarcopenia. Isometric and dynamic strength begins to decline at age 50 and correlates significantly with the type II muscle fiber atrophy (Larsson, Grimby, & Karlsson, 1979). When comparing older participants with younger adults, Nilwik et al. (2013) found that the mean muscle fiber size was about 20% smaller, type II muscle fiber size was substantially smaller, and older adults had lower percentage of type II fibers as well as the percentage of muscle area occupied by type II muscle fibers.

## 2.2-Older adult’s response to resistance training

Structured resistance training is important because it causes increases in skeletal muscle size and strength (Ahtiainen et al., 2016). This would benefit older adults because of the steady decrease in muscle mass with age, although they may receive less benefit from this type of training.

Churchward-Venne et al. (2015) found that there were no nonresponders to resistance training in older adults. Data showed after interventions of 12 and 24 weeks of resistance training to a group of 110 older adults, that although there was a large variability among the response of the participants,  they had improvements in the following variables (showed in average increased percentage for 12 and 24 weeks of intervention): lean body mass (12 weeks: 1.8%, 24 weeks: 2.3%), type I muscle fiber size (12 weeks: 8%, 24 weeks: 9%), type II muscle fiber size (12 weeks:

17%, 24 weeks: 23%), muscle strength (12 weeks: 23%, 24weeks: 35%), and physical function (12 weeks: 8.2%, 24 weeks: 17.8%).

Resistance training is a commonly accepted treatment for sarcopenia, but it has been shown that despite the positive effects of resistance training in older adults, the response is not as effective as that in young adults. After resistance training interventions, young participants show significant increase (5-6.2%) in muscle CSA, and older adults show lower to no increase (0-2.5%) (Greig et al., 2011; Raue et al., 2009). Also there is a difference in the growth in fiber type. After a resistance training protocol young participants had greater growth (40%) in type II myofibers compared to their older counterparts (19.5%), and were also the only ones who experienced type I myofiber growth (Kosek et al., 2006; Kosek & Bamman, 2008).

Acute hypertrophic response is impaired in older adults. Testing gene expressions by muscle biopsies hours after a resistance training intervention, show differences between old and young adults. Some mRNA genes have shown to significantly increase (TIMP, ACTC1) and some to decrease (REDD1, GDF8) as an acute response to resistance training in young adults, however this gene expressions show no significant difference before and after resistance training in older adults (Dennis et al., 2008; Greig et al., 2011).

Strength increase is also different between young and old populations. LaRoche et al. (2008) found that with 8 weeks (24 sessions) of isokinetic resistance training comparing old participants versus young, experimental versus control, and pre training versus post training, there was a trend ($p = 0.06$) in the three-way interaction for greater improvement in the young training (+16%) compared with the other groups: old training (+7%), young control (-4%), and old control (+6%), when measuring peak knee extensor torque. Supporting this there are other studies that show around a 10% difference in the increase in strength between old and young adults after a resistance training intervention, suggesting a blunted response of older adults to resistance training (Greig et al., 2011; Raue et al., 2009). Additionally, many studies have shown less improvements in older adult population, and even if this lower response is not significant, there is a trend to have a diminished response (e.g. (Bickel, Cross, & Bamman, 2011; Mayhew, Kim, Cross, Ferrando, & Bamman, 2009).

Not only is the strength increasing effect between older and younger adults different, but the cause seems to be different as well. Young men have greater increase in muscle mass, there are identified differences with older adults in gene expression that make young men have a greater magnitude of hypertrophic response to exercise (Dennis et al., 2008; Stec, Mayhew, & Bamman, 2015), helping them increase their strength. On the other hand, older men's improvement in strength is caused by improved muscle activation, with a lower muscle hypertrophy during the initial weeks of training when compared to young men (Walker & Hakkinen, 2014).

While the increase in strength in older adults may benefit the daily living activities, not being able to increase or maintain muscle mass might present a problem. Muscle mass relative to body height is inversely associated with all-cause mortality in older adults, being the all-cause mortality risk significantly higher in the lowest muscle mass index quartile compared to the highest muscle mass index quartile (Srikanthan & Karlamangla, 2014).

## 2.3-Muscle stiffness: a possible cause for the blunted response to resistance training

Stiffness is the relationship between the deformation of a body and a given force. In the human body it can be described from the level of a single muscle fiber, to the entire body (Butler, Crowell, & Davis, 2003). Muscle stiffness is the muscle's ability to resist an external force that modifies its shape, and is measured in N/m (Agyapong-Badu et al., 2016).

Muscle stiffness can be measured *in vivo* in different ways: the free-oscillation technique, passive-elastic stiffness, and ultrasound shear wave elastography. The free-oscillation technique requires a probe with an accelerometer, and stiffness is calculated as a ratio between the force applied (the mass of the probe multiplied by the positive peak of the damped acceleration) and the muscle deformation (the double integer of the acceleration signal) (Ditroilo et al., 2012). Passive-elastic stiffness can be calculated with a dynamometer as the ratio of the change in the passive resistive force to the change in the ankle angle through the last ½ of the full stretch range of motion (Gajdosik, Vander Linden, McNair, Williams, & Riggin, 2005). Ultrasound shear wave elastography, is calculated by the rate at which a tissue responds to an impulsive excitation, and the speed at which shear waves propagate away from the region of excitation (Palmeri, Wang, Dahl, Frinkley, & Nightingale, 2008).

A hypothesis to the cause for the impaired response of the aged muscle to resistance training could be the increase in muscle stiffness. It is known that muscle stiffness is greater in older population (Agyapong-Badu et al., 2016; Ditroilo et al., 2012; Palmer & Thompson, 2017).

The structure likely causing this increase in stiffness is the ECM, which is stiffer in old muscle (Gao, Kostrominova, Faulkner, & Wineman, 2008). Excessive ECM deposition in aged muscle is correlated with an increase in muscle stiffness (Lacraz et al., 2015). Increase in ECM components such as hydroxyproline and advanced glycation end-products are seen with increased modulus, as well as being correlated with an increase in collagen deposition (Lacraz et al., 2015; Wood et al., 2014).

Engler et al. (2004) showed that increased stiffness in the ECM cells affects the interaction with the environment, causing cells to have a reduced striation. Changes in ECM mechanical properties have the potential to influence the ability of the muscle to respond to changes in external loading and the force transmission to the skeleton (Wood et al., 2014). ECM is a mechanoreceptor that couples mechanical information from outside the cell with intracellular biochemical events, this process of converting mechanical energy into biological events is known as mechanotransduction (Hornberger & Esser, 2004).

Hornberger & Esser (2004) suggest that mechanotransduction could ultimately regulate protein synthesis. And this could be through the functional contribution of the integrin-associated focal adhesion kinase (FAK) in the modulation of load-induced hypertrophy response of the muscle (Klossner, Durieux, Freyssenet,

& Flueck, 2009). FAK is involved in the early steps of mechanotransduction in striated muscle (Durieux, Desplanches, Freyssenet, & Fluck, 2007). In aged rat's muscle pressure loading decreased the extent of FAK phosphorylation when comparing it with young muscle (Rice et al., 2007). The decrease in phosphorylation in aged muscle indicates it is not receiving the signal in the same way as the young muscle, instead is receiving a weaker signal, and this could be caused by the increased stiffness of the ECM.

These changes to the mechanical properties of the muscle could explain the blunted response of older adults to resistance training. As this is affecting protein synthesis, and protein synthesis modulates muscle's hypertrophy.

## 2.4-Stretching: potential intervention to enhance the response to resistance training

Stretching has shown to increase flexibility (range of motion) in older adults even after only one repetition (Feland, Myrer, & Merrill, 2001). When comparing static stretching with proprioceptive neuromuscular facilitation (PNF), PNF stretching has shown to have greater effects on range of motion (Funk, Swank, Mikla, Fagan, & Farr, 2003), in both trained and untrained individuals (Hindle, Whitcomb, Briggs, & Hong, 2012).

PNF stretching uses the body's neuromuscular reflex pathways to cause relaxation in the muscle. Tension developed while contracting and stretching a muscle facilitate relaxation through 2 mechanisms: reciprocal inhibition and autogenic inhibition (Ninos, 1996). With reciprocal inhibition the voluntary contraction of the antagonist muscle being stretched leads to reduced activation in the targeted muscle (Sharman, Cresswell, & Riek, 2006). In autogenic inhibition the tension in the muscle during the contraction elicits activity in the Golgi tendon organs, which inhibits contraction of the muscle and facilitates relaxation (Ninos, 1996; Sharman et al., 2006).

There are 3 main PNF stretching techniques: contract-relax, agonist-contract, and contract-relax-contract. These techniques facilitate relaxation by the following pathways respectively: autogenic inhibition, reciprocal inhibition, and both autogenic and reciprocal inhibition (Ninos, 1996). The contract-relax method consists of a static stretch performed by the clinician, followed by an isometric contraction of the muscle being stretched, and finally an additional static stretch by the clinician. The agonist-contract technique is characterized by a contraction of the agonist muscle against a resistance, while simultaneously stretching and relaxing the antagonist muscle (Olivo & Magee, 2006). The contract-relax-contract technique has the clinician passively moving the extremity until resistance is felt, an isometric contraction of the antagonist muscles is followed by a contraction of the agonist muscles against resistance, and ends with relaxation of the extremity (Surburg & Schrader, 1997).

Stretching interventions have also shown to significantly decrease muscle stiffness (acute effect of ~14% decrease) (Akagi & Takahashi, 2014; Hirata et al., 2017; Nakamura et al., 2014; Taniguchi et al., 2015). One theory for this effect is the lengthening of muscle fascicles (Fowles, Sale, & MacDougall, 2000). When fascicles are lengthened, the muscle is placed in a different point in the passive torque curve, when tested at the same joint angle before and after stretching, the muscle is effectively at a shorter muscle length, resulting in a decrease in muscle stiffness.

Hibbert (2016) concluded with her training investigation that having older adults participate in a long term stretching intervention prior to a resistance training intervention, significantly increased their response to resistance training. Three groups were defined in the investigation: young women (young RT only) that did 8 weeks of resistance training, old women (old RT only) that did 8 weeks of resistance training, and old women (old stretch + RT) that performed 8 weeks of PNF stretching followed by 8 weeks of resistance training. Hibbert (2016) found that there was a significant difference between both the old women's groups in the percent change for the gastrocnemius volume and the platarflexor torque, both being higher in the old stretch + RT group. For muscle volume, old RT only had a ratio of change (posttest/pretest) of 1±0.06, while old stretch + RT had a ratio of change of 1.11±0.14. For plantarflexor torque the ratios of change were 1.05±0.39 and 1.32±0.21 for the old RT only and old stretch + RT respectively. These results showed that the old stretch + RT group had a better response to resistance training than the old RT only group. Muscle stiffness decreased with the 8 weeks of stretching, however this decrease was not statistically significant. It is not clear if the old stretch + RT responded better to exercise because of our hypothesis, that stretching decreased muscle stiffness (even if it was not statistically significant) and improved the response to resistance training, or simply because overall this group had 8 weeks of physical activity (regardless the type of physical activity) allowing improvement through some other unknown mechanism that primed the muscles for better response.

### 2.5-Summary

In summary, we know that muscle mass and function loss is a big problem in older adults affecting their physical function. Resistance training interventions have been widely investigated, and the effect they have in this population have shown to be positive but blunted compared to young adults. Greater muscle stiffness in older adults could be aiding to cause this blunted response to resistance training, and as it has been shown that stretching can decrease muscle stiffness, it may benefit older adults. We hypothesize that performing a stretching intervention prior to a resistance training program will enhance the response to resistance training more than resistance training alone in older adults. With this knowledge, training programs to counteract the symptoms of sarcopenia could be modified to become more beneficial.

## Chapter 3. Methods

### 3.1-Participants

We recruited 16 women (average age, 75.44 years) that were randomly divided in 2 groups: resistance training + resistance training (RT + RT), and stretching + resistance training (S + RT). Randomization of the group assignment was done before participant's recruitment. Exclusion criteria includes: Parkinson's disease, stroke, peripheral artery disease, cancer, diabetes, osteoarthritis in the lower extremities, high blood pressure, significantly overweight (BMI>32).

Participant's level of physical activity was measured during their first visit. The self-administered Physical Activity Scale for the Elderly (PASE) test was used. This instrument contains items about self-reported occupational, household, and leisure activities over a one-week period (Washburn, Smith, Jette, & Janney, 1993). A higher score indicates a higher physical activity level.

All participants provided written informed consent for their participation in the investigation. They were aware that their participation in the study was voluntary and they could choose to withdraw at any moment. The East Carolina University Institutional Review Board approved all experimental procedures.

### 3.2-Instruments

To measure the isokinetic plantarflexor torque participants performed 1 set of 5 repetitions of maximal isokinetic plantarflexion at 60 degrees per second in an isokinetic dynamometer (HUMAC NORM Testing & Rehabilitation System, CSMI Medical Solutions, Stoughton, Massachusetts). Participants were laying down in supine position with their legs straight, and their right foot strapped to the dynamometer. They had the opportunity to practice the protocol with 5 repetitions at a 50% effort, to get familiarized with the movement.

The muscle volume of both lateral and medial gastrocnemius was collected using a b-mode ultrasound (Aixplorer, SuperSonic Imagine, France). Ultrasound has been validated by Infantolino, Gales, Winter, & Challis (2007) as an accurate method for estimating muscle volume. Analysis in 8 young participants was done to determine this study tester's reliability measuring muscle volume. The test re-test ICC (SEM) values were 0.97 (7.7) and 0.91 (5.04) for the medial and lateral gastrocnemius respectively. The protocol consisted of collecting a series of 6 equidistant panoramic cross sectional images of each muscle (Figure 1). The first step was to mark the most distal and proximal part of the muscle, then take off 1 cm on each side and place a mark (blue marks on Figure 1). Measure the length between the last 2 marks, and divide the muscle in 5 intervals of the same width (red marks on Figure 1), which determined the 6 places where the cross sectional images were taken from.

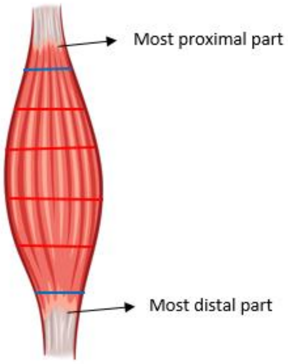

**Figure 1.** Diagram of protocol for muscle volume measurement.

Muscle stiffness was measured using ultrasound shearwave elastography. Elastography has been shown to be a reliable method for determining muscle stiffness (Eby et al., 2013). . This study tester's reliability with muscle stiffness was measured in 8 young participants. The ICC (SEM) values for the medial and lateral gastrocnemius stiffness were 0.15 (12.09) and 0.07 (11.36) respectively. The scale was set for the maximum to be 100kPa, and the probe was placed in the middle of the muscle between the third and the fourth mark. Each image was analyzed using a circular region of interest with a 2 mm diameter taken in the middle of the muscle. The value for each muscle was determined by the average of 3 images.

### 3.3-Procedures

Participants attended the East Carolina University Biomechanics Laboratory for strength, volume, and muscle stiffness testing, prior to any type of intervention (week 0), after the pre-intervention (week 8) and after the intervention (week 16) (Figure 2).

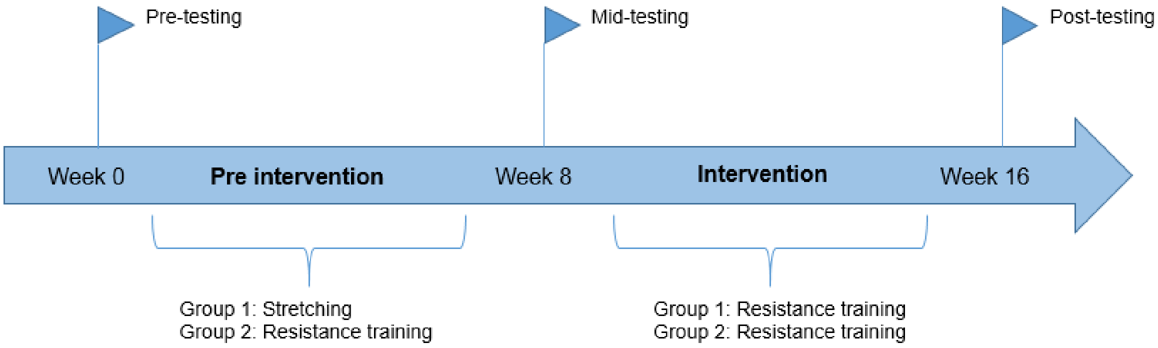

**Figure 2.** Study design.

For the RT + RT group the pre-intervention and the intervention had the same characteristics. Participants attended the FITT building (Fitness, Instruction, Testing and Training facility) 3 days a week for 8 weeks to perform a general lower extremity strength training program, with at least one rest day between sessions. The sessions had a 5-minute warm-up on a cycle ergometer, and 3 sets of 10 repetitions of 6 resistance training exercises: knee flexion (Figure 3), knee extension (Figure 4), leg press (Figure 5), calf press (Figure 6), band-resisted plantarflexion (Figure 7), and band-resisted dorsiflexion (Figure 8).

On the first day of training participants were tested for 1 repetition maximum (RM) for each exercise after familiarization and warm-up. Participants were allowed their preferred rest period, up to one minute, between repetitions. The intensity for the training sessions were determined as percentages of the 1RM. In a meta-analysis by Steib, Schoene, & Pfeifer (2010) training at high intensity showed to produce the greatest benefits in maximal muscle strength, where optimum range was from 60% to 80% of 1 RM. The 8 weeks interventions had changes in the intensity every 2 weeks as followed: first 2 weeks they performed the exercises at 50, 60 and 70% of their 1RM; the next 2 weeks it increased to 60, 70, and 80% of their 1RM; at the beginning of the following 2 weeks maximal testing took place to adjust their 1RM and they trained at 50, 60, and 70% of their new 1RM; and the last 2 weeks it increased again to 60, 70, and 80% of their 1RM (Hibbert, 2016). This protocol was done for the pre intervention and the intervention. All training sessions were supervised.

**Figure 3.** Knee flexion.

**Figure 4.** Knee extension.

**Figure 5.** Leg press.

**Figure 6.** Calf press.

**Figure 7.** Band-resisted plantarflexion.

**Figure 8.** Band-resisted dorsiflexion.

For the S + RT group, the resistance training intervention was the same as described above for the RT + RT group. For the stretching intervention, participants performed the contract-relax method of PNF stretching during 8 weeks, 3 days per week. The protocol consisted of 30 seconds of passive stretching, where the participant was passively moved to the end range of motion, then was asked to push against the stretch for 10 seconds, and ended with 30 seconds of passive stretching again, taking the stretch to a new end point. The following muscles were stretched bilaterally: hamstrings (Figure 9), piriformis (Figure 10), quadriceps (Figure 11), gastrocnemius (Figure 12), and hip extensors (Figure 13).

**Figure 9.** Hamstrings PNF stretching.

**Figure 10.** Piriformis PNF stretching.

**Figure 11.** Quadriceps PNF stretching.

**Figure 12.** Gastrocnemius PNF stretching.

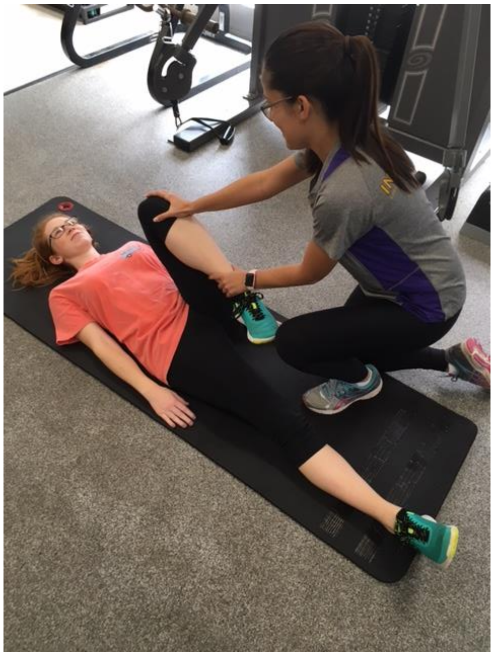

**Figure 13.** Hip extensors PNF stretching.

## 3.4-Data processing

Images collected with the ultrasound were analyzed using image processing software (Osirix Imaging Software, Pixmeo, Bern, Switzerland; ImageJ, U. S. National Institutes of Health, Bethesda, Maryland) to determine the CSA of the muscle. Muscle volume was calculated adding the volume of each interval of the muscle, averaging the CSA of each end of the sections and multiplying it by the width of the interval. The volume of the 1 cm left off at the end of each side of the muscle was calculated as a cone. All this was done with a formula (Figure 14) in MatLab R2016a.

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

**Figure 14.** Muscle volume calculation- CSA S*n* is the cross sectional area of the corresponding slice, sw is the interval width.

## 3.5-Statistical analysis and study design

Statistical significance level was set *a priori* to $p<0.05$. 2 x 2 mixed-model ANOVAs were performed to determine differences following the interventions between time, groups, and possible time*group interactions for the dependent variables: isokinetic plantarflexor torque and muscle volume. One comparison was made between the pre and post-test of both groups, to determine the effect of the

16 weeks of pre-intervention with the intervention. Additionally to compare between equal length resistance training interventions, the first 8 weeks of the RT + RT group (pre-intervention)was compared with the last 8 weeks of the S + RT group (the resistance training portion). All the statistical analysis were conducted using SPSS Statistics v.23 (SPSS Inc., Chicago, Illinois). This study design is categorized as quasi-experimental.

# Chapter 4. Results

Out of the 16 recruited participants two (one from each group) were unable to complete the 16 week intervention, so were excluded from the statistical analysis. The reasons why they were unable to complete the study were injuries and illness unrelated to the intervention of the present study. The adherence (sessions attended) average was 89.29±8.87% for the RT + RT group, and 95.24±3.34% for the S + RT. There were no significant differences between the groups in the demographic information variables at baseline (Table 1). Resistance training volume load values are presented in Table 2 for the 16 weeks of RT + RT group and the resistance training portion of the S + RT group.

Table 1. Participant's demographic information. Values presented as mean ± standard deviation.

| Group | n | Age (years) | Height (cm) | Weight (kg) | BMI (kg/m²) | PASE score |
|---|---|---|---|---|---|---|
| RT + RT | 7 | 78.43±9.20 | 157.6±6.47 | 67.06±11.03 | 26.91±3.40 | 140.66±106.25 |
| S + RT | 7 | 73.14±3.98 | 159.93±5.48 | 65.11±8.30 | 25.52±3.76 | 115.63±73.36 |

Table 2. Resistance training intervention volume load for each exercise by group. Values presented as mean ± standard deviation.

| | | Weeks 1 & 2 | Weeks 3 & 4 | Weeks 5 & 6 | Weeks 7 & 8 | Weeks 9 & 10 | Weeks 11 & 12 | Weeks 13 & 14 | Weeks 15 & 16 |
|---|---|---|---|---|---|---|---|---|---|
| Leg curl | RT+RT | 697.86 ± 179.86 | 801.43 ± 203.67 | 788.57 ± 144.82 | 918.57 ± 166.68 | 901.43 ± 211.14 | 1045.71 ± 251.25 | 987.14 ± 257.92 | 1154.29 ± 301.21 |
| | S+RT | | | | | 625.71 ± 101.14 | 732.86 ± 119.82 | 777.14 ± 151.41 | 905.71 ± 180.26 |
| Leg extension | RT+RT | 633.57 ± 263.01 | 740.57 ± 309.80 | 757.86 ± 219.96 | 886.43 ± 251.82 | 872.14 ± 187.55 | 1017.14 ± 227.36 | 967.14 ± 192.16 | 1130 ± 224.50 |
| | S+RT | | | | | 565.71 ± 102.24 | 675.71 ± 97.31 | 738.57 ± 87.97 | 850.71 ± 126.83 |
| Leg press and calf press | RT+RT | 1544.28 ± 550.93 | 1914.29 ± 556.14 | 1795 ± 345.27 | 2082.86 ± 415.56 | 1900 ± 350.67 | 2211.43 ± 417.75 | 2088.57 ± 496.47 | 2411.43 ± 552.25 |
| | S+RT | | | | | 1571.43 ± 210.98 | 1827.14 ± 260.88 | 1760 ± 211.03 | 2027.14 ± 292.10 |

Volume load was calculated as: sets x repetitions x load (lb)

### 4.1- Isokinetic plantarflexor torque

There was no significant difference (p=0.625) in torque between the groups at baseline. Mean values for each group in the different time points are displayed on Table 3, and individual values for each participant on Figure 15. There was no significant time by group interaction (p=0.578) when comparing the entire training period, there was no main effect of groups (p=0.985), and there was no main effect of time (p=0.893). The percent change distribution from post to pre-test for each group is displayed on Figure 16.

A significant time by group interaction (p=0.040) was found when comparing the first 8 weeks of RT + RT and the last 8 weeks of S + RT. This analysis was done to see the changes in the resistance training portion of the S + RT after performing a pre-intervention of stretching, however the results are driven by a drop in mid-test values.

Table 3. Ankle plantarflexor torque for pre, mid and post-testing. Values presented as mean ± standard deviation.

| Group | Pre-test torque (Nm) | Mid-test torque (Nm) | Post-test torque (Nm) |
|---|---|---|---|
| RT + RT | 47.51 ± 15.84 | 34.86 ± 16.72 | 52.34 ± 15.12 |
| S + RT | 51.49 ± 13.74 | 36.86 ± 13.53 | 48.53 ± 14.78 |

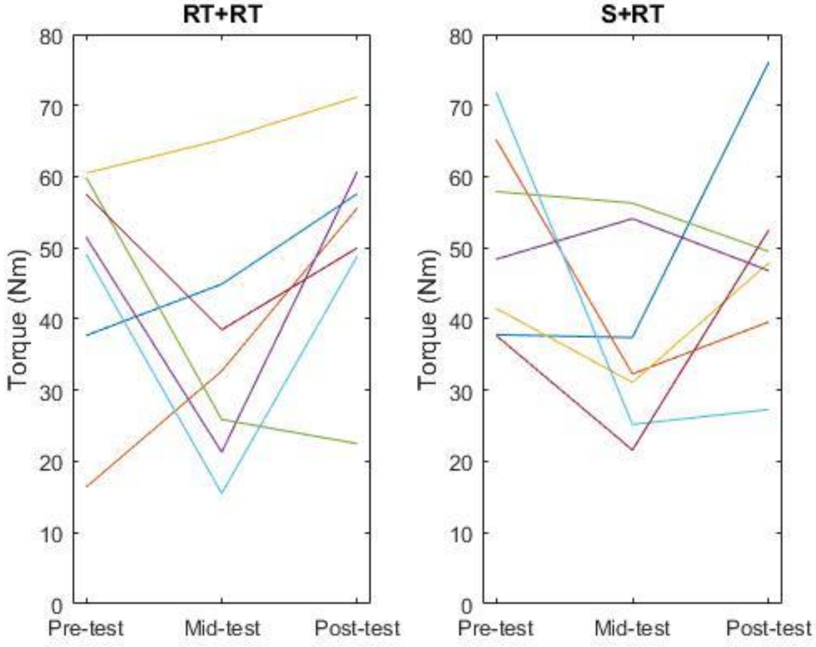

**Figure 15.** Ankle plantaflexor torque by group. Each line represents the value of each individual participant and the change it had in time from pre to post-test. The data to the left is from the RT + RT group that performed 16 weeks of resistance training. The data to the right is from the S + RT group that performed 8 weeks of stretching (from pre to mid-testing) followed by 8 weeks of resistance training (from mid to post-testing).

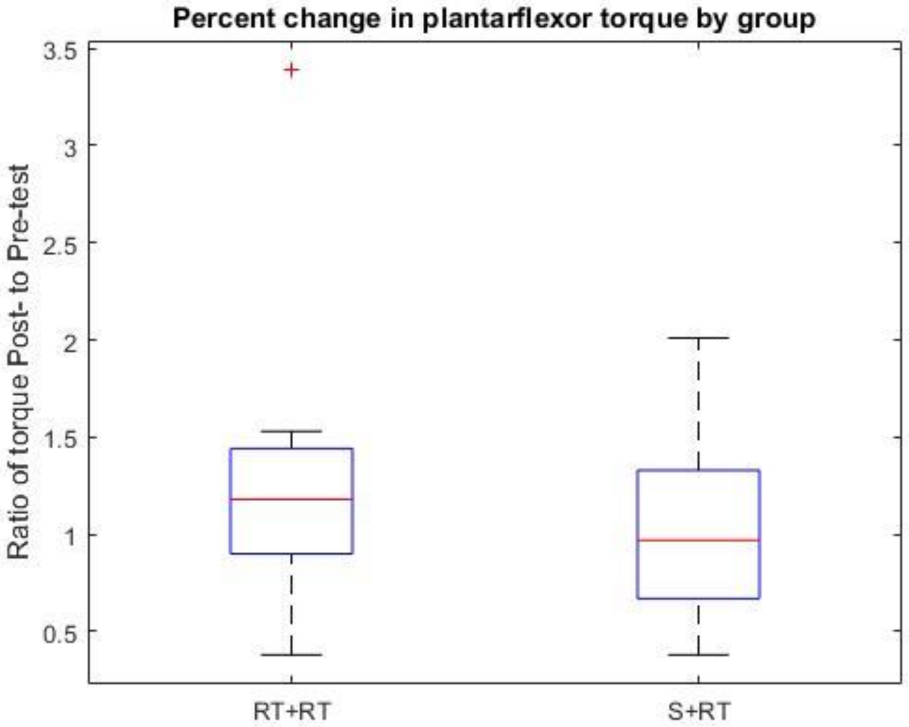

**Figure 16.** Ratio of post-test/pre-test of torque by group. The box represents the middle 50% of the data (IQR). The red line represents the median, and the lines extending from the top and bottom of the boxes represent the maximum and minimum values respectively. The cross at the top of the plot is an outlier, defined as a value more than one and a half times the IQR from the top of the box.

## 4.2- Gastrocnemius muscle volume

There was no significant difference (p=0.802) in muscle volume between the groups at baseline. Mean values for each group in the different time points are displayed on Table 4, and individual values for each participant on Figure 17. There was no significant time by group interaction found (p=0.503) when comparing the entire training period, there was no significant main effect of groups (p=0.679), and there was no significant main effect of time (p=0.119). The distribution of percent changes from post to pre-test for each group is displayed on Figure 18. The time by group interaction was not significant (p=0.882) either when comparing the first 8 weeks of RT + RT with the last 8 weeks of S + RT (resistance training portion), there was no significant main effect of groups (p=0.71), and there was no main effect of time (p=0.35).

Table 4. Gastrocnemius muscle volume for pre, mid, and post-testing. Values presented as mean ± standard deviation.

| Group | Pre-test volume (cm³) | Mid-test volume (cm³) | Post-test volume (cm³) |
|---|---|---|---|
| RT + RT | 197.47 ± 49.45 | 203.61 ± 36.47 | 206.97 ± 44.17 |
| S + RT | 192.39 ± 17.59 | 191.86 ± 17.65 | 196.35 ± 17.95 |

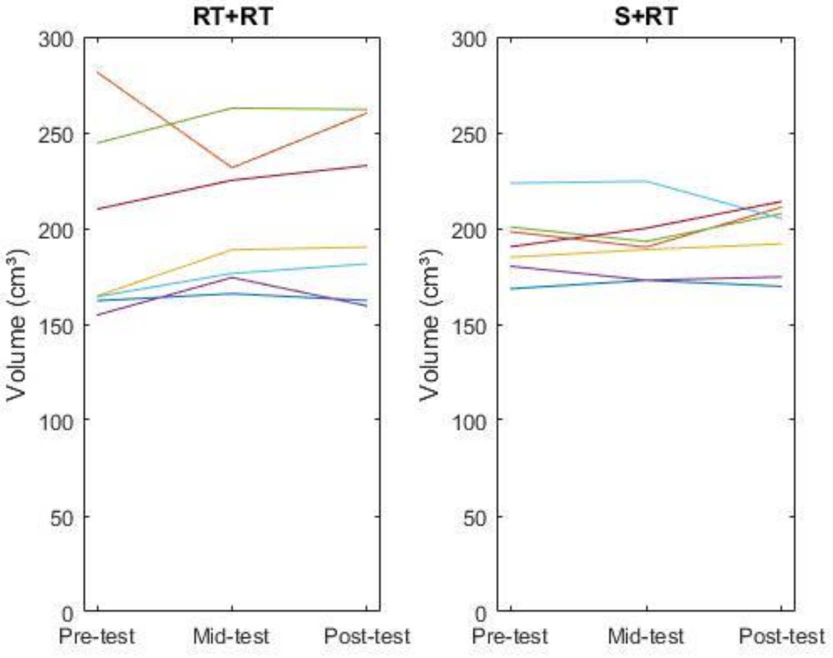

**Figure 17.** Gastrocnemius combined muscle volume by group. Each line represents the value of each individual participant and the change it had in time from pre to post-test. The data to the left is from the RT + RT group that performed 16 weeks of resistance training. The data to the right is from the S + RT group that performed 8 weeks of stretching (from pre to mid-testing) followed by 8 weeks of resistance training (from mid to post-testing).

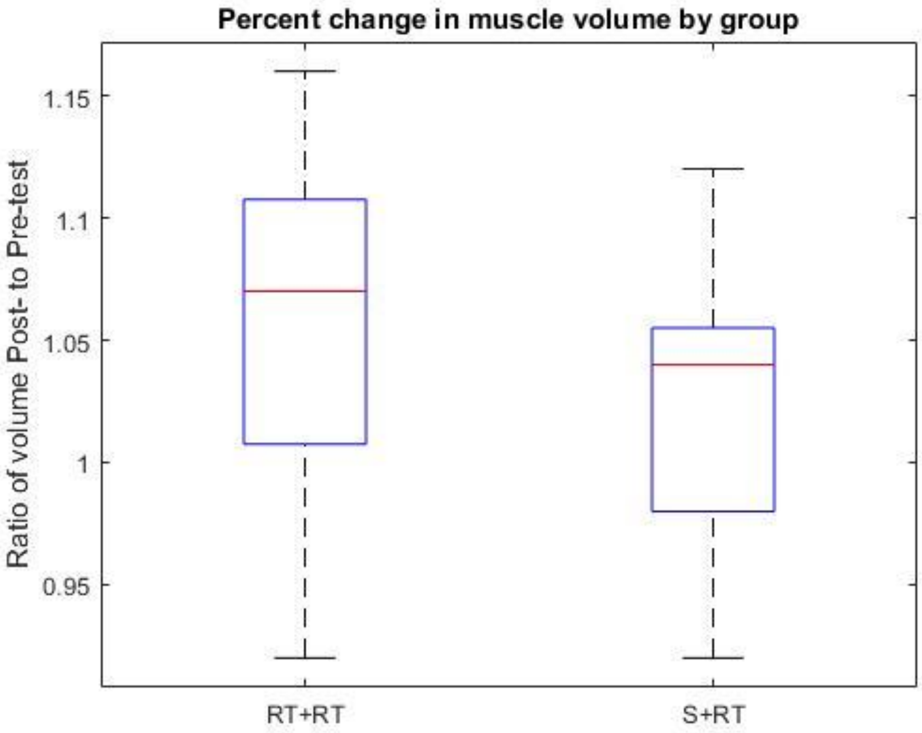

**Figure 18.** Ratio of post-test/pre-test of muscle volume by group. The box represents the middle 50% of the data. The red line represents the median, and the lines extending from the top and bottom of the boxes represent the maximum and minimum values respectively.

## 4.3- Muscle stiffness

Gastrocnemius muscle stiffness values are presented on Table 5. As a result of a software malfunction, pre-test data for stiffness is currently unrecoverable therefore it is not reported in this document. For the medial gastrocnemius there was no significant time by group interaction found (p=0.968) when comparing the mid-test with the post-test, either was there a main effect of time (p=0.30) or group (p=0.584). When looking at the lateral gastrocnemius there was no significant time by group interaction either (p=0.534) nor main effect of time (p=0.675) or group (p=0.427).

Table 5. Gastrocnemius muscle stiffness for mid and post-testing. Values presented as mean ± standard deviation.

| Group | Medial gastrocnemius stiffness (kPa) | | Lateral gastrocnemius stiffness (kPa) | |
|---|---|---|---|---|
| | Mid-test | Post-test | Mid-test | Post-test |
| RT + RT | 20.52 ± 6.96 | 17.65 ± 4.10 | 15.83 ± 5.84 | 16.14 ± 8.79 |
| S + RT | 22.31 ± 10.36 | 19.65 ± 8.74 | 19.47 ± 4.89 | 17.87 ± 6.70 |

# Chapter 5. Discussion

The present study quantified plantarflexor torque, and combined gastrocnemius muscle volume in older adult population, to determine if a pre-intervention of stretching followed by a intervention of resistance training would have a better effect increasing those variables than a pre-intervention of resistance training followed by the same resistance training intervention. With the present findings we observe that both protocols have the same effect (no significant differences) on plantarflexor torque and muscle volume when comparing the 16 weeks of resistance training against the combination of 8 weeks of PNF stretching followed by 8 weeks of resistance training. The only significant time by group interaction found was when comparing torque values of the pre-intervention of RT + RT group with the intervention of S + RT group.

The non-difference observed in the 16 week period shows that the participants had the same effect on muscle volume and platarflexor torque independently if they performed 16 or 8 weeks of resistance training. However independently of the group there was no significant main effect of time for either variable. The time by group significant interaction was found because the torque values for the first 8 weeks of resistance training of the RT + RT group decreased, while the torque values for the 8 weeks of resistance training preceded by 8 weeks of stretching in the S + RT group increased.

Even though the changes found in the present study were non-significant, the percent change in torque observed in the current study are similar to previous findings. The RT + RT group had an average percent change in the 16 weeks (48 sessions) of training of 35.8% (ES 0.2). In a previous study older adults showed a percent change between 23-35% when performing between 24 and 72 sessions of resistance training (Churchward-Venne et al., 2015). Also a 25.9% increase in strength was seen in older women over 60 years with a 20-24 week training protocol (Ahtiainen et al., 2016). Although it is important to note that the 35.8% increase seen in this study is being highly increased by an outlier data point. When looking at the median the percent increase is 17.9%, which is still similar to the values seen in the studies mentioned before.

When looking at 8 week interventions (24 sessions) the results are similar to the percent change found in the 16 weeks (8 weeks of resistance training) of the S + RT. The percent increase in torque during the 16 weeks of S + RT was 5.27% (ES -0.11), which is similar to the 7% found in an 8 week intervention (LaRoche et al., 2008). Also in a 12 week (30 sessions) resistance training program an increase in peak torque during leg flexion of 10.4% was seen, and this was in a male young adult population (Glowacki et al., 2004).

The percent change in muscle volume of the current study was 5.64% (ES 0.58) and 2.22% (ES 0.29) for the RT + RT group and S + RT group respectively. In a previous study were older adults performed 12 weeks (36 sessions) of high-intensity resistance training program, muscle size measured with a CT scan showed no increase (Raue et al., 2009). Also in a 16 week resistance training study when thigh lean mass was measured with a DXA, older adults had an increase of 4.2% (Bickel et al., 2011). A factor possibly explaining this very low to non-response in load-mediated hypertrophy is the presence of nonresponder participants. Previous studies have shown biochemical differences identified in some participants that could explain a no response to an exercise program. After a 16 week resistance training protocol using a cluster analysis based on the magnitude of myofiber hypertrophy, 17 out of 66 participants were categorized as nonresponders, indicating there was a difference in factors that influence the myofiber downstream of the mechanical loading in these participants (Bamman, Petrella, Kim, Mayhew, & Cross, 2007).

Another factor possibly explaining the low increase in muscle volume is the fact that all the participants of the present study are women. In previous research a trend has been shown that older women have a lower response to increasing muscle volume than older men after a resistance training protocol of 9 weeks (27 sessions) (Ivey et al., 2000).

The results of the present study show important differences from the findings of Hibbert (2016). Hibbert's study found a large increase in torque (32%) and volume (11%) with an experimental group protocol same to the one in this study with 8 weeks of stretching followed by 8 weeks of resistance training. When our findings correspond to 5.27% and 2.22% for torque and volume respectively. A possible explanation of this difference is the fact that the participants from the present study where over average on their physical activity level according to their gender and group age, which could have affected their level of response. Norms for the PASE established that for general population women from ages 70-75 have mean values of 89.1±55.5 and ages from 76-100 have mean values of 62.3±50.7, meanwhile our participants have a mean of 128.2±88.7 (Washburn et al., 1993). Overall physical activity has a significant effect on older women’s muscle strength (Rantanen et al., 1999), which could explain why participants from the present didn’t get significant increases in the studied variables.

The time by group significant interaction found in the torque values we believe correspond to a limitation of the present study. The interaction was found when comparing mid to pre-testing from the RT + RT group against mid to post-testing from the S + RT. The values of the platarflexor torque during the mid-testing seem to be affected by an unknown variable, because most of the values are lower than the pre and post-testing for both groups, and this does not match the increase or maintenance expected from the pre-interventions.

A limitation of the training protocol of the present study was the increase in load. Participants would train 2 weeks at 50, 60, and 70% of the 1RM and the following 2 weeks at 60, 70, and 80% of the 1RM. After those 4 weeks when the 1RM was assessed again they would repeat the same protocol. In some cases the increase in 1RM was small enough such that instead of increasing they would decrease or maintain the weight lifted as the 60, 70, and 80% of the previous 1RM was bigger than the 50, 60, and 70% of the new 1RM. Some participants may not have been completely comfortable performing the 1RM testing, which could have explained the low increases in 1RM. A possibility would be to use prediction equations, which have shown moderate to high predictive validity on machines in older adults (Knutzen, Brilla, & Caine, 1999).

The effort given by the participants during the strength assessments is a limitation identified in this study. The fact that there was a low mid-testing could explain this as the change in torque wasn't expected based on previous literature. Also the low increases seen in some participants with the 1RM testing suggest a low effort. Participants were verbally encouraged to give their maximal effort during the strength assessments, however those efforts could be improved. Motivation in the RT + RT group could have been also affected by the fact that this group of participants performed the same exercises during 48 sessions, and could have seemed monotonous.

Another limitation of the present study was that the 1RM for each machine used in the intervention wasn't assessed at the end of the intervention. There is 1RM data of the beginning of the intervention and 4 weeks after, however 1RM was not assessed at the end of the entire intervention, so this data cannot be used to show the improvement in strength after the intervention. Also for the group that had stretching as a pre-intervention no 1RM was collected during this period, while this data was collected for the other group as their pre-intervention was resistance training.

The calf press exercise performed in the intervention was performed in a leg press machine. This limited the participants to have the same 1RM for both exercises, as the maximum amount of weight that they were able to leg press had to be used as the maximum for the calf press, because to perform the calf press exercise they had to push the plate first so that they could extend completely their legs.

Adherence was lower than past studies conducted in the laboratory. A possible explanation is the time of the year when the study took place, which affected the adherence of the participants to the training sessions. Because of the length of the study for most participants at least 8 out of the 16 weeks of trainings were during the summer, which made it difficult to attend 3 days per week when other activities like family trips took place. However the statistical analysis was ran excluding participants that had an attendance lower than 90% and the findings were the same.

Future studies looking in this type of research should consider including ways to asses maximal effort during strength testing in older adults. A possibility would be to record muscle activation with electromyography, to ensure that a maximum contraction is being recorded during the maximal testing. Other approach would be to assess sincerity of effort which can be done with the use of coefficient of variation, to control for the change in effort in different strength measurements. However current measurements as the coefficient of variation have shown questionable results, because of the high error rates and low stability (Shechtman, Anton, Kanasky Jr, & Robinson, 2006). Other recommendation for future research involving long term treatments, is to make an effort to periodize the training, which could improve participant's motivation.

In conclusion based on our findings performing a stretching intervention prior to a resistance training program does not enhance the response to resistance training more than resistance training alone in older adults. The effect seen with 16 weeks of resistance training is the same as 8 weeks of stretching followed by 8 weeks of resistance training. However these findings may have been influenced by a low effort when performing the strength assessments, which is something that need to be considered with future research.

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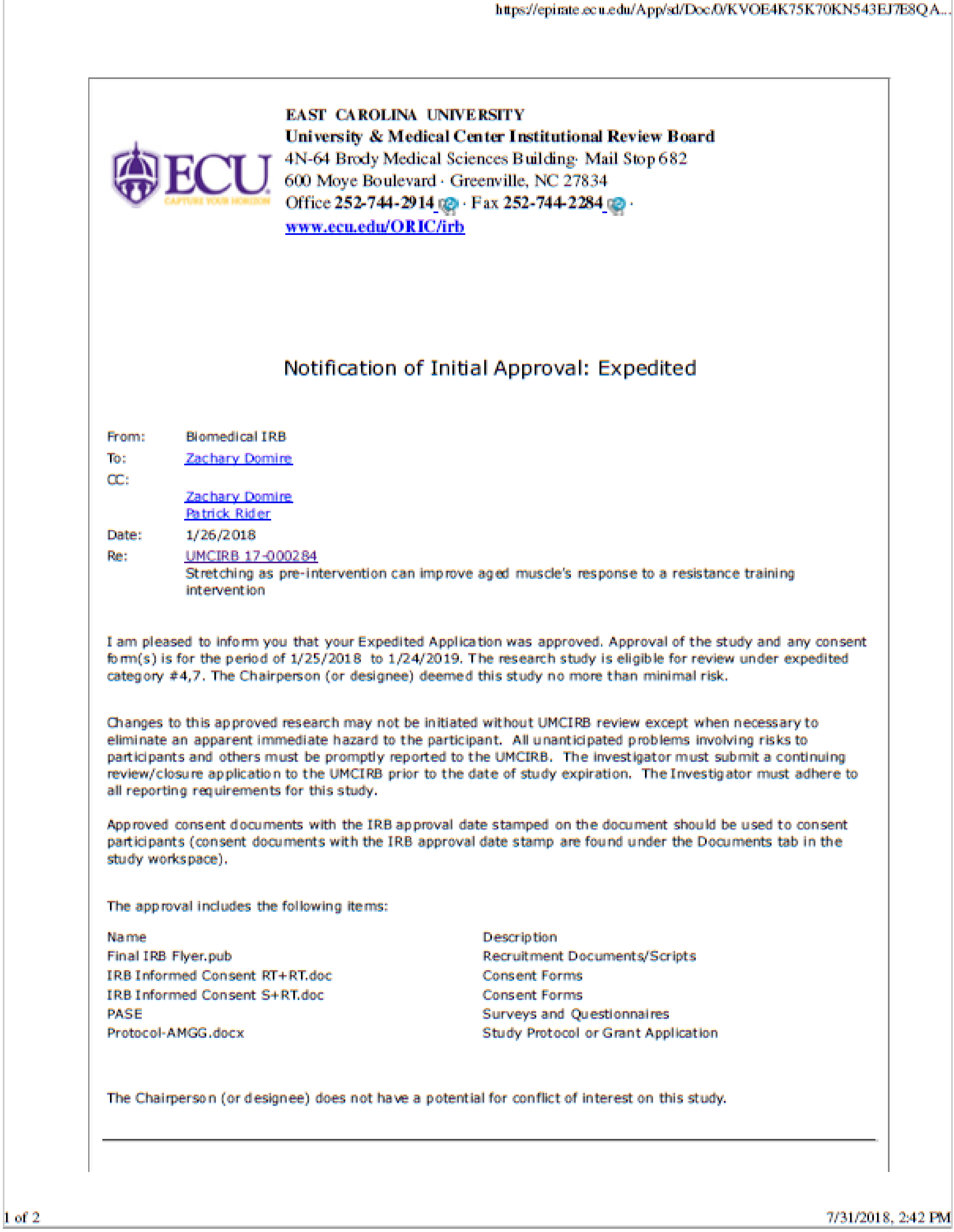
# Appendix A: Institutional Review Board Approval Letters



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**EAST CAROLINA UNIVERSITY**
**University & Medical Center Institutional Review Board**
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Office **252-744-2914** · Fax **252-744-2284** ·
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## Notification of Initial Approval: Expedited

From: Biomedical IRB
To: Zachary Domire
CC:
Zachary Domire
Patrick Rider
Date: 1/26/2018
Re: UMCIRB 17-000284
Stretching as pre-intervention can improve aged muscle's response to a resistance training intervention

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 1/25/2018 to 1/24/2019. The research study is eligible for review under expedited category #4,7. 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 |
|---|---|
| Final IRB Flyer.pub | Recruitment Documents/Scripts |
| IRB Informed Consent RT+RT.doc | Consent Forms |
| IRB Informed Consent S+RT.doc | Consent Forms |
| PASE | Surveys and Questionnaires |
| Protocol-AMGG.docx | Study Protocol or Grant Application |

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

1 of 2

7/31/2018, 2:42 PM