EFFECTS OF A SIX WEEK GASTROCNEMIUS PNF STRETCHING INTERVENTION ON STRUCTURAL PROPERTIES OF MUSCLE AND NEURAL ADAPTATIONS OF MUSCLE IN YOUNG WOMEN

by

Alex E. Semanderes

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Director of Thesis: Dr. Zachary J. Domire

Major Department: Kinesiology

Muscle injuries account for nearly one-third of sport medicine clinic visits in the United States (Woods et al, 2007) and amass financial burden on both athletes and their respective sports clubs. It is unclear whether stretching prevents injury, but it is practiced due to the common notion that the ability to move through a full range of motion (ROM) with ease makes it less likely to experience muscular injury as a result of rapid/extreme movements (Akagi et al, 2013a&b; Zakas etal, 2006; Haskell et al, 2007). The physiological characteristics which are thought to adapt as a result of stretching include: cross-sectional area (CSA) of muscle and muscle strength, muscle fascicle length, material stiffness, structural stiffness, and neuromuscular activity. The purpose of this research is to assess physiological changes related to structural and material properties of muscle as well as neural adaptations of the gastrocnemius as a result of a 6 week PNF stretching program in young women. Similar studies involve solely men or a mix of men and women – none involve exclusively young women; we will study women to determine if adaptations previously observed in men occur in a different fashion. We hypothesized that after a 6 week PNF stretching intervention, ankle range of motion will increase in the participants as a result of increases in fascicle length, decreases in CSA, decreases in material stiffness, and decreased muscle activation.
A total of 8 subjects between 18 and 31 years of age were recruited for this study. Each subject underwent PNF stretching which targeted the gastrocnemii in their right leg (experimental leg) while their left leg remained un-stretched (serving as internal control) for a total of 6 weeks with a minimum of 16 stretching sessions. Pre-test and post-test measurements included: material stiffness, CSA of muscle, and muscle fascicle length using ultrasound imaging; structural stiffness and isokinetic strength using a dynamometer, and neural activity using EMG electrodes. The factors of time (pre-test and post-test) vs. group (stretch and control) were tested with a mixed model repeated measures on time and between group comparisons ANOVA ($P < .05$), in addition to regression analyses performed on various characteristics.

ROM significantly increased by about 9° for the treatment leg; subjects also had a significant cumulative increase of 1mm in the fascicles of the gastrocnemius in the treatment leg. There were no changes in CSA, strength, material stiffness, structural stiffness, and muscle activation of the experimental leg. In conclusion, a stretching intervention has a clear effect on increasing ROM, but a full understanding for the physiological mechanisms increasing ROM must be further examined. Increases in gastrocnemius fascicle length slightly contribute to increases in ROM, but not enough to fully explain a significant increase in ROM. It is likely the soleus is the calf muscle responsible for increasing/limiting ROM in the ankle, however, since no analytical measures were taken on this muscle, we have no information on the magnitude each physiological mechanism plays in restricting ROM. By building off this research and comprehensively monitoring adaptations in the soleus after chronic stretching, we can continue to understand the effects of stretching and tailor better stretching interventions.
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IN YOUNG WOMEN

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by

Alex Semanderes

APPROVED BY:

DIRECTOR OF THESIS: __________________________________________
Zachary Domire, PhD

COMMITTEE MEMBER: __________________________________________
Anthony Kulas, PhD

COMMITTEE MEMBER: __________________________________________
Patrick Rider, MS

CHAIR OF THE DEPARTMENT OF KINESIOLOGY: ______________________
Stacey Altman, JD

DEAN OF THE GRADUATE SCHOOL: _________________________________
Paul J. Gemperline, PhD
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Chapter I. Introduction

Injuries to skeletal muscle account for over 30% of sport-related injuries seen in sports medicine clinics across the United States (Woods et al, 2007). These injuries generate financial losses to athletes and individuals and also carry the potential of costing sports teams millions of dollars (Ivarsson et al, 2015). It is unclear whether stretching prevents injury, but it is practiced due to the common notion that the ability to move through a full range of motion (ROM) with ease makes it less likely to experience muscular injury as a result of rapid/extreme movements (Akagi et al, 2013a&b; Zakas et al, 2006; Haskell et al, 2007). Despite the controversy of whether stretching is actually effective in preventing injury, it has been a long-practiced technique incorporated into sport practice and activities of daily living for many individuals (Young & Behm, 2003). There is a need to determine what physiological adaptations occur as a result of stretching and whether these adaptations are effective in preventing injury.

It is well established chronic stretching results in a higher range of motion (Smith et al, 1994; Sharman et al, 2006), however stretching may not be effective in preventing injury (Shrier, 1999; Herbert 2002), causing concern for the practicality of stretching in terms of preventing injury. Moreover, it is still unclear why this range of motion is increased as a result of stretching over a prolonged period of time. Elements believed to influence increased ROM include changes in passive muscle properties which include the structural and material architecture both within and surrounding fascicles. In addition to passive muscle properties, decreases in muscle activation as a result of stretching is another characteristic which may explain increases in ROM.

Stiffness of a muscle is a factor related to passive muscle properties that may influence ROM. A less stiff muscle would give a greater ROM while a stiffer muscle allows for less ROM. The equation shown below is used to determine factors involved in the stiffness of a muscle:
\[ K = \frac{F}{\Delta L} \]

Where \( K \) is stiffness of muscle, \( F \) is the force exerted on muscle, and \( \Delta L \) is the change in fascicle length of muscle.

The equation for material stiffness is dissected to determine what measurements are needed to quantify stiffness of a muscle:

\[ \lambda = \frac{\sigma}{\varepsilon} \]

Where \( \lambda \) is the material stiffness of muscle, \( \sigma \) is the stress placed on muscle, and \( \varepsilon \) is the strain placed on muscle.

The following equations will be substituted into the material stiffness equation to eventually isolate the variable of stiffness:

\[ \sigma = \frac{F}{CSA} \]

\[ \varepsilon = \frac{\Delta L}{L} \]

Where CSA is the cross sectional area of muscle and \( L \) is the length of muscle.

When the appropriate substitutions are made the new equation for material stiffness is as follows:

\[ \lambda = \frac{(F \div CSA) \div (\Delta L \div L)}{L} \]

The new material stiffness equation is then simplified to isolate stiffness:

\[ \lambda = \frac{F \cdot L}{CSA \cdot \Delta L} \]

\[ \lambda = \frac{(K \cdot L)}{CSA} \]
\[ K = (\lambda \cdot CSA) ÷ L \]

Since CSA, fascicle length, and material stiffness can impact the stiffness of a muscle, the influences chronic stretching has on these characteristics as well as structural stiffness will be examined for this study. The numerator and denominator of the equation have inverse effects on the stiffness (K); increases in fascicle length results in less stiffness while increases in material stiffness and CSA will result in more stiffness. An additional characteristic that will be examined in order to explain why ROM increases as a result of stretching is neural activity within the stretched muscle; decreases in muscle activation may change an individual’s comfort level when completing a full ROM effectively increasing ROM.

PRT attempts to quantify the ease at which a muscle moves through a passive range of motion and is an outcome measure of structural stiffness of muscle. In the ankle, PRT is measured by moving the foot through a passive ROM at a set speed and recording the force required to move the foot at this speed; higher structural stiffness will require more force to move the foot through its ROM, resulting in greater PRT. Literature which supports stretching causes an increase in structural stiffness at the chronic level of stretching (Rees et al, 2007; Gajdosik et al, 2005) is contradicted by literature which claims structural stiffness decreases or does not change at both the acute and chronic level (Nakamura et al, 2012; Mahieu et al, 2009; Mahieu et al, 2007) and only complicates the role stretching plays on the structural stiffness of muscle. Since the effects of chronic stretching on structural stiffness are unknown, this thesis research will attempt to clarify what physiological adaptations from stretching contribute to decreased structural stiffness and increased ROM.

The first possible element which may explain ROM changes is the cross-sectional area of the muscle which results from stretching. Altering the width of the muscle would have an
influence on the structural properties, or ease at which a muscle is able to move through a full range of motion. Reduction in cross sectional area would reduce structural stiffness. A wider muscle would be more difficult to move through a full range of motion which implies stretching may reduce muscle cross sectional area (Akagi et al, 2013b).

Moreover, measures in strength changes are a direct result of the CSA changes of the muscle; decreases in muscle size lead to decreases in strength. It is well documented that muscle strength does not change as a result of a chronic stretching protocol (Konrad et al, 2014; Akagi et al, 2013b). No evidence exists to contradict these findings by Konrad et al and Akagi et al. Monitoring muscle strength before and after a chronic stretching could help determine what changes occur to CSA and if these changes have an impact on ROM. The degree of change in muscle size must be studied in order to determine its impact on ROM at the end of a chronic stretching programs; however, there are more adaptations which may contribute to change in ROM at the end of a chronic stretching program.

Another adaptation involved with structural properties of the muscle which may be responsible for increased ROM includes sarcomerogenesis. Sarcomerogenesis is a muscle adaptation which is an increase in fascicle length by adding sarcomeres in series (Brockett et al, 2001). Evidence showing capability of sarcomerogenesis as a result of eccentric exercise has been shown in rabbits (Zollner et al, 2012; Butterfield and Herzog, 2006) as well as in humans (e Lima et al, 2015; Brockett et al, 2001) which may explain in-part why stretching increases range of motion. Additionally, stretching regimens have also been shown to increase ROM with the presence of sarcomerogenesis (Freitas et al, 2015; Blazevich et al, 2014). On the other hand, there is evidence indicating ROM may increase despite no sarcomerogenesis (Konrad et al, 2015; Nakamura et al, 2012), which may indicate changes in muscle fascicle length are not solely
responsible for increasing ROM. Attempting to quantify changes seen in muscle fascicle length brought on by stretching may open doors to seeing the influence, if any, muscle fascicle length has on ROM and possibly injury prevention.

An additional factor which may increase ROM are decreases in material stiffness. Material properties influence the passive movement of muscle and are influenced by the organization of connective tissue (Kovanen et al, 1984) and the function of intracellular titin (Nakamura et al, 2012). Previous studies have attempted to quantify changes to passive material properties within the muscle using ultrasound elastography in order to explain changes in ROM arising from stretching. There is evidence these material stiffness properties are altered through both acute (Akagi et al, 2013a; Nakamura et al, 2014) and chronic (Akagi et al, 2013b) stretching regimens. However, other evidence states there are no changes in modulus (material stiffness) as a result of chronic bouts of stretching (Konrad et al, 2014; Nakamura et al, 2012; Mahieu et al, 2009). Quantifying modulus may give insight into the changes in the structure of proteins controlling passive movement of the muscle which could help explain why an increased range of motion is observed after chronic stretching.

The last element to explore is neural adaptations involved with a chronic stretching program. Decreased motor neuron excitation as a result of desensitization of stretch reflex through stretching has been a speculated mechanism for the ability to tolerate a wider range of motion (Konrad et al, 2015; Akagi et al, 2013b; Hayes et al, 2012). However, this muscle activation in response to stretching can be directly monitored with the use of electromyogram (EMG). Evidence supported by EMG states: muscle activation decreases as a result of a chronic stretching program (Konrad et al, 2015; Hayes et al, 2012; Gajdosik et al, 2005; Guissard et al...
Without evidence to refute these findings, duplicating the quantification of changes may help further supporting findings and claims found in previous research.

All in all, there is no definitive answer as to what causes an increased range of motion, all four of the aforementioned ideas may play some part in the impact stretching has on the musculoskeletal system. Additionally, there is no literature using the equation $K = \frac{ACSA}{L}$ to comprehensively assess changes in muscle associated with stretching (CSA, muscular strength, sarcomerogenesis, material stiffness, structural stiffness and neural activity within the muscle) as a means for explaining why stretching for a chronic period of time will lead to an increased range of motion. Since one mechanism is not solely responsible for the adaptations brought on by stretching, comprehensively assessing these mechanisms as a whole may help us explain why increased range of motion as a result of stretching and whether these adaptations are helpful in preventing injury.

Statement of Purpose

The purpose of this thesis is to assess physiological changes related to structural and material properties of muscle as well as neural adaptations of the gastrocnemius as a result of a 6 week PNF stretching program in young women. Similar studies involve solely men or a mix of men and women – none involve exclusively young women; we will study women to determine if adaptations previously observed in men occur in a different fashion.

Hypothesis

After a 6 week PNF stretching intervention, ankle range of motion will increase in the participants as a result of increases in fascicle length, decreases in CSA, decreases in material stiffness, and decreased muscle activation.
**Significance**

Increasing range of motion is used as an injury preventative measure in many trained and untrained individuals. It is not fully understood why this increased range of motion is evident after prolonged stretching interventions, but the purpose of this study will attempt to explain why these changes occur though a novel, comprehensive observation of passive resistive torque, CSA, muscle fascicle length changes, muscular strength, material stiffness (modulus), and neural properties before and after a stretching intervention. It is worth exploring physiological changes in musculature as a result of prolonged stretching interventions in order to determine if women adaptations behave similarly to past studies. These new findings will hopefully give insight on whether stretching is useful for prevention of injury; it is important to see what changes stretching incurs, if any, to the gastrocnemius in order to understand prevention by linking the physiological changes to desired benefits.

**Delimitations**

1. Subjects in this study will be female.
2. Subjects will have a Body Mass Index of less than 30 kg/m2.
3. Subjects will not have a history of pain or injury associated with the lower limbs.
4. Subjects will be 18-40 years of age.
5. The study only represents the population of Greenville, North Carolina.
6. The only extremities measured are the left and right lower leg.

**Limitations**

1. Lack of random sampling and random assignment may allow for bias.
2. Results from this study cannot be generalized to all muscles in the human musculoskeletal system.

3. Results from this study cannot be generalized to males.

**Operational Definitions**

1. Range of motion (ROM) - degree of movement performed by a joint in all possible directions.

2. Passive range of motion - measures the angle of a joint when moved under a certain amount of constant force.

3. Structural stiffness - resistance of muscle elongation during limb movement. Connective tissue, fascicle length, and muscular strength play a role in the ability of a muscle to contract in both eccentric and concentric manners.

4. Material stiffness - measure of the amount of force needed to pull muscles apart without taking size and shape into consideration.

5. Passive resistance - measure of resistance of passive movement within a muscle to quantify stiffness of a muscle as a whole body.

6. Ultrasound elastography - Generation of a shear wave in a muscle through and measurement of wave velocity; measurements of wave velocity are an indirect measure of modulus.

7. Modulus - slope of the elastic region on a stress-strain curve. It is a material property of normalized stiffness to assess organization of tissue within muscle.

8. Static stretch - performing and holding a given stretch while remaining motionless.

9. Isometric contraction - contraction of a muscle, usually against resistance, while the joint angle remains constant.
10. Proprioceptive neuromuscular facilitation (PNF) - stretching of a muscle both passively and actively. First, the subject is stretched passively by another person for 30 seconds followed by a 6 second break. Next, the person being stretched is instructed to resist the stretch being performed resulting in an isometric contraction for at least 3 seconds. Lastly, the person is stretched passively again (usually past the original range of motion) for at least 15 seconds.

11. Younger women - Women aged 18-40 years; women in this age range will be recruited for the study.

12. Muscle spindle fiber - fibers located within a muscle which detect stretch. When stimulated, these relay information to the reflex center to contract other sarcomeres to prevent injury to the muscle.
Chapter II. Review of the Literature

Introduction

Muscle injuries account for nearly one-third of sport medicine clinic visits in the United States (Woods et al, 2007) and amass financial burden on both athletes and their respective sports clubs. There is evidence supporting stretching regimens as beneficial in terms of preventing injury during exercise due to an extended range of motion. The ability to move through a full range of motion with ease decreases the likeliness of an individual to experience muscular injury as a result of rapid/extreme movements (Akagi et al, 2013a&b; Zakas et al, 2006). There is also controversy, however, in whether or not these benefits result in reductions of strength and performance in an individual in addition to the skepticism of stretching’s capabilities of reducing injury risk. In order to determine whether stretching is beneficial in terms of preventing injury, we must explore multiple mechanisms which may be responsible for increasing range of motion. In determining how much each respective mechanism may be responsible for increasing ROM, we will be able to understand how stretching prevents injury.

The first factors influencing ROM to be explored involve structural properties contained in the equation \( K = (\lambda \cdot \text{CSA}) \div L \), which is the equation for structural stiffness. Changes in structural properties of muscle include changes in: the measurements of structural stiffness, the CSA (which is directly related to strength), sarcomerogenesis (L), and material stiffness (\( \lambda \)). As CSA increases, the stiffness of the muscle increases; inversely, the presence of sarcomerogenesis would decrease structural stiffness. Material stiffness aims to quantify the ability of a muscle’s ability to pull apart without taking the size and shape of the muscle as a whole into consideration. Factors influencing material stiffness are proteins in connective tissue (Akagi et al, 2013a&b) and the protein titin within the muscle fascicles (Nakamura et al, 2012). These structural properties have an influence on the structural stiffness of the muscle, which is measured by PRT.
The last mechanism which may be responsible for increasing joint ROM are changes in neural activity within a muscle; a decrease in neural activity may be a factor in increasing ROM of a joint (Konrad et al, 2015; Akagi et al, 2013b). The proposed methods for inducing an increased ROM, which are stated above, will be examined through different types of stretching in order to pinpoint adaptations responsible for increased ROM with the ultimate goal of determining whether these adaptations prevent injury. The benefits of PNF stretching over static stretching will also be explored in addition to the case of whether stretching is effective in preventing injury. One mechanism may not be entirely responsible for changes in ROM making it pertinent to examine multiple factors which may induce changes in musculature which are responsible for increasing flexibility. After these mechanisms are explored, the most effective stretch type will be determined in addition to whether stretching in general is an activity which will significantly reduce the risk of injury. Due to the fact most health professionals recommend stretching and there are studies which show stretching prevents injury, the stance of stretching being beneficial in relation to injury prevention will be taken.

*The Influence of Stretching on Injury Prevention*

Stretching before athletic events is encouraged by all types of health professionals in order to reduce risk of injuries and optimize performance (Thacker et al, 2004; Akagi et al, 2013a&b; Konrad et al, 2015). However, there is evidence that suggests stretching may put you at greater risk for injury (Shrier et al, 1999). Individuals regularly performing all types of stretches before participating in high intensity exercise do not have a significant reduction in experiencing injury which prevents further participation in exercise compared to non-stretched individuals (Pope et al, 2000; Jacobs & Berson, 1986). Possible reasons (in addition to decreased neural activity) behind an insignificant reduction of injury as a result of stretching may be that
increased muscle plasticity will decrease the amount of energy a muscle can absorb and unequal length of muscle fascicles makes muscles more likely to become injured during eccentric exercise (Shrier et al, 1999). On the contrary, there is evidence both PNF and static stretching significantly reduces risk of experiencing muscular injury versus not stretching before participating in high intensity exercise as reported by high level endurance athletes (Ekstrand et al, 1983; Bixler & Jones, 1992; Wilber et al, 1995).

Moreover, there are less serious, non-debilitating muscular injuries associated with exercise, such as muscle soreness or pain, which may be influenced by a stretching intervention. Stretching before exercise may be effective in significantly decreasing experiencing these minor pains associated with exercise (Howell, 1984; Devries, 1961). On the contrary, minor pains may still be present post-exercise even if an individual stretches beforehand according to other surveys (Johansson et al, 1999).

Since static and PNF stretching’s influence on experiencing injury is contradictory, it is important to examine how the specific physiological adaptations of PNF stretching differ from those of static stretching; determining these specific differences in physiological adaptations between the types of stretching may possibly determine a better understanding of physiological mechanisms associated with injury. By conducting the proposed study at hand, information added to the knowledge of the mechanisms which predispose an individual to injury as a result of exercise may be helpful in creating productive stretching programs.

**Structural Adaptations of Muscle Associated with Stretching**

All of the elements (CSA, sarcomerogenesis, material stiffness) of structural properties of muscle each have a role in the structural stiffness (quantified by PRT) of the muscle. Passive
resistive torque, measured via dynamometer, quantifies the ease at which a muscle moves through a passive range of motion (Figure 1). In the ankle, PRT is measured by moving the foot through a passive ROM at a set speed and recording the force required to move the foot at this speed; higher structural stiffness will require more force to move the foot through its ROM, resulting in greater PRT. The resistance of this force may be tracked throughout the ROM or at the end of ROM to determine differences in torque between subjects in an attempt to quantify structural stiffness. Change in torque divided by change in angle determines the slope of the line, if the muscle is stiffer the slope will be higher. Unlike material stiffness, structural stiffness takes the muscle’s entire shape and size into consideration in order to determine how stiff a muscle is while in motion.

![Graph showing change in torque vs. ankle angle.](image)

(Konrad et al, 2014)

**Figure 1**: Example of passive resistive torque of the gastrocnemius starting at 0° and moving to 25° of dorsiflexion to demonstrate structural stiffness. The amount of force required to move the ankle increases as it approaches its maximum dorsiflexion ROM.

Studies have demonstrated muscle stiffness remains unchanged or decreases as a result of chronic stretching, while at the same time increasing ROM (Konrad et al, 2016; Blazevich et al,
Conversely, it is possible to increase ROM while increasing passive resistive torque through long-term stretching program (Rees et al, 2007; Gajdosik et al, 2005); however, a possible explanation for no change seen in structural stiffness of the muscle, may be explained by change in the Achilles tendon’s structural stiffness, a structure which is separate from the gastrocnemius muscles (Konrad et al, 2015). Since literature is mixed about the effects of stretching on structural stiffness, this could be a partial explanation for why we see changes in ROM; other factors associated with muscle stiffness may help explain why ROM increases, namely changes in CSA.

The first factor to be examined as part of structural adaptations of muscle includes changes in CSA as a result of chronic stretching. Studies which examine CSA at the end of a chronic stretching program show there is no change in CSA as a result (Freitas et al, 2015; Akagi et al, 2013b; Nakamura et al, 2012); there are no other studies which refute these claims. Since changes in CSA are directly related to changes in muscular strength, another method of examining changes in CSA are examining changes in muscular strength as a result of a chronic stretching program.

Evidence supports there is no change in muscle strength as a result of a chronic stretching regimen (Konrad et al, 2015; e Lima et al, 2015; Konrad et al, 2014; Akagi & Takahashi, 2013b; Mahieu et al, 2009). Additionally, pairing PNF stretching with a resistance training program may be more effective at strength gain than resistance training alone (Arazi et al, 2012). On the other hand, there is evidence stretching lowers muscle strength, but as a result of an acute stretching protocol (Sa et al, 2016; Akagai et al, 2013a), suggesting stretching may produce muscle fatigue if it is done prior to exercise. Since CSA’s effect on increasing ROM as a result of stretching is
ambivalent, other factors may be responsible for increasing ROM and decreasing stiffness such as muscle length.

The next structural property to examine is sarcomerogenesis (L), or an increase in serial sarcomere number, which is an adaptation within the muscle fascicles as a product of stretching (Figure 2 & 3). Changes within the muscular fiber components over an extended period of time are able to be measured using ultrasound imaging or through extraction of the muscle from a euthanized animal. Eccentric exercise in animals over a chronic period of time results in sarcomerogenesis (Peixinho et al, 2014; Butterfield & Herzog, 2006); although this study does not involve the use of stretching to illustrate sarcomerogenesis, it is proof the concept exists in mammals. Since it does exist in mammals it is plausible to conclude this adaptation will occur in humans.

Figure 2: Depiction of sarcomeres in series.
As a result of adding a sarcomere at the Z-line, the product is sarcomerogenesis:

|-------Sarcomere-------|Z-Line|-------Sarcomere-------|Z-Line|-------Sarcomere-------|

**Figure 3: The addition of a sarcomere in series, demonstrating sarcomerogenesis.**

Indirect evidence of sarcomerogenesis exists due to the fact eccentric exercises are capable of adding sarcomeres (Brockett et al, 2001) via a shift to the right in the stress strain curve of human subjects after chronic stretching. Additionally, there is direct evidence lengthening of fascicles as a result of a chronic stretching program (Freitas et al, 2015; Blazevich et al, 2014); however, lengthening of fascicles does not always mean sarcomerogenesis, other means such as lengthening of the fascicles themselves may be an explanation for this. Lengthening of the muscle fascicle allows for greater force generated due to higher contractile speed, indicating this adaptation associated with stretching may not have a negative effect on athletic performance. On the other hand, ROM may also increase despite no change in fascicle length (Konrad et al, 2015; Nakamura et al, 2012), indicating sarcomerogenesis may not be solely responsible for increasing ROM.

Without definitive proof, it is important to add evidence which may or may not support various findings of muscle fascicle length change as a result of stretching in order to help determine what is responsible for increasing ROM in human muscle. Additionally, since controversy already exists as to what happens to muscle fascicles as a result of stretching, other areas of muscle adaptations must be explored.
The next step in examining the structural adaptations of muscle and its consequences on ROM is examining changes in material stiffness ($\lambda$) as a result of a chronic stretching program. Elastic modulus is modifiable because endurance trained athletes are likely to experience an increase in material stiffness through increased cross-linking of collagen fibers (Kovanen et al, 1984). A higher shear modulus within the muscle is also to be expected when females are compared to male counterparts (Eby et al, 2015), indicating there may be a difference in adaptations to muscle stretching according to gender. An increase in cross-linking of collagen within a muscle generates more material stiffness and in turn decreases ROM. Stretching may inhibit the cross-linking of collagen prevalent in endurance athletes, which in turn would increase the range of motion and may make it less likely for those who participate in endurance training to experience injury as a result of rapid/extreme movement. Additionally, decreased water content within the muscle as a result of stretching may be a mechanism which explains the increased range of motion (Akagi et al, 2013b). Changing tissue organization and content is shown to have an effect on ROM and may be helpful of indicating injury prevention.

Material stiffness may be measured via ultrasound elastography and is useful in determining changes in the tissue quality of a muscle. Monitoring these changes are useful in understanding whether stretching is indicative of injury prevention. Studies show stretching at chronic levels does yield a decrease in material stiffness measured through ultrasound elastography (Akagi et al, 2013b) and increases ROM; however, there is no other evidence to confirm or refute these findings. It is important to further examine material stiffness through ultrasound elastography in addition to all other structural properties mentioned in this document in order to obtain a better representation of what stretching does at the connective tissue and passive muscle movement levels. Moreover, the proposed study at hand may help determine
whether adaptations of stretching on women are different than those in men and exactly how much material stiffness contributes to increasing ROM along with the other comprehensively monitored muscle parameters monitored for this study. As mentioned previously, material stiffness is more than likely not the only factor which may impact ROM; examining other factors associated with increased ROM are important to explore.

**Adaptations in Neural Activity of Muscle Associated with Stretching**

Muscle spindle fibers protect a muscle from stretching too far in order to prevent injury. The electrical activity within a muscle as it relates to muscle spindle activity may be measured via electromyography (EMG) to determine muscle spindle reflex. Static stretching causes a decrease in reflex activity (Nielsen et al, 1993) which indicates decreased neural input during acute bouts of stretching. This helps explain why stretching acutely increases ROM. Moreover, static stretching for a chronic period of time shows a significant decrease in H-reflex electrical amplitude paired with an increased ROM (Guissard et al, 2004). Other studies shows there is no significant change in EMG electrical activity, (Gajdosik et. al, 2005; Konrad et al, 2015) or H-reflex ratios (Hayes et. al, 2012) after chronic stretching. Similar studies have attributed increased neuromuscular tolerance as a mechanism of increasing range of motion, but EMG was not used to show definite decreases in neuromuscular activity (Akagi et al, 2013b; Hayes et al, 2012).

It is pertinent to accurately measure sensory input activity of muscles to determine whether it is a contributing factor for increasing ROM. Muscle spindles are a protective mechanism to prevent over-stretching and more information needs to be learned about them in order to assess whether they must be conditioned to increase ROM and prevent injury. Further
examining EMG output may help support previous evidence indicating neural activities role in increased ROM and ultimately injury prevention.

**Benefits of PNF Stretching Compared to Static Stretching**

Despite the controversy in which factors determine increased ROM as a result of stretching, it is important to justify choosing one form of stretching over another. There are multiple methods used to acquire desired gains in ROM; two common productive (in terms of increasing ROM) stretches are static (most common) and PNF stretching (Lucas et al, 1984; Sharman et al, 2006). Static stretching involves moving a joint to maximal ROM against some type of resistance and holding it (in an isometric fashion) for about 30 seconds in order to stretch a targeted muscle. PNF stretching involves passively having a limb moved (usually by another individual) to a maximal ROM and holding it for 15 seconds, followed by a period of rest; next the person being stretched is moved into maximal ROM again, but are instructed to contract the target muscle in the opposite direction of the force moving them into maximal ROM. A resting period after the contract period is given, and the subject is moved into maximal ROM again and held there for 15 seconds. Evidence supports using PNF stretching over static stretching in that is more effective in multiple areas.

PNF is not only proven to be more effective in terms of rate of increasing ROM (Tanigawa et al, 1972), but also in terms of greater increases in joint ROM (Etnyre et al, 1986; Wallin et al, 1985). Moreover, PNF produces greater increases in both passive ROM (Ferber et al, 2002; Feland et al, 2002) and active ROM (Etnyre et al, 1986; Spernoga et al, 2001). A possible reason for this is the decrease in muscle activation as a result of the methods incorporated in PNF, namely the contracting phase which is not incorporated into the static

Another evident difference between static and PNF stretching is at end ROM, PNF stretched muscles yield greater passive torque measures (Magnusson et al, 1996) than muscles that have been stretched statically. A possible explanation for this greater ROM are changes in the structural properties of muscles (increased structural stiffness) that are evident in PNF stretches, but not static stretches. However, passive resistance throughout the whole ROM of a muscle are similar in both static and PNF stretching.

Since PNF stretching is proven to increase both active and passive ROM at a greater rate and magnitude than static stretching, it is best to use PNF for the purposes of this study. The end goal is to have a greater ROM of motion in order to theoretically avoid injury (ACSM, 1998). Despite having this common conception of injury prevention published and widely accepted, there is controversy as to whether stretching prevents injury as a result of physical activity.

Summary

In summary, stretching increases range of motion with little doubt, however, the mechanisms which are responsible for this increased ROM are not completely understood. Injury is something humans have dealt with since the beginning of time and it has continually costed people financially, physically, and mentally. In order to have a high quality of life we must avoid injury and in order to do so we must learn how to avoid injury. By exploring the mechanisms contingent of stretching we can learn how to prevent these injuries from happening and employ safe, effective stretching regimens.
Based on the review of literature structural properties of muscle \( K = \frac{ACSA}{L} \) and neural activity changes within muscle may play collaborative roles in increasing ROM and hopefully this study can establish changes expected to occur as a result of PNF stretching. Due to the nature of the study, we may see differences in the way women adapt to stretching since most previous studies measure changes in solely men. As a result, stretches which have been considered effective may actually be proven to be harmful and cause a revolution leaning toward safe stretching. People who consider stretching to be harmful may be persuaded to participate in stretching before activity.

Taking all these findings and ideas into consideration, it is important to determine which effects are responsible for increasing range of motion within a joint and whether or not these adaptations can affect exercise performance. It is important to prevent injury during athletic performance while maintaining the properties that allow an athlete to perform optimally in order to fully receive benefits of exercise. The findings mentioned in this paper are important in terms of developing methods which determine proper stretching routines for younger women and hopefully warming up for exercise will be changed for the better as a result of this study. At the end of the study, it is likely one factor will not be singled out in terms of responsibility for increasing ROM; taking a step closer to determining what role structural properties and neural activity of muscle have in relation to ROM will allow individuals to employ safe stretching practices to avoid injury, ultimately preventing individuals from spending large sums of money to address exercise injuries.
Chapter III. Methods

Design

This study aimed to determine the effects a 6-week PNF stretching program has on CSA, muscular strength, material stiffness, structural stiffness, and muscle activation. We hypothesized PNF stretching would cause increases in fascicle length, decreases in material and structural stiffness, decreases in CSA, and decreases muscle activation.

Subjects

8 subjects between 18 and 31 years of age were recruited for this study. Subjects had their right leg stretched (experimental leg) while the left leg remained un-stretched to serve as a control leg (internal control). Participants were recruited via social media and flyers placed around town/campus. In order to be considered for this experiment the participants must have had a Body Mass Index below 30 kg/m² and would have been excluded if any of the following criteria pertain to the participant: history of lower body injury or musculoskeletal dysfunction, diabetes, cancer, blood pressure above 160 mm/Hg, neurological illness, peripheral artery disease, and heart disease. Procedures involved throughout the experiment were approved by the ECU Internal Review Board; participants read and/or had informed consent documents explained to them and signed them after gaining an understanding of what is expected as a result of this experiment.

Descriptive statistics for subjects of this study are found in Table 1. The 8 participants were an average of 22.38 ± 3.7 years of age with an average BMI of 23.86 ± 3.4. All subjects were part of both the control and treatment groups; the left leg served as the control/unstretched leg while the right leg was the treatment/stretched leg. Two participants were physically active (5+ hours of physical activity per week) for the 6-week intervention period and were instructed
to exercise prior to PNF stretching on days assigned to report to the laboratory. The remainder of
the subjects did not engage in strict physical activity programs, but did exercise sporadically
throughout the 6-week period and were instructed to always report to the lab after physical
activity on assigned stretching days. No significant differences were observed in weight, height,
and BMI across the subjects.

Table 1: Mean and standard deviation values of subject characteristics.

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.38 ± 3.66</td>
<td>66.61 ± 11.66</td>
<td>1.68 ± 0.03</td>
<td>23.86 ± 3.38</td>
</tr>
</tbody>
</table>

Measurement Reliability

In order to determine the accuracy of repeated trials before the experiment began, 3
subjects were measured twice on different days to determine cross sectional area and fascicle
length at 25%, 33%, 50%, 67%, and 75% of the aponeurotic tendon length for the lateral and
medial gastrocnemius of the treatment leg. Intraclass class correlation coefficients were
determined for each subject and verify accurate/reliable repeatability of measurements (Tables 2
& 3).

Table 2: ICC for Fascicle length. Fascicle length measurements were taken in pilot subjects
across several different days.

<table>
<thead>
<tr>
<th>Site</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral Gastrocnemius</td>
<td>0.95</td>
</tr>
<tr>
<td>Medial Gastrocnemius</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 3: ICC for Cross-Sectional Area. CSA measurement were taken in pilot subjects
across several different days.

<table>
<thead>
<tr>
<th>Site</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral Gastrocnemius</td>
<td>0.97</td>
</tr>
<tr>
<td>Medial Gastrocnemius</td>
<td>0.98</td>
</tr>
</tbody>
</table>
**Instrumentation**

The Aixplorer Supersonic ultrasound system (Supersonic Imagine, Aixplorer, Bothell, WA) was used in order to obtain gastrocnemius muscle fascicle length, cross sectional area, and muscle volume; Ultrasound measurements were processed with OsiriX Lite Dicom Viewer (Pixmeo, Bernex, Switzerland) on a desktop iMac Computer (Apple, Inc.; Cupertino, CA). Additionally, ultrasound elastography on the same system (Aixplorer Supersonic) was used to assess material stiffness of the gastrocnemius muscle bellies. Aquasonic Ultrasound Gel (Parker Laboratories, Aquasonic 100, Fairfield, NJ) was used on the ultrasound probe as an image enhancer and lubricant. Before dynamometer testing, Trigno Wireless EMG electrodes (Delsys, Inc., Natick, Massachusetts) were placed on lateral and medial gastrocnemius muscle bellies to measure muscle activation during passive range of motion and maximal voluntary contraction (MVC) testing. Qualysis Track Manager (QTM) (Qualysis Medical AB, Göteborg, Sweden) was used to capture EMG data which was analyzed with Visual 3D (C-Motion, Germantown, MD). The HUMAC dynamometer (Computer Sports Medicine, Inc. [CSMI], Stoughton, Massachusetts) was used in order to assess ankle range of motion and isokinetic strength for each individual. Microsoft Excel (Microsoft Corporation, Redmond, Washington) was used to organize and compute data obtained from OsiriX Lite Dicom Viewer, Trigno Wireless EMG electrodes, and the HUMAC dynamometer. These measurements were performed pre- and post-stretching protocol.

**Assessment Protocol**

**Ultrasound Imaging**

The initial visit to the ECU Biomechanics Laboratory for each participant involved imaging of the lateral and medial gastrocnemius in each leg in order to assess muscle fascicle
length, cross sectional area, and volume. This was done with participants in the prone position at 0 degrees of flexion/extension at the knee, hip, and ankle while the ultrasound imaging probe was used to image each region of interest in the gastrocnemii (the Aixplorer Supersonic will be set to “B mode” for this portion of the experiment). The joint angle for the ankle was measured via goniometer in order to ensure accurate, consistent measurements for pre- and post-protocol data comparison. Before each session, the subject number was entered into the patient’s information database while ensuring anonymity. Afterwards, a panoramic image (Figure 4) was collected to determine the origin and insertion of each gastrocnemius, which will then be split into 5 equidistant sections (starting 1 cm in from the proximal and distal ends, for a total of 6 cross-sectional image regions) in order to take cross-sectional images through each gastrocnemius (Figure 5).

Figure 4: Panoramic image taken of the entire lateral gastrocnemius.
Two panoramic images were taken of the entirety of each gastrocnemius to measure muscle length as well as assess the length of fascicles at region of interest. Afterward, 2-3 cross-sectional images (Figure 6) were taken at each of the markers (equidistant from one another).

Figure 6: Cross sectional image of the lateral gastrocnemius.
While the participant was in the same position, ultrasound shear wave elastography (SWE mode) videos were taken via the imaging probe in the medial and lateral gastrocnemius of each leg in order to assess material stiffness of the muscle bellies. This was done by placing the probe at the marked halfway point of the measured muscle length while lining the top of the region of interest box with the most superficial point of the gastrocnemius; after proper placement of the probe (Figure 7) and region of interest (Figure 8), a 10 second clip was recorded to measure material stiffness. There was a total of two 10-second clips for this portion of the protocol.

Figure 7: Ultrasound probe placed at the middle of the muscle for SWE measurements.
Dynamometer and EMG Measurements

Dynamometer and EMG equipment were used in conjunction with each other to obtain muscle activity data. Prior to testing done on the dynamometer, EMG surface electrodes were placed (Figure 9) on the lateral and medial gastrocnemius muscle bellies of both legs to assess muscle activity while performing the dynamometer tests. Data from EMG was gathered while participants moved through a passive range of motion and again while performing isokinetic strength testing.
Before participants were moved to the dynamometer, the chair attached to the dynamometer was adjusted to a 180 degree plane for the participant to be tested on. The participants were then instructed to lay flat in the prone position on the dynamometer chair (Figure 9) to assess range of motion, passive torque, and isokinetic strength of the left and right gastrocnemii. The participants placed their right (experimental) leg into the dynamometer lever which was used to measure range of motion; this was done by calibrating the machine to each individual’s end range of plantarflexion and dorsiflexion. After calibrating the dynamometer and acquiring ROM for each participant, the computer attached to the dynamometer was set to run continuous passive motion (CPM) tests incorporating con/con and 15/15 setting (15 degrees per second) for 3 sets of 5 repetitions with 10 seconds rest between each set. This passive range of motion was done to quantify structural stiffness of the gastrocnemius by recording amount of
torque (Nm) needed to passively move the ankle through a full range of motion. After this data was gathered, the computer was then programmed to run isokinetic testing with the speed of 15 degrees per second. There were 2 sets of 3 repetitions with ten seconds of rest between each set for this portion of the protocol; this measurement was used to assess strength of the calf muscle groups. After measurements were done in the right leg, the same protocol was used in the left (control) leg (internal control will be used for this experiment).

**Stretching Protocol**

The methods for this experiment were devised in order to assess the physiological adaptations that occur within a 6-week PNF stretching protocol. PNF stretching was chosen over static stretching due to a significantly greater increase in range of motion when the two types of stretching are compared (Sharman et al, 2006). Stretching over a course of five or more weeks shows a significant increase in range of motion when compared to non-stretching control groups, however the proposed mechanism for this increased range of motion is due to a decrease in “sensation” within the stretched muscle (Nakamura et al, 2014; Akagi et al, 2013). After a week of processing all data, each subject will report to the ECU Biomechanics Laboratory to begin the PNF stretching protocol.
The first stretching session, and each session thereafter, required each participant to go through PNF stretching as follows: participants laid prone on the dynamometer table while the knee remained at a 180° angle for the entirety of the stretch; the person performing the stretch moved the participant’s ankle to maximal dorsiflexion (without discomfort) and held it there for 15 seconds, after which the participant was given a 5-second break. Next, the participant’s ankle was moved to maximal dorsiflexion (without discomfort) again and was instructed to push her foot against the force of the stretcher’s hand while remaining in the same maximal dorsiflexion position; this isometric muscle contraction was held for 6 seconds followed by a 5 second rest. Lastly, the ankle was moved into maximal ROM (without discomfort) again and the stretcher held the ankle in this position for 15 seconds. These sets were performed 5 times, with 30 seconds rest between sets, and were only performed in the right leg (experimental group) while the left gastrocnemii remained un-stretched. This stretching protocol was performed 3 times a
week for six weeks. The control leg will remain un-stretched and will be compared to the experimental leg pre-and post-protocol.

Figure 11: Timeline for experimental protocol.

Each participant was required to participate in at least 16 of 18 of the stretching sessions (absence of 2 in-lab sessions allowed). Baseline and Post-test measurements were taken by the same investigator in order to eliminate measurement error; however, 1 PNF stretching session was performed by the research aide.

At the end of the 6-week stretching program, participants came in to the ECU Biomechanics Laboratory to have their gastrocnemii imaged via ultrasound in the same fashion as the pre-experimental protocol assessments. Additionally, the same tests performed with the HUMAC dynamometer and EMG during pre-experimental protocol were done. All data gathered from the dynamometer was saved to an external USB thumb drive, which was used to transfer data to a computer for further data processing. After data processing, statistical analyses was used to compare changes across control and stretching groups.
Compliance

Each subject was permitted to miss 2 stretching sessions for the entire 6-week intervention to be included in the data analysis. All subject met the criteria for inclusion in this study.

Data Processing

Images gathered via ultrasound imaging sessions in both medial and lateral gastrocnemius from both left and right legs were transferred into the database on a desktop Mac in the laboratory. The images were opened and assessed with Osirix Lite Dicom Viewer. First, each of the six cross sections were traced using the closed polygon tool to obtain cross-sectional area (Figure 12).

![Figure 12: Trace of transverse image of the gastrocnemius to determine CSA.](image)

Next, the open polygon tool was used to trace muscle fascicle length at 25%, 33%, 50%, 67%, and 75% of the length of the deep aponeurotic tendon for each of the 2 images. The following images depict the measurements used for this experiment.
First, the deep aponeurotic tendon was traced to determine total length of the gastrocnemius (Figure 13).

Figure 13: Tracing of the deep aponeurotic tendon to obtain muscle length which will be used to divide muscle into 25%, 33%, 50%, 67%, and 75% segments.

25% of the length of the deep aponeurotic tendon was measured first as depicted in Figure 14:

Figure 14: Tracing of the deep aponeurotic tendon to determine the point at 25% of muscle length.
Muscle fascicle tracing began at approximately 25% of deep aponeurotic tendon length as shown in Figure 15:

**Figure 15:** Tracing the deep aponeurotic tendon to reach 25% of the length is highlighted by the green line. The blue line is the tracing of the fascicle at 25% length of the gastrocnemius.

These steps were repeated until all fascicle lengths are taken (25%, 33%, 50%, 67%, and 75% of deep aponeurotic tendon). The final fascicle length measurement, depicted in Figure 16, was done for two images and fascicle lengths were averaged based on their location:

**Figure 16:** Tracing of fascicles at 25% (blue), 33% (dark yellow), 50% (orange), 67% (green), and 75% (light yellow) of the muscle.
The data gathered via OsiriX Lite Dicom Viewer was transferred into Microsoft Excel in order to compute averages for the muscle fascicle lengths as well as each respective cross-sectional area. Figure 17 illustrates how volume was calculated:

![Diagram of volume calculation](image)

**Figure 17:** The volume of each section will be calculated and added together to obtain total volume of each gastrocnemius.

Fascicle lengths of all 5 measurement sites within a single gastrocnemius were summed together and used as the variable for analysis (4 total for each subject). Material stiffness was assessed via Aixplorer Supersonic by opening the shear wave elastography video clips on the computer screen attached to the Aixplorer Supersonic. The video clip was moved to the midway point (about the 55th frame out of 110 frames) to account for video buffering which occurs while taking the measurements and to ensure measurements are stabilized. A 5 mm diameter circle (Akagi et al, 2013b) centered in the region of interest box for each image was used and the average value (in kPa) was taken for both sets of videos taken pre- and post-experimental protocol. These numbers were saved into an Excel datasheet for each subject.

EMG data was processed using Visual 3D (V3D); EMG signals were high-pass filtered (Butterworth) at 10 Hz and sampled at a frequency of 1000 Hz (Konrad et al, 2015). Measurements were taken after the 3rd CPM cycle to avoid the conditioning effect (Konrad et al, 2015; Mahieu et al, 2009). Root means square filters were used to eliminate noise, but this
caused quiet readings to become artificially inflated. The minimum value of the EMG data was subtracted from the maximum value in order to eliminate the artificial noise. Maximum values of EMG amplitude during CPM testing was divided by the maximum value of EMG amplitude during isokinetic testing to determine muscle activation percentage. This ratio was used for pre-testing and compared to data processed for post-testing in order to determine changes in muscle activity which may be a result of PNF stretching.

Data obtained via dynamometer was transferred as an Excel spreadsheet with graphs. Range of motion was placed in an Excel sheet for both pre- and post-experimental measures in order to assess any changes. Continuous passive movement data and charts were also transferred into an Excel workbook to compare pre- and post-experimental changes of torque. The slope between 5° and 10° of dorsiflexion on the PRT chart generated from CPM testing (after the 3rd cycle) was used to determine structural stiffness of the ankle. Lastly, peak isokinetic force recorded during dynamometer testing was entered into the participant’s Excel workbook and pre- and post-experimental differences of torque were assessed at the end of the protocol.

Statistical Analysis

This experiment aimed to quantify changes in ankle range of motion and gastrocnemius fascicle length, material stiffness, passive range of motion, isokinetic strength, and muscle activation during muscle contraction and passive ROM across time for each group involved in the experiment. Additionally, changes in these categories were compared across groups for this study; for statistical analyses of variables, the factors of time (pre-test and post-test) vs. group (stretch and control) were tested with a mixed model repeated measures on time and between group comparisons ANOVA with a P-value set at < .05 in order to determine any significant changes as a result of this experiment. Regression analyses were performed between ROM and
each individual physiological factor to determine if any changes in the physiological characteristics we measured have a relationship with the change in ROM. With 8 experimental subjects, large effect sizes (~0.9) will be needed in order to achieve a power of 0.8. However, changes that would result in smaller effect sizes would not be large enough to be physiologically meaningful.
Chapter IV. Results

This study was conducted to determine the effects a 6-week PNF stretching program has on the muscular architecture, stiffness, strength, ROM, and neuromuscular activity of the gastrocnemius. We hypothesized PNF stretching would increase fascicle length, decrease material and structural stiffness, have no effect on strength, and desensitize neuromuscular responses which would increase the ROM in the ankle. This chapter contains the following sections: 1) ROM changes, 2) Stiffness, 3) CSA, 4) Fascicle Length, and 5) neuromuscular changes.

Range of Motion

The two types of ROM analyzed in this study were total range of motion and dorsiflexion range of motion; joint angle readings taken from dynamometer tests were used to determine both types of ROM.

Total Range of Motion

Total ROM indicates the magnitude of motion in both dorsiflexion and plantar flexion movements. Figure 18 shows the degree of change in ROM in the stretched leg for every subject with exact numbers listed in Table 2 along with data from the control leg. ROM in the treatment leg increased significantly from $53.13 \pm 6.62^\circ$ to $61.88 \pm 7.02^\circ$ while no significant differences were observed in control leg as ROM measurements went from $53.38 \pm 6.67^\circ$ to $52.38 \pm 8.09^\circ$ (Figure 19; significant interaction effect for group and time, p= 0.02). There was a significant main effect for time (p= 0.03), but no group main effects (p= 0.11).
Figure 18: Individual pre-test and post-test ankle range of motion (ROM) in the treatment leg for each subject.

Table 4: Individual pre-test and post-test ankle range of motion (ROM) for the treatment leg and control leg for each subject. Units are in degrees.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment Leg ROM</th>
<th>Control Leg ROM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
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</tbody>
</table>
Figure 19: Average of the combined pre-test and post-test ankle range of motion (ROM) for the treatment and control legs. # indicates significant interaction effect (p =0.02).

Dorsiflexion Range of Motion

Figure 20 displays each subject’s pre-test and post-test dorsiflexion ROM. Dorsiflexion ROM for the treatment group did not significantly change as the group average went from 28.38 ± 4.63° to 24.13 ± 6.79° and in the control average dorsiflexion ROM did not significantly change going from 31.13 ± 4.22° to 29.88 ± 2.36° (Figure 21). There was no group by time interaction (p= 0.328), main effect for time (p= 0.530), or group main effect (p= 0.17).
Figure 20: Pre-test and post-test dorsiflexion range of motion (ROM) in the treatment leg for each subject.

Table 5: Dorsiflexion range of motion (ROM) for the treatment leg and control leg for each subject. Units are in degrees.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment Leg ROM</th>
<th>Control Leg ROM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
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</table>
Figure 21: Average of the combined pre-test and post-test dorsiflexion range of motion (ROM) for the treatment leg and control leg.

**Stiffness**

**Structural Stiffness**

Pre-test and post-test structural stiffness for the treatment leg was 0.485 ± 0.30 Nm/° and 0.444 ± 0.17 Nm/°, respectively; pre-test and post-test structural stiffness for the control leg was 0.295 ± 0.07 Nm/° and 0.258 ± 0.085 Nm/°, respectively (Figure 22). Structural stiffness of the treatment leg decreased by about 40% and by about 42% in the control leg indicating a main effect for time (p= 0.029); however, no group main effect (p= 0.396) and no group by time interaction (p= 0.957) was present.
Figure 2: Structural stiffness of the control and treatment leg before and after the PNF stretching intervention. The slope of torque versus change in angle (5° - 10° of dorsiflexion) is used to quantify structural stiffness. * Indicates main effect for time.

Material Stiffness

In the treatment leg, the average right lateral gastrocnemius (RLG) material stiffness went from 18.09 ± 4.09 kPa to 20.41 ± 6.68 kPa while the right medial gastrocnemius (RMG) material went from 17.13 ± 4.74 kPa to 19.09 ± 3.07 kPa. Figure 23 displays the group averages of material stiffness for each gastrocnemius. In the control legs, left lateral gastrocnemius (LLG) material stiffness average began at 18.01 ± 3.69 kPa and ended at 21.59 ± 5.91 kPa and left medial gastrocnemius (LMG) modulus went from 18.09 ± 4.58 kPa to 19.54 ± 4.76 kPa. There was no group main effect (p= 0.599), no main effect for time (p= 0.077), and no group by time interaction (p= 0.879).
Table 6: Pre-test and post-test material stiffness values for the treatment leg of each subject. Right lateral gastrocnemius (RLG) and right medial gastrocnemius (RMG) material stiffness values are in kPa.

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Table 7: Pre-test and post-test material stiffness values for the control leg of each subject. Left lateral gastrocnemius (LLG) and left medial gastrocnemius (LMG) material stiffness values are in kPa.

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Cross Sectional Area & Strength

To determine average cross sectional area (CSA), areas obtained from the 6 cross section images were added together for each subject then averaged as a group (Figure 24). RLG CSA pre-test averages went from $25.15 \pm 4.18 \text{ cm}^2$ to $25.60 \pm 5.33 \text{ cm}^2$ in post-test measurements; the RMG CSA pre-test average was $47.98 \pm 9.38 \text{ cm}^2$ and the post-test average was $48.87 \pm 11.15 \text{ cm}^2$. In the control leg, LLG CSA pre-test averages was $24.99 \pm 3.35 \text{ cm}^2$ and the post-test average was $23.53 \pm 3.46 \text{ cm}^2$; the LMG CSA pre-test average went from $49.53 \pm 10.30 \text{ cm}^2$ to a post-test average of $48.45 \pm 10.44 \text{ cm}^2$. There was no group effect ($p=0.364$), no main effect for time ($p=0.291$), and no group by time interaction ($p=0.122$) for CSA.

Isokinetic strength was also assessed to determine if there were differences in CSA after 6 weeks of PNF stretching. Figure 25 displays peak muscle torque during isokinetic testing for each leg; the treatment leg peak isokinetic force pre-test average was $95.56 \pm 34.78 \text{ Nm}$ and the post-test average was $88.11 \pm 14.73 \text{ Nm}$. The control leg peak isokinetic force average was...
initially $80.10 \pm 31.28$ Nm and was $72.84 \pm 22.94$ Nm for the post-test average. There was a group main effect ($p=0.025$), but there was no main effect for time ($p=0.054$), and no group by time interaction ($p=0.981$) for CSA.

**Figure 24:** Combined average for the total cross sectional area (CSA) in the treatment leg (RLG & RMG) and control leg (LLG & LMG). The average CSA was determined for each of the 6 cross section sites for the group as a whole, then all 6 group averages were added together to determine the combined CSA of each muscle.
Fascicle Length

Figure 25: Group averages of peak isokinetic muscle torque in the treatment and control leg.

In the treatment leg, the RLG fascicle length average significantly increased from 26.86 ± 3.37 mm at baseline and t0 27.36 ± 3.30 mm after post-testing; RMG fascicle length at baseline was 28.08 ± 2.23 mm and significantly increased to 28.63 ± 2.71 mm. In the control leg, LLG pre-test and post-test fascicle length average was 28.13± 2.17 mm and 28.23 ± 2.27 mm, respectively; LMG pre-test fascicle length average was 27.64 ± 1.88 mm and was 28.62 ± 1.56 mm after post-testing. There was a main effect for time (p= 0.004) and a significant group by time interaction (p= 0.033), but there was no group main effect present (p= 0.159).

Each subject’s fascicle length at each measurement site in the RLG (Table 8), RMG (Table 9), LMG (Table 10), and LLG (Table 11) are displayed below. A regression analysis of
change in ROM versus change in fascicle length were done for the treatment leg (Figure 27) and the control leg (Figure 28), however none of these analyses explain the variability in the data.

Table 8: Fascicle length (mm) for each subject at each measurement site in the right lateral gastrocnemius (RLG).

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Table 9: Fascicle length (mm) for each subject at each measurement site in the right medial gastrocnemius (RMG).

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Table 10: Fascicle length (mm) for each subject at each measurement site in the left lateral gastrocnemius (LLG).

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Table 11: Fascicle length (mm) for each subject at each measurement site in the left medial gastrocnemius (LMG).

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Figure 26: Average total fascicle length for the treatment leg (RLG & RMG) and the control leg (LLG & LMG).

Figure 27: Change in range of motion (ROM) vs. net change in fascicle length in treatment leg.
Neuromuscular Adaptations

Normalized EMG activation means for each muscle are displayed in Figure 29; peak EMG voltage during CPM testing was divided by peak EMG voltage during isokinetic strength testing to determine muscle activation percentage. RLG averages decreased from $7.23 \pm 4.05\%$ to $6.69 \pm 4.87\%$; RMG averages decreased from $6.69 \pm 2.42\%$ to $5.72 \pm 4.69\%$. LLG averages decreased from $6.69 \pm 4.38\%$ to $6.21 \pm 3.63\%$ and LMG activation decreased from $6.76 \pm 2.60\%$ to $6.27 \pm 4.58\%$. There was no group main effect ($p=0.628$), no main effect for time ($p=0.807$), and no significant group by time interaction ($p=0.932$).
Figure 29: Normalized muscle activation percentage for the treatment leg (RLG & RMG) and the control leg (LLG & LMG). Peak EMG voltage during CPM testing was divided by peak EMG voltage during isokinetic strength testing to determine muscle activation percentage.

**Summary**

Our 6-week PNF stretching program resulted in a significant increase in total ROM (group by time interaction, p= 0.02; main effect for time, p=0.03) for the stretched leg compared to the control leg, with few changes in muscle architecture or neural properties. PNF stretching also a significant main effect for time on the ROM and fascicle length of the treatment leg. No significant changes were observed in CSA/strength, stiffness, fascicle length, and neuromuscular changes.
Chapter V. Discussion

The purpose of this research was to assess physiological changes of muscle architecture and neural adaptations of the gastrocnemius as a result of a 6-week PNF stretching program performed on young women. We hypothesized an increase in ROM would be dependent on changes in the following muscle properties: material stiffness, structural stiffness, CSA, muscle fascicle length, and neural adaptations. In this chapter, we will discuss our methodology, results, and hypotheses in relation to similar research studies. This chapter will be divided as follows: 1) Influence of PNF Stretching Interventions on ROM, 2) Physiological Impact of PNF Stretching on Muscle, 3) Future Considerations, and 4) Conclusions.

Influence of PNF Stretching Intervention on ROM

Increases in ROM resulting from PNF stretching has been well documented in the past, however, there is no consensus as to which physiological adaptations are responsible for this increased ROM. In our study ROM in the treatment leg increased significantly from 53.13 ± 6.62° to 61.88 ± 7.02° while no significant differences were observed in control leg as ROM measurements went from 53.38 ± 6.67° to 52.38 ± 8.09°. Our study has produced results similar to previous research (Konrad et al, 2014; Mahieu et al, 2009) in that ROM significantly increased by 9 degrees from a chronic PNF stretching program. The goal of our study was to determine which physiological adaptations from PNF stretching contribute to an increased ROM; in doing so we have confirmed previous findings and shed some light on which physiological adaptations may play a role in increasing ROM.

Total ROM and Dorsiflexion ROM

Chronic PNF stretching programs significantly increase total ankle ROM (Konrad et al, 2015; Mahieu et al, 2009) accompanied by significant increases in dorsiflexion ROM (Akagi et al, 2013b; Nakamura et al, 2012; Mahieu et al, 2007); these two outcomes are also present after
chronic static stretching programs (Akagi et al, 2013b; Nakamura et al, 2012; Mahieu et al, 2007). These studies were the basis for our hypothesis that we would see in an increase in total ROM with the assumption that an increase in dorsiflexion ROM would be responsible for this increase. We did see a significant increase in total ROM, but no changes occurred in the dorsiflexion direction; instead there was an increase in plantarflexion ROM possibly due to psychological factors in terms of subjects being more comfortable with dynamometer testing during post-testing. If this were the case, increases in plantarflexion ROM in the control leg would be observed, but since this did not happen it is unlikely psychological factors played a role.

The absence of an increase in dorsiflexion ROM may be due to inconsistently placing subjects in the anatomical neutral position while testing on the dynamometer, thus creating bias. Since increases in dorsiflexion ROM were expected from a stretching intervention (Konrad et al, 2015; Mahieu et al, 2009; Akagi et al, 2013b; Nakamura et al, 2012; Mahieu et al, 2007), an attempt at correcting the placement of anatomical neutral was done by using 0 force on the PRT vs. angle charts generated by CPM testing as the neutral position for each subject. This corrective measure did not result in significant changes in dorsiflexion ROM, but this correction may not be appropriate due to the fact the stretching intervention may have altered anatomical neutral position for each subject it is likely an error in placement of anatomical neutral has prevented observable differences of pre-test and post-test dorsiflexion ROM.

**Intervention Protocol**

Previous studies examine the effects of stretching on all male samples (Nakamura et al, 2014; Akagi et al, 2014b; Konrad et al, 2014) with few studies examining a sample with roughly half women (Konrad et al, 2015; Mahieu et al, 2009). Our all-female internal control sample is
the first of its kind in chronic PNF stretching literature. This was done to compare what differences may occur in a study sample of all females with other study samples which include all or mostly males.

Similar studies (Konrad et al, 2014; Mahieu et al, 2009) implemented 6 weeks of PNF stretching with significant changes in ROM, however our study involved more comprehensive analyses of physiological adaptations in order to detect something that may have gone unnoticed in previous studies. Our study implemented a 6-week PNF stretching intervention with each set of PNF stretching including: a 15-second stretch-and-hold, a 6-second contract-and-hold, and another 15 second stretch-and-hold for a total of 5 times per session, 3 times per week for a total of 15 sets per week. This was done in order to replicate the Konrad et al (2014) intervention which included a total of 16 sets per week; 15 was chosen in our study in order to evenly distribute sets across the week to prevent an imbalance.

Implementation of internal control in this study allowed for the analysis of 16 different legs, which is small compared to previous literature; the number of subjects in the Akagi et al (2013b) study was 38, which is the smallest sample size of studies similar to ours. Subject characteristics from previous research included an average age of about 23 year and active but not elite athlete type individuals which is identical to the subjects in our study (average age of about 22.38). Subjects were recruited by flyers placed in Ward Sports Medicine Building on a first-come-first-serve basis, leaving the possibility open for underrepresentation of the entire female population. There is a small possibility that the length, frequency, and subject recruitment in our protocol limited our intervention and prevented observation of more statistically significant findings, but this is unlikely due to the fact significant increases in ROM were present at the end of the study.
Physiological Impact of PNF Stretching on Muscle

CSA and muscle strength do not significantly change as a result of stretching (Akagi et al, 2013b; Konrad et al, 2015; e Lima et al, 2014; Freitas et al, 2015; Nakamura et al, 2012), which is confirmed by our findings. An increase in CSA would ultimately mean an increase in strength, however since CSA remained unchanged strength was unaltered as well. The lack of change in gastrocnemius volume, CSA, and strength from PNF stretching suggest other physiological elements which were not assessed may have an influence ROM.

Ambiguous findings from past research related to decreased muscle activation make measurement of electrical output of the gastrocnemius and important factor to measure. Previous research suggests decreased muscle activation due to chronic stretching increases range of motion (Akagi et al, 2013b; Hayes et al, 2012), but since muscle activation was not directly measured this is only a speculation. H-reflex measurement techniques, which is different from EMG but is used to directly measure muscle activation, suggests no changes in muscle activation occur with 6 weeks of stretching (Hates et al, 2012), but with 8 weeks of stretching there may be a decrease in muscle activation (Guissard et al, 2004). This may suggest that at least 8 weeks of stretching is necessary to observe changes in muscle activation, however using EMG to track changes in electrical output of muscle after 8 weeks of stretching suggests there is no change in muscle activation (Gajdosik et al, 2005) and contradicts this notion. It may be necessary to measure muscle inhibition rather than muscle activation in order to determine any neuromechanical changes to muscle after a chronic stretching program.

Average RLG activation percentage during pre-testing was 7.23% and decreased to 6.69% during post-testing; RMG activation percentage during pre-testing was 6.70% and decreased to 5.72% during post-testing. Small EMG voltage values during pre-testing suggests
gastrocnemius muscle activation does not prevent the ankle from moving through a full ROM; since there were small voltage values initially, it was unlikely muscle activation would have an effect on passive ROM at the end of the study. Also, there was a possibility of variable placement of the EMG electrodes on subjects from pre-test to post-test; however, this error was minimized through electrode placement guidelines.

There was a no significant change in material stiffness at the end of our intervention which is the opposite of results in previous studies (Akagi et al, 2013b); since our protocol did not modify material stiffness, it is unlikely it is a limiter of ROM. Structural and material stiffness were expected to decrease in response to the stretching intervention, but the structural stiffness significantly decreased while the material stiffness of the gastrocnemius did not change, indicating there was no training effect. Decreases in structural stiffness have been previously reported (Mahieu et al, 2009; Mahieu et al, 2007), however, this change does not assess physical adaptations such as material stiffness or muscle volume because structural stiffness is an outcome measure. Hormones, such as estrogen and progesterone, released at different levels throughout the menstrual cycle may have interfered with the material stiffness of the gastrocnemius; these hormones have been documented to either decrease the structural stiffness of muscle and ligaments (Eilling et al, 2007; Park et al, 2009; Sarwar et al, 1996; Heitz et al, 1999) or have no effect on muscle stiffness (Bell et al, 2012) or ligament laxity (Belanger et al, 2004). However, it is not known if chronic stretching may interfere with these hormones and the effects they have on joint and muscle stiffness. Our study did not monitor any menses related factors during our protocol, therefore no definite answer can be given on whether menstrual hormones effect material and structural stiffness of the gastrocnemius.
Footwear worn leading up to pre-test and post-test measures may have influenced our stiffness measurements and interfered with our expected findings. There may have been a seasonal effect where subjects wore high heels at a higher frequency during the warmer months of post-testing as opposed to wearing flat-soled shoes during the colder months of pre-testing. Frequent wearing of high heels would cause gastrocnemius muscles to remain shortened for a long period of time throughout the day, possibly negating the effects the stretching protocol has on material and structural stiffness.

Similar increases in fascicle length have been recorded in previous studies (Freitas et al, 2015; Blazevich et al, 2014), however the cumulative increase of 1 mm in fascicle length does not fully explain the increase in ROM observed in our study. The equation below explains how plantar flexors with a moment arm of approximately 40 mm (Maganaris et al, 2000) coupled with the same 1-mm fascicle length increase found in our study generated an increase in ROM by approximately 1.4°; since the increase in ROM of our subjects was almost 9°, there appears to be some other physiological adaptations responsible for the remaining 7° of increased ROM.

\[
\text{Moment Arm} = \Delta \text{Fascicle Length} \div \Delta \text{Joint Angle}
\]

\[
40 \text{mm} = 1 \text{ mm} \div \Delta \text{Joint Angle}
\]

\[
\Delta \text{Joint Angle} = 1 \div 40
\]

\[
\Delta \text{Joint Angle} = 0.25 \text{ radians or } 1.43^\circ
\]

None of the physiological changes we observed are able to fully explain why ROM increases after chronic PNF. A possible explanation, based on the equation above, is that adaptations occurred in the soleus making the soleus is the primary limiter of ankle ROM.
Biarticular muscles are established as limiters of ROM which is why the gastrocnemius was chosen as our focus; this may have prevented us from seeing any impact the soleus has on ROM, but it is possible to calculate what impact the soleus has on ROM with the same equation used above. Optimal fascicle lengths in the gastrocnemius are about 5.5 cm (Abe et al, 2000; Lichtwark et al, 2007), while the optimal fascicle length for the soleus is about 3 cm (Panizzolo et al, 2013); The equation below explains how moving the ankle from 0° to 8.75° of plantarflexion would cause all plantar flexor muscle fibers to stretch by about 6 mm.

\[
\text{Moment Arm} = \frac{\Delta \text{Fascicle Length}}{\Delta \text{Joint Angle}}
\]

\[
40 \text{mm} = \frac{\Delta \text{Fascicle Length}}{0.15 \text{ Radians}}
\]

\[
\Delta \text{Fascicle Length} = 40 \times 0.15
\]

\[
\Delta \text{Fascicle Length} = 6 \text{mm}
\]

This 6-mm fascicle shortening, though identical in each muscle, effects each muscle differently; as the gastrocnemius fascicles lengthen by 11% from 5.5 cm to 4.9 cm, the soleus lengthens by 20% from 3 cm to 2.4 cm. Since the soleus fascicles experience a larger percentage of change than the gastrocnemius fascicles during this movement, the soleus will move further along the passive force length curve and therefore, could be a larger limiter of ankle ROM than the gastrocnemius. Another possible mechanism for increased ankle ROM is an increase in the compliance of the Achilles tendon. In reference to the equation above, the Achilles tendon would have to stretch about 6mm in order to achieve the increase in ROM that resulted from our intervention. Given an approximate 18 cm Achilles tendon length (Rosso et al, 2012) and a typical tendon breaking strain of around 10% (Rigby et al, 1959), this increase in length is highly
unlikely. However, it is possible tendon adaptations could partially contribute to the increased ROM.

The tibiofibular interosseous membrane is another physiological component closely related to the stability of the ankle joint (Kennedy et al, 2000; Hanson et al, 2004). If our subject’s ROMs were adequate due to physiological adaptations in the gastrocnemius already being at their maximum threshold, the tibiofibular interosseous membrane may have adapted in a way to increase our subject’s ROM. Additionally, the manner of PNF stretching may have altered ligaments of the talus such as the posterior and anterior talofibular ligaments ultimately shifting the position of the talus in relation to other bones thus altering ROM. However, it is unlikely the ligaments associated with ROM of the ankle played a role in our study since no subjects engaged in stretching of the calf prior to and outside of our study.

Future Considerations

Future studies must focus on the physiological adaptations of the soleus and tibiofibular interosseous membrane to confirm their roles as limiters of ankle ROM. It may be useful to perform a similar chronic PNF stretching study in subjects with known restrictions/deficits in ROM in order to determine whether the soleus and/or tibiofibular interosseous membrane play roles in limiting ROM; using subjects with restriction/deficits in ROM may also be useful in assessing adaptations in the gastrocnemius as a result of stretching. Additionally, in-depth monitoring of footwear and the menstrual cycle for the entirety of future studies is necessary to document the influence heel height and hormones (estrogen and progesterone) have in structural and material stiffness in women. Knowledge of these aforementioned physiological adaptations, or lack thereof, and footwear habits would help us understand how PNF stretching increases ROM of the ankle and should be monitored in future studies.
Future studies should monitor changes in fascicle length, CSA, stiffness, and muscle activation of the soleus in order to determine the role it plays as a limiter of ROM. Though imaging of this muscle is difficult to achieve via ultrasound, alternative measures may need to be used to document change in the soleus. Since EMG was unable to produce numbers large enough to indicate any potential changes in muscle activation, monitoring pain tolerance with questionnaires should be considered in the future to determine what role pain threshold plays in ROM.

An additional factor which should be assessed in future stretching protocols is the pennation angle of fascicles; changes in pennation angle alter the direction of forces placed on the muscle and bone which could provide insight on stretching’s effectiveness on preventing injury. It may be beneficial to study sports teams which have already incorporated stretching programs into their practices to document pre-season and post-season physiological changes which may explain increases in ROM in addition to the impact stretching has on exercise injuries. Menstrual cycle stages should also be monitored in future studies involving women; changes in ligaments are well documented, but no studies observe the relationship long-term stretching and menstrual hormones have on each other. Data from this study can be used to construct more accurate and comprehensive techniques to monitor changes in the calf muscles to help determine what causes a change in ROM in the ankle.

The results of this study may not transfer to other muscles or joints due to varying fascicle lengths, pennation angles, and overall muscle volume of muscles throughout the body. Lack of random assignment in this study is another factor which may not allow our results to be generalized other populations. We chose to experiment on the gastrocnemius because the calf is commonly stretched across all levels of athletes and calf strains are frequent and of high clinical
interest. Our results indicate stretching may not be effective in preventing gastrocnemius injury due to the lack of significant changes in our results. However, since it is likely changes occur in the soleus, stretching may reduce the risk of injury to the calf in general as two out of three calf injuries occur at the junction of the medial gastrocnemius and soleus (Bright et al, 2017).

Conclusions

In conclusion, our stretching intervention was a success due to the increase in ankle ROM, but a full understanding for the physiological mechanisms increasing ROM must be further examined. Based on previous studies, possible contributors to increases in ankle ROM are decreased muscle activation (Guissard et al, 2004) and decreases in material stiffness (Akagi et al, 2013b), but this was not evident in our study as a result of our intervention. Increases in gastrocnemius fascicle length may slightly contribute to increases in ROM, but not enough to fully explain a significant increase in ROM. It is possible that the soleus is responsible for increasing/limiting ROM in the ankle, however, since no analytical measures were taken on this muscle, we have no information on the magnitude each physiological mechanism plays in restricting ROM. As a result, the role stretching plays in preventing injury is still ambiguous, but we have taken a few steps closer to help determine the effectiveness of stretching on injury prevention. By building off this research and comprehensively monitoring physiological changes in the soleus which stem from stretching, we will increase our understanding of muscle and joint biomechanics to ensure the safety of individuals participating in exercise.
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Young, W. B., & Behm, D. G. (2002). Should static stretching be used during a warm-up for strength and power activities?. *Strength and Conditioning Journal, 24*(6), 33; 33.


Notification of Initial Approval: Expedited

From: Social/Behavioral IRB
To: Zachary Domire
CC: Zachary Domire
     Patrick Rider
Date: 2/24/2017
Re: UMCIRB 15-001545
Effects of a Six Week Gastrocnemius PNF Stretching Intervention on Structural Properties of Muscle and Neural Adaptations of Muscle in Young Women

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 2/23/2017 to 2/22/2018. The research study is eligible for review under expedited category #4,6. 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:

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<td>Email Flyer</td>
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The Chairperson (or designee) does not have a potential for conflict of interest on this study.