EFFECTS ON PLANTARFLEXOR STRENGTH, CALF GIRTH, AND DORSIFLEXION RANGE OF MOTION WHEN STATIC STRETCHING IS IMPLEMENTED DURING TWO WEEKS OF IMMOBILIZATION

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Effects of Plantarflexor Strength, Calf Girth, and Dorsiflexion Range of Motion When Static Stretching is Implemented During Two Weeks of Immobilization

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ABSTRACT

This study examined the effects of chronic static stretching on dorsiflexion range of motion (ROM), calf strength, and calf girth when immobilized for two weeks. Thirty-six North Dakota State University students participated in this study and were divided into one of three groups: control group (CG), experimental group (EG), and experimental stretching group (ESG). All participants completed a pre-test of calf girth, strength, and dorsiflexion ROM. After the pretest, both experimental groups wore an immobilizer for two weeks. In addition, the ESG stretched for 10 minutes, twice daily. Data analysis indicated significant differences were found between groups for post-test measures of girth (F_{2,31}=6.50, p=0.0048), dorsiflexion ROM (F_{2,31}=29.06, p<0.0001), and strength (F_{2,31}=6.74, p=0.0041). Post hoc testing indicated significant increases in dorsiflexion ROM and calf strength in the ESG and significant decreases in dorsiflexion ROM and calf strength in the EG. Also, the EG lost more girth than the ESG.
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### TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>CHAPTER I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Research Questions</td>
<td>2</td>
</tr>
<tr>
<td>Research Hypothesis</td>
<td>2</td>
</tr>
<tr>
<td>Assumptions</td>
<td>2</td>
</tr>
<tr>
<td>Limitations</td>
<td>3</td>
</tr>
<tr>
<td>Delimitations</td>
<td>3</td>
</tr>
<tr>
<td>Organization of Literature</td>
<td>3</td>
</tr>
<tr>
<td>Definitions of Terms</td>
<td>3</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>5</td>
</tr>
<tr>
<td>CHAPTER II. LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td>Databases and Keywords Searched</td>
<td>6</td>
</tr>
<tr>
<td>Stretching Terminology</td>
<td>7</td>
</tr>
<tr>
<td>Hypertrophy</td>
<td>9</td>
</tr>
<tr>
<td>Atrophy</td>
<td>11</td>
</tr>
<tr>
<td>Range of Motion</td>
<td>12</td>
</tr>
<tr>
<td>Chronic Stretching Effects When Added To Other Techniques</td>
<td>13</td>
</tr>
<tr>
<td>Chronic Stretching Effects With Limited Motion</td>
<td>15</td>
</tr>
<tr>
<td>Stretching Theories Examined in Animal Studies</td>
<td>19</td>
</tr>
</tbody>
</table>
Summary…………………………………………………………………………………..25

CHAPTER III. METHODS………………………………………………………………26
Experimental Design……………………………………………………………………26
Subjects…………………………………………………………………………………..26
Procedures………………………………………………………………………………27
Instrumentation………………………………………………………………………..30
Statistical Analysis…………………………………………………………………….31

CHAPTER IV. MANUSCRIPT…………………………………………………………32
Abstract…………………………………………………………………………………..33
Introduction……………………………………………………………………………34
Methods…………………………………………………………………………………35
Results…………………………………………………………………………………..37
Discussion……………………………………………………………………………..38
Conclusion…………………………………………………………………………….43
Acknowledgments……………………………………………………………………43
References…………………………………………………………………………….43
Tables………………………………………………………………………………….46
Figures………………………………………………………………………………….47

CHAPTER V. CONCLUSION/CLINICAL APPLICATION…………………………49
REFERENCES………………………………………………………………………..56

APPENDIX A. IRB APPROVAL LETTER………………………………………61
APPENDIX B. INFORMED CONSENT…………………………………………62
APPENDIX C. PARTICIPANT RECRUITMENT EMAIL…………………………66
APPENDIX D. DATA SHEET ........................................................................................................... 67
APPENDIX E. RECORDED DATA ............................................................................................. 68
APPENDIX F. TIME LOG ............................................................................................................. 69
APPENDIX G. REHABILITATION PROGRAM .......................................................................... 70
APPENDIX H. STATISTICS ........................................................................................................ 71
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Payments per Subject</td>
<td>27</td>
</tr>
<tr>
<td>2. Schedule</td>
<td>28</td>
</tr>
<tr>
<td>3. Stretching Protocol</td>
<td>29</td>
</tr>
<tr>
<td>4. Stretching Protocol</td>
<td>46</td>
</tr>
<tr>
<td>5. Girth, Dorsiflexion, and Strength (Mean ± SD)</td>
<td>46</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Girth</td>
<td>47</td>
</tr>
<tr>
<td>2. Dorsiflexion</td>
<td>47</td>
</tr>
<tr>
<td>3. Plantarflexion</td>
<td>48</td>
</tr>
</tbody>
</table>
CHAPTER I. INTRODUCTION

Stretching has long been an essential aspect of athletic performance. The effects of acute static stretching have been greatly studied and have shown a decrease in muscle performance. However, chronic stretching, which is defined as a stretching program done over several days to weeks and emphasizes the long-term or chronic effects of stretching, has been shown to improve performance. More specifically, chronic stretching has increased muscle strength and endurance over time. The ability to increase muscle strength through a low-intensity component of an exercise program such as static stretching could be ideal for post-operative athletes. Furthermore, the maintenance of muscle strength is crucial in the return-to-play progression of the initial stages of rehabilitation for injured athletes.

Stretching can enhance the hypertrophic effect when implemented with a resistance training program. Furthermore, stretching has been shown to increase strength in humans while activity is restricted. Although not the primary purpose of static stretching, longer periods of static stretching may result in stretch induced hypertrophy. Animal studies that have induced static stretching to a muscle have noted muscle hypertrophy after the static stretch had been continuously applied for long periods of time. These animal studies are not applicable to human studies due to the duration of stretch that is induced (up to 24 hours per day), therefore, human research is needed to analyze the effects of chronic stretching on strength during immobilization.

Finally, immobilization can cause a dramatic decrease in muscle size after two weeks. This decrease in muscle size after immobilization can be a predictor of the return-to-play progression. Athletes must return to normal muscle size and strength before returning to play.
Decreasing the amount of atrophy and the amount of strength lost due to immobilization by static stretching could be ideal for individuals that are immobilized.

**Research Questions**

1. Does static stretching of the plantarflexor muscles during two weeks of immobilization result in a decrease in atrophy?
2. Does static stretching of the plantarflexor muscles during two weeks of immobilization result in a reduction in loss of muscular strength?
3. Does static stretching of the plantarflexor muscles during two weeks of immobilization result in a reduction in loss of dorsiflexion ROM?

**Research Hypothesis**

1. It is hypothesized that static stretching during two weeks of immobilization of the plantarflexors will result in a decrease in atrophy, a reduction in loss of muscle strength, and a reduction in the loss of dorsiflexion ROM.

**Assumptions**

1. The Cybex II Isokinetic Dynamometer is a valid measurement of muscular strength.
2. Strength gains after two weeks of stretching are strictly due to the static stretching program.
3. Strength gains are due to the stretch induced muscle hypertrophy and not another factor.
4. Verbal cues for stretching will create the same tension in stretches with each person.
5. All subjects are creating the same amount of tension in the muscle with a stretch.
6. The data from this study can be implemented in the clinical setting with injured athletes.
Limitations

1. Stretching cannot be induced for several hours a day, as demonstrated in animal studies, to receive the same fast onset of stretch induced hypertrophy; however, several sessions of static stretching for two-weeks may have the same effect.

2. Atrophy and the decrease in strength of an injured athlete are closely compared to subjects who are immobilized without an injury.

Delimitations

1. Subjects did not possess previous injury to the calves.

2. Healthy subjects were chosen in this study.

3. Stretching was induced twice daily for two weeks with the duration approximately twenty minutes a day; Thirty seconds was spent on gastrocnemious and soleus five times.

Organization of Literature

Chapter I: Introduction

Chapter II: Review of literature

Chapter III: Methodology

Chapter IV: Manuscript, includes the results, to be submitted for publication

Chapter V: Conclusion/ Clinical Application

Definitions of Terms

Active—Dynamic flexibility.\(^8\)

Acute Stretching—Stretching for a single bout or for a short duration. Acute stretching will focus on the acute effects of stretching.\(^2\)

Atrophy—The loss in muscle mass or strength due to a decrease in physical activity.\(^9\)
Constant-Angle Stretching—Static stretch that is held at the same point for a period of time.¹⁰

Constant-Torque Stretching—Static stretch that is kept at constant resistance.¹⁰

Chronic Stretching—Stretching for an extended period of time usually consisting of weeks. Chronic stretching focuses on the chronic effects of stretching or a stretching program.²

Cybex II Isokinetic Dynamometer—Equipment used to measure strength isokinetically.⁸

Healthy—A person who does not suffer from any health related illnesses and does not have a history of lower leg injury.

Injury—Any damage or harm done to the body.⁸

Isokinetic—a movement that takes place at a constant speed.⁹

Long-Term Stretching—See Chronic Stretching

Hypertrophy—Increase in cell size.⁹

Muscle Hypertrophy—Increase in muscle size.⁹

Muscle Strength—The ability for a muscle to create a force with maximal effort.⁸ The amount of force that can be produced using the Cybex II Isokinetic Dynamometer will indicate muscle strength.

Non-Taxing Exercise—An activity that puts a minimal amount of stress on the body.

Passive—Static flexibility.⁸

Physically Active—Someone who works out or participates in a strength training program for a minimum of one to a maximum of seven days per week.

Post-operative—The time immediately after a person who has undergone a surgery.

Range of Motion (ROM)—The amount of motion received at a joint in various action.⁸
Resistance Training Program—A program consisting of several exercises that create a resistance for a muscle or muscle group with the goal to increase muscle strength.  

Return-to-Play Progression—The development of a subject throughout a rehabilitation process to return to normal physical activities or to return to a sport.  

Static stretching—Elongation of a muscle to the point where there is a discomfort and then holding this position for a length of time.  

Strain—Deformation of muscular tissue.  

Stretching—Elongating a muscle.  

Stretching Program—A number of stretching exercises that are applied several times a week for several weeks targeting certain muscles.  

Stretch Induced Hypertrophy—A theory for why there is an increase in strength due to a stretching program.  

**Abbreviations**  
Hours (hr), Minutes (min), Seconds (s), Weeks (wk)
CHAPTER II. LITERATURE REVIEW

This literature review discusses the effects of chronic static stretching on ankle strength and atrophy after two weeks of immobilization and is organized by the following topics:

- Databases and Key Words Searched
- Stretching Terminology
- Hypertrophy
- Atrophy
- Range of Motion
- Chronic Stretching Effects When Added to Other Techniques
- Chronic Stretching Effects with Limited Activity
- Stretching Theories Examined in Animal Studies
- Summary

**Databases and Keywords Searched**

The following databases were searched in writing this literature review: National Library of Medicines; Pub med (Medline and EBSCO) and sport discus (SPORTDiscus). Journal articles written in English between the years 1927 and 2011 were searched. Additional references were collected by a careful analysis of the citations of others’ research. The following key words were used:

2. Atrophy  Performance 8. Performance  13. Stretch Induced-
Effects  6. Long-Term  10. Static stretching

6
Stretching Terminology

Categories. Stretching has long been an important aspect of athletics. Stretching is the state of a muscle being extended or elongated. There are two categories of stretching that are based on the period stretching is induced: acute stretching and chronic stretching. Acute stretching is performing a bout of stretch on a muscle for a short period of time.\(^2\) Acute stretching is termed this way because it examines the short-term effects of stretching. Chronic stretching is defined as a stretching program done over several days to weeks and emphasizes the long-term or chronic effects of stretching.\(^2\) These two classifications of stretching have very different acute and chronic effects and are important to analyze. In a recent meta-analysis,\(^2\) 21 articles were analyzed on the acute effects of different types of stretching. All of these articles found negative effects on performance: force, torque, and jump. In fact, these negative results of acute stretching can last up to 30 minutes.\(^{11}\) Other research has shown that the negative effects of acute stretching can be counteractive when the proper warm up was implemented.\(^{12}\) Although many negative effects of acute stretching on physical performance have been noted, the effects of chronic stretching have only recently been studied. This literature review will focus on the chronic effects of stretching and more specifically the effects on muscular strength and muscle size.

Types. There are three main types of stretching: proprioceptive neuromuscular facilitation (PNF), ballistic, and static. PNF stretching is the most taxing on the body and requires assistance by an additional person. This technique involves a combination of active and passive stretching.\(^{13}\) In contrast, ballistic stretching consists of a repetitive bouncing movement when the muscle is in an elongated position.\(^{13}\) Ballistic stretching has been known to create a greater environment for injury.\(^{14}\) Finally, static stretching consists of the muscle being elongated
slowly and then being held for 6-60 s. This stretching technique is more frequently used because it is easy to use and is least commonly associated with injury. This method has proven to be an effective method of stretching to increase joint ROM, however, no studies have been done on what stretching method is best to increase muscle performance.

*Types of Static Stretching.* Static stretching is used most frequently, but is a loosely defined term that implies no change in motion or angle to a joint when a stretch is given. Static stretching can be done individually and consistently, and has a small chance of injury making it the best choice for stretching a large group of people. A static stretch can be held at the same point for a period of time which is termed constant-angle stretching. Constant-angle stretching is the elongation of a muscle until discomfort (maximal ROM) and then holding the stretch. A factor in decreasing maximal ROM is the stretch reflex. Muscle spindles are located in the muscle itself and are responsible for sensing a change in muscle length. When a muscle is drastically elongated, the muscle fibers respond to prevent damage as well as the golgi tendon organs. The golgi tendon organs are located in the tendons associated with muscle and are responsible for detecting changes in muscle tension. Once a stretch is held for a certain length of time, the golgi tendon organs are inhibited, and the amount of torque is changed. For this reason, a muscle can be slightly stretched further after approximately seven seconds, increasing the maximal ROM. Stretching that is kept at a constant resistance is entitled constant-torque stretching. This type of stretch provides the greatest amount of resistance throughout the static stretch. In addition, constant-torque stretching has been shown to decrease musculotendinous stiffness. For this reason, constant-torque static stretching may be the best type of static stretching to use when looking for the greatest results.
**Duration.** Another important aspect of stretching is the duration of the stretch. Static stretch is most effective for increasing muscle flexibility at 30 s as opposed to 0, 15, or 60 s.\(^ {15}\) Also, the amount of times per week a stretch is induced is important when looking at chronic stretching. It has been suggested that stretching should be performed three times a week with a 36 to 48-hour rest period in between each session.\(^ {16}\) However, other research states that a stretch should be performed at least three times a week, or more preferably, daily.\(^ {14}, 15\) Additionally, stretching should be performed four to five times in each session for each muscle or muscle group. Although the duration of stretching has been studied greatly with the goal of an increasing range of motion (ROM), stretching duration, with the objective to increase strength via hypertrophy, is yet to be studied.

**Hypertrophy**

Generally, strength is directly correlated with the size of a muscle. Hypertrophy is defined as an increase in cell size.\(^ {9}\) The actin and myosin contractile proteins increase in size and number resulting in hypertrophy.\(^ {17}\) In eccentric training, muscle cells also add sarcomeres longitudinally, thus adding length to the muscle fiber as well.\(^ {18}\) There are three main components of skeletal muscle hypertrophy: satellite cells, immunology, and growth factor proteins. Satellite cells assist in the growth, maintenance, and repair of damaged muscle tissue and increase the size and number of actin and myosin within a muscle fiber. Satellite cells are activated when the muscle fiber receives any form of trauma, overload, or damage, which in turn can develop as a result of injury, resistance training, or, arguably, stretching.\(^ {4}\)

The inflammatory response also plays an important part with muscle hypertrophy. Inflammation generally follows trauma with the purpose of containing and repairing body tissue and cleaning up waste products.\(^ {9}\) Neutrophils are one of the first cells to appear at the injury site
in the inflammatory process. Additionally, neutrophils have been known to aide in muscle regeneration. Neutrophils can be helpful during phagocytosis by eliminating debris and stimulate satellite cells to the area. Finally, growth factors, such as insulin-like growth factors (IGF), are involved in muscle hypertrophy. These growth factors are very specific proteins that aide in the inflammatory process. Hypertrophy can be observed when a trauma is induced on a muscle. The main hypertrophic trauma induced on a muscle is experienced through strength training. It can be argued that stretching, although not as traumatic as strength training, is a mild form of trauma to the muscle sparking the inflammatory response and resulting in hypertrophy.

Along with hypertrophy, muscle force is also an important factor in strength. Muscle force can be explained through the length tension velocity relationship. This relationship explains that length and velocity are the two main factors in force. Length depends on the number of cross bridges the actin and myosin have in contact with each other. Velocity is dependent on the speed the cross bridges can move. Without full ROM, maximum force is difficult to obtain. For this reason, the strength induced muscle hypertrophy theory may be due to a physiological change in muscle size or possibly a change in muscle length.

**Measuring Muscle Strength.** Muscle strength is a fairly loose term and can be measured in several different ways. Isokinetic testing has been used in sports related facilities since the 1960s. Some authors have found isokinetic testing to be reliable while others have found relatively low reliability coefficients. However, a well-defined test protocol for isokinetic testing in ankle plantarflexion and dorsiflexion provides a procedure that is reliable for quantifying muscle function. Peak torque, work, and power are all measurements that can be obtained using the Cybex II Isokinetic Dynamometer. However, significant differences in scores when using the Cybex II Isokinetic Dynamometer have been observed to be due to differences in

age and weight.\textsuperscript{24} According to Holmback et al.,\textsuperscript{25} peak torque is commonly used to determine maximal isokinetic strength.

\textbf{Atrophy}

Muscle atrophy is a decrease in muscle size resulting from disuse. The reduction in muscle protein synthesis is believed to be the initial cause of atrophy during disuse of the muscle.\textsuperscript{9} Secondly, atrophy can occur due to the increased muscle protein breakdown.\textsuperscript{9} Muscle atrophy can also result from disuse, such as immobilization or bed rest. Muscle atrophy is more predominant in the early phases of immobilization. Some muscles demonstrate greater rates of atrophy than others during immobilization. Many studies have looked at atrophy by looking at muscle cross-sectional area. Muscle cross-sectional area of the thigh and calf decreased at approximately 2-3\% each week during the first month of bedrest.\textsuperscript{26} Cross-sectional area was reduced in the plantarflexor muscle by 3.2\% (3,976/4,518 mm\textsuperscript{2}) while knee-extensor muscles decreased 3.6\% (6,650/7,347 mm\textsuperscript{2}) and hip-extensor muscles by 2.3\% (8,728/8,931 mm\textsuperscript{2}) during bed rest for one month. In a study by Stevens et al.\textsuperscript{7} where the ankle was immobilized for seven weeks, dorsiflexors atrophied 18.9\% while plantarflexors atrophied 24.4\% respectively. Most of the atrophy happened in the first two weeks equaling 9.6\% dorsiflexion and 14.1\% plantarflexion.\textsuperscript{7} Resistance exercise provides the muscle with an overload stimulus and promotes an increase in protein synthesis resulting in hypertrophy.\textsuperscript{9} Without an overload to the body, there is an increase in muscle protein breakdown resulting in atrophy.

\textbf{Immobilization.} Immobilization is usually done by casting or bracing. Immobilization in a muscle’s shortened position is often done after injury to aid in the healing process, but results in atrophy.\textsuperscript{27} This atrophy can usually be reversed after returning to activity. However, observed immobilized stretching of rat hind legs led to a 7\% increase in muscle protein synthesis\textsuperscript{27}, which
may be the reason for stretch-induced muscle hypertrophy. An increase in muscle protein synthesis is seen during hypertrophy. Because it is unethical to immobilize a human being in a stretched position, bouts of stretching throughout a long period of time has similar effects as immobilizing a muscle in a lengthened position.4

**Measuring Atrophy.** Muscle size can be measured various ways. The gold standard for measuring muscle size is observed through Magnetic Resonance Imaging (MRI). Because an MRI is expensive and time consuming, another way to measure muscle size is through girth measurements. Girth measurements measure the circumference of a muscle area. Although girth measurements do not solely measure muscle (e.g. bone, adipose tissue, and skin), they have been observed to be a reliable predictor of atrophy.28 Girth measurements are also significantly correlated with measures of muscle cross sectional area.28 Depending on the tape measure used to measure girth, the reliability of the measurement can increase. Tape measures that have a spring-loaded handle make every measurement of girth have the same amount of tension while pulling around the muscle. Girth measurements are suggested after surgery to analyze differences between muscle size in the involved and uninvolved body segments.28

**Range of Motion**

The area through which a joint can move freely without pain is defined as its range of motion (ROM).8 An individual can have normal ROM or they can be hypermobile or hypomobile. Hypermobile means that a joint has an excessive amount of motion more than normal. A hypomobile joint has a lack of motion compared to normal ROM. Range of motion can be improved through several different stretching techniques as previously discussed. Chronic stretching can increase joint ROM by 8° in healthy muscles.29 It is unknown if the amount of motion a joint has is a predictor of the amount of strength that can be produced.
**Measuring Range of Motion.** A goniometer is an instrument that measures joint ROM. There are three parts to the goniometer: 1) a stationary arm, 2) a movement arm, and 3) a protractor/fulcrum. When measuring the motion of a joint, the stationary arm does not move while the movement arm is used to determine the angle of motion. The fulcrum is the moving point that is aligned with the joint, while the protractor displays degree/joint angle; a number that is often considered the gold standard for measuring joint ROM.

**Chronic Stretching Effects When Added To Other Techniques**

Several researchers have found greater strength gains in subjects who add stretching to additional training programs.\(^3\)\(^{,30-31}\) Kokkonen et al.\(^3\) matched 16 men and 16 women to partners of the same gender and similar 1RM s for knee extension. Subjects were randomly assigned to a weight-training group or to a weight-training stretching group. Both groups completed a weight training program which consisted of knee flexion, knee extension, and leg press. The weight was determined by the pre-test 1RM, and previously measured by using the Nautilus knee flexion and extension machine (Paramount Fitness Corp., Los Angeles, CA USA) and the Paramount plateloaded bilateral 45º leg press machine (Paramount Fitness Corp., Los Angeles, CA, USA).\(^3\) In addition to the weight training program, those chosen for the experimental stretching group came in on two additional days per week when they did not complete resistance training and participated in a 30 minute stretching program. Stretches were included that were specific to the hamstrings, quadriceps, adductors, abductors, external and internal rotators, plantar flexors, and dorsiflexors. After eight weeks of training, the subjects completed a post-test. Strength gains were seen in both groups for all three 1RM tests. However, 1RM for knee extension in the weight training and stretching group (781 ± 238 N) was significantly greater than the weight training group (733 ± 247 N). This significant difference was also seen in the 1RM leg press
Kokkonen et al. concluded that strength gains in early-phase novice lifters can be enhanced by adding a stretching program to a weight training program.

Dintiman examined the effects of several different combinations of flexibility, sprint, and weight training programs on ROM, leg strength, and running speed. This experiment consisted of 145 subjects divided into five groups: Experimental Group A (sprint training and flexibility training program), Experimental Group B (sprint training and weight training), Experimental Group C (sprint training, flexibility training and weight training program), Control Group I (sprint training), and Control Group II (inactive). Each subject ran the 50yd-dash for time, recorded leg strength using a dynamometer, and had several flexibility measurements taken. The appropriate stretching, strength, and sprint training programs were added to each group for a total of eight weeks; after eight weeks several post-tests were performed. Strength gains in all the groups were found (Group A = 28.20 lb, Group B= 137.30 lb, Group C= 154.50 lb, Control I= 35.20 lb) compared to the inactive group (Control II= 6.40 lb). The group with all three programs (stretching, strength, and sprint) had the largest increase in leg strength. This information is important to note for athletes with no restrictions. According to this study, the best approach to improve leg strength in sprinters is to add all three of these aspects to their programs. Although strength gains in the sprint training and stretching group were not greater than the experimental groups and Control Group I, strength gains were much greater in Group A than the Control Group II. That being said, there was an increase in performance when stretching alone was the intervention compared to the inactive group. Also, as previously stated, the Experimental Group A had a larger improvement (0.42s) than the Control Group I (0.33s) and Control Group II (0.06s) on sprint speed. Groups involving stretch training (Experimental Groups A and C) had
a significantly great increase in ROM in trunk flexion and extension, shoulder flexibility, and ankle flexibility than the other groups.

Finally, unlike other research results, Wilson et al. composed a study involving stretching and strength in the upper extremity. Sixteen male powerlifting subjects were used for an eight-week period to measure muscle stiffness and rebound and purely concentric bench press performance. All subjects participated in a pre-test for flexibility, series elastic component stiffness, rebound bench press, and purely concentric bench press performance. The subjects were split into two groups: a control group and an experimental group. Both groups participated in an eight-week training program, but twice a week the experimental group also performed several stretches such as the chest stretch, or a pole stretch that lasted a total of 10 to 15 minutes each session. After eight weeks, a post-test was performed and data were compiled. There was a significant difference in musculotendinous stiffness between pre- and post-tests in the experimental group at 70% (pre= 18,271 ± 4,090 N·m$^{-1}$, post 16,038 ± 3,603 N·m$^{-1}$) and in the maximal load condition (pre= 18,271 ±3,535 N·m$^{-1}$, post= 16,965 ±3,129 N·m$^{-1}$). Although average resultant velocity produced was not stated to be significant, an increase in rebound bench press has been shown an improvement from pre (0.288 ± 0.04 m·s$^{-1}$) to post (0.264 ± 0.05 m·s$^{-1}$) compared to the control group pre (0.218 ± 0.05 m·s$^{-1}$) and post (0.218 ±0.06 m·s$^{-1}$) data. Knowing that an increase in performance can be seen when stretching is added to other programs, it is important to look at stretching without a resistance program to see if it has the same effect on strength.

**Chronic Stretching Effect With Limited Activity**

Many healthy subjects do not need to depend on stretching to increase strength when resistance training is adequate. For some, however, resistance training may not be an option. The
elderly, injured, or post-operative patients may not be able to physically participate in resistance training and may need a low-intensity component like stretching to develop strength. The evidence disparity between stretching in combination with strength training or stretching alone has resulted in several authors looking at the effects that stretching alone has on strength.\textsuperscript{32-34} Kokkonen et al.\textsuperscript{32} gathered 38 subjects and split them up into various groups: stretching group and no stretch group (11 females and 8 males in each group). Although physical activity was not strictly limited, subjects could not participate if they were physically active more than 60 min/d for three times per week. All subjects performed several pre-tests for strength, strength endurance, 20-m sprint, vertical jump, and standing long jump; the pre-test data were collected over a course of three days. The stretching group participated in a 10-week stretching program that lasted 40 min and was performed three times per week. There were a total of 15 exercises that focused on the lower body. After the 10-week period was over a post-test was completed.

The post-test results presented a significant improvement over the pre-test score in the stretching group for sit and reach, standing long jump, vertical jump, 20-m sprint, knee flexion and extension 1RM, and knee flexion and extension endurance. There were no significant differences in the control group for any variables. Differences in strength between and within groups were observed. Within the stretching group, an increase in 1RM for knee flexion by 15.3\% (51.0 ± 14.1kg/44.7 ± 14.5kg) and extension by 32.4\% (82.0 ± 25.8 kg/ 63.8 ± 25.8 kg) was observed. Within the control group, knee flexion increased 3.3\% (47.0 ± 14.4 kg/46.1 ± 15.1 kg) and knee extension increased 2.8\% (71.0 ± 20.8 kg/ 69.7 ± 21.5kg). The difference between the stretching group and control group for knee flexion (CI of 95\% = 12.4 ± 5.8\%) increased as well as knee extension (CI of 95\% = 29.6 ± 13.2\%).\textsuperscript{32}
Large increases in endurance were also noted in this study. Within the stretching group, an increase in knee flexion endurance by 30.4% (22.3 ± 4.7kg/17.2 ± 3.4kg) and extension by 28.5% (23.7 ± 4.7 kg/ 18.5 ± 3.1 kg) were observed. Within the control group, knee flexion endurance changed -0.9% (19.3 ± 4.4kg/19.5 ± 4.1kg), and knee extension endurance changed -0.1% (16.6 ± 3.6 kg/ 18.6 ± 2.7 kg). Increases in strength and endurance for knee extension and flexion were increased drastically over 10 weeks of stretching.\textsuperscript{32}

Handel et al.\textsuperscript{33} also investigated stretching with a focus on the effects on maximum torque. Testing took place prior to the stretching program, at four weeks, and at the end of the eight-week program. The stretching program consisted of contract-relax stretches for a total duration of 10 min. An isokinetic dynamometer was used to measure torque. A remarkable 21.6% increase was seen in torque under the eccentric load with both knee extensor and flexor groups. Eccentric load increased 12.9% more than the concentric load.

In another study\textsuperscript{34} that looked at the best way to improve hamstring flexibility through stretching, isokinetic peak torque was evaluated after the increase of hamstring flexibility. Nineteen subjects stretched one leg using static stretching, and the other using PNF stretching five days a week for three weeks. Greater ROM gains were seen in PNF stretching (9.5°) than static stretching (8.0°). Isokinetic measures increased significantly at 60°/s and 120°/s eccentrically and at 120°/s concentrically. This indicates that there is a striking increase in isokinetic torque eccentrically and concentrically after PNF and static stretching of the hamstrings.

There have been two studies that have not found significant improvement in muscle performance by using a long-term stretching program.\textsuperscript{35,36} Bazett-Jones et al.\textsuperscript{35} used 21 subjects in a six-week static stretching program. Pre-tests consisted of bilateral knee ROM, 55-m sprint
time, and vertical jump; these tests were conducted again at three weeks and at six weeks. The stretching protocol consisted of four repetitions of static hamstring stretching on each leg for 45s. No significant differences were found in any of the four variables. The authors suggest that the performance improvements were not found due to the fact that ROM was not increased.

Secondly, LaRoche et al. observed the effects of four weeks of static or ballistic stretching on 29 males on hip extension. Pre and post measurements were taken on peak torque, rate of torque development, work, and peak torque angle. Subjects were divided into three groups including a ballistic group, static group, and control group. Both of the stretching groups participated in ballistic or static stretches for three days per week for four weeks, equaling a time of ten minutes per session. If there was an increase in strength due to stretching, both stretching groups would be significantly different than the control group; however, this was not the case. Peak torque increased in the static group (5.4 ± 19.0%), ballistic group (7.8 ± 12.7%), and control group (6.1 ± 17.9%). Rate of torque development increased in the static group (4.8 ± 22.7%), ballistic group (3.6 ± 28.0%), and control group (9.7 ± 24.0%). Also, work increased in the static group (3.9 ± 7.0%), ballistic group (14.7 ± 27.4%), and control group (9.7 ± 9.5%). Although there was not a significant difference, increases were seen within each group. More importantly, a decrease in strength was not seen in these groups which means stretching could possibly maintain strength. This study also did ballistic stretching which is not suggested. The population that has been looked at in each of these articles on stretching and strength varies across gender, age, and physical capabilities relating to several people. These gains in strength may be due to physiological changes in the muscle that are supported by the basic science evidence on stretch-induced hypertrophy.
**Stretching Theories Examined in Animal Studies**

**Stretch Induced Muscle Hypertrophy.** In terms of research, several animal studies have investigated stretch-induced hypertrophy.\(^\text{4,6,27-42}\) Goldspink\(^6\) looked at the role of passive stretching in slowing muscle atrophy. More specifically, Goldspink looked at protein synthesis being one of the main contributors to stretch induced hypertrophy. The combination of stretching and electrically stimulating muscles was examined for muscle protein synthesis. Muscle protein synthesis was seen to have about a 7% increase per day with stretching alone and a 9% increase per day with stretching and electrical stimulation.

In another study by Goldspink,\(^27\) the authors used young growing male rats and immobilized one leg flexed (soleus lengthened and extensor digitorum longus shortened) or extended (soleus shortened and extensor digitorum longus lengthened) and used the other leg as a control. Goldspink\(^27\) then measured protein synthesis. Each rat was in the casting for six hours, 24 hours, or seven days. A significant increase in atrophy is noted after immobilizing a muscle in a shortened position. While immobilized in a lengthened position, there is a stimulation of protein synthesis and an induced growth of muscle. The rapid growth in the rat soleus muscle was due to increased rates of protein synthesis which induced the growth of muscles held in the lengthened position. In order to guarantee protein synthesis, the muscle needs to have a prime loading force against the muscle.\(^37\) When this tension is produced, an increase in protein synthesis promotes hypertrophy.

Leterme et al.\(^38\) also did a similar study on immobilization of the soleus in a lengthened position during non-weight bearing (NWB) activity. Male Wistar rats were divided into three groups. The control group was weight bearing while the other two groups were NWB. Of the two NWB groups, one group was strictly NWB while the other was NWB with the hind legs
immobilized into dorsiflexion for the entire study (14 days). The NWB immobilized stretching group not only prevented the loss of force output but also prevented the loss of muscle mass. The NWB and stretching group increased muscle wet weight/body weight by 5% (0.44 ± 0.04mg/g) compared to the control group (0.42 ± 0.01mg/g), which the NWB group had a significant change of -26% (0.31 ±0.02mg/g) less than the control group. Stretching not only maintained the muscle wet weight/body weight ratio but also had an increase. This difference could be due to the additional sarcomeres that are added to both ends of the muscle after long-term periods of stretching are induced. When the numbers of sarcomeres are increased, the muscles ability to produce more force increases, thereby increasing strength.

It is known that atrophy is the physiological, biochemical, and histochemical change towards faster-twitch muscle fibers having decreases in strength.\textsuperscript{38} According to Leterme et al.\textsuperscript{38}, when the soleus was placed under a stretch during NWB activity, there was a greater percentage of Type I muscle fibers activity (86.7 ± 2.3) than NWB mice that were not placed under a stretch (73.4 ± 2.0). It was also shown to have a decrease in Type IIA muscle fibers from the NWB and stretch induced group (7.5 ± 2.1%) to the NWB group (23.7 ± 2.1%). Repression of the fast-twitch muscle fibers was seen as well as the slow-twitch muscle fibers being activated during extensive stretching. When comparing type I to type IIA-IIX, the NWB and stretching group (93.7 ±2.0%) and control group (92.7 ±2.4%) were most similar in type I muscle fibers versus the NWB group (78.9 ± 2.2%). Stretching had shown to decrease the change of different fiber types, thereby hindering atrophy. Therefore, if there are no gains in strength, there could be maintenance of strength and muscle size through NWB activity when stretching is implemented. If immobilization in a stretched position for an extended period of time maintains muscle types
In turn maintaining strength in rats, stretching for multiple long periods of time could possibly have the same effect on humans.

Immobilization in a stretched position has had similar effects when stretching is implemented for several times throughout time. Coutinho et al.\textsuperscript{39} looked at the effects of stretching for 40 minutes every three days for a three-week period on muscle weight, length, serial sarcomere number, and fiber area. Eighteen Wistar rats were placed in three different groups: A) left soleus immobilized in shortened position, B) same as group A, with the addition of a 40-minute stretch, and C) the non-immobilized soleus was only stretched. Group C, the non-immobilized stretching group, had an increase in length (5±2%), serial sarcomere number (4±4%), and fiber area (16±44%) when comparing the left stretched soleus to the non-stretched soleus. The immobilized group A had a decrease in weight (44±6%), length (19±7%), serial sarcomere number (23±15%), and fiber area (37±31%). The immobilized stretching group B also had a reduction but had milder muscle fiber atrophy compared to group A with 22±40% fiber area reduction compared to 37±31%. The stretching group had a decrease in atrophy compared to the non-stretching immobilized group.

A separate study was done on four chicken wing muscles that experienced stretching for 5 weeks but were not immobilized.\textsuperscript{4} The two muscles mainly focused on were the anterior latissimus dorsi and the patagialis. Weight of the anterior latissimus dorsi increased 81% from the control (0.170 ± 0.014g) to the experimental group (0.309 ± 0.015g). This was the same for the patagialis with an increase of 63% from control (0.159 ± 0.006g) to experimental (0.260 ± 0.007g) groups. Length of the anterior latissimus dorsi increased 24% from the control (2.5 ± 0.0cm) to the experimental group (3.1 ± 0.1cm). This was the same for the patagialis with an increase of 22% from control (2.7 ± 0.1cm) to experimental (3.3 ± 0.1cm) groups. Length
changes were virtually fulfilled at one week. Cross-sectional muscle growth was fulfilled at five weeks. Muscle cross-sectional area of the anterior latissimus dorsi increased 54% from the control (4.77 ± 0.42mm$^2$) to the experimental group (7.37 ± 0.56mm$^2$). This was the same for the patagialis with an increase of 49% from control (7.22 ± 0.48mm$^2$) to experimental (10.77 ± 0.60mm$^2$) groups. Mitochondrial enzyme proportions were unchanged in the anterior latissimus dorsi but were altered in the stretched patagialis. Stretching of chicken wings resulted in muscle growth.

Besides possible structural changes to the muscle during stretch induced muscle hypertrophy, there are cellular effects of stretch induced muscle hypertrophy. As previously stated, neutrophils are one of the first cells to appear in the inflammatory process. These cells have been known to appear at the first sign of injury and weaken muscle degeneration. Additionally, neutrophils have also been known to aide in muscle regeneration. Neutrophils can be helpful during phagocytosis by eliminating debris and stimulating satellite cells to the area.$^4$ If these proteins can be seen after stretching, the inflammatory process is taking place and with inflammation comes hypertrophy. Pizza et al.$^{40}$ studied the effects of stretching and isometric contractions on neutrophil involvement. Seventy-one male mice were split into four groups: control, passive stretching, isometric contractions, and lengthening contractions. All four groups had the same surgical procedure and the three groups were compared to the control group to rule out the fact that increase in neutrophils could be due to the procedure and not the treatment. The control group had shown no increase in neutrophils. All three groups saw an increase in neutrophil concentrations and macrophage concentrations most significantly after three days of a stretching program. This means that neutrophils, a main component of inflammation, are seen in the area being stretched after long periods of stretching.
Other authors have looked at hormonal changes in the body during hypertrophy. More specifically, insulin-like growth hormone I (IGH-I) has been looked at after chronic bouts of stretching. Yang et al.\textsuperscript{41} looked at the effect of six days of immobilization in a stretched position. One leg of several rabbits immobilized the tibialis anterior and extensor digitorum longus in an elongated position while the soleus was immobilized in a shortened position. The opposite leg of each of the rabbits was used as the control group. Changes in muscle mass between groups were significant (P < 0.01). The extensor digitorum longus increased 19.3\% (2.5 ± 0.04g vs 3.0 ± 0.1g) as well as the tibialis anterior with a 33\% increase (2.4 ± 0.04g vs 3.2 ± 0.2 g).

Additionally, a decrease in muscle mass of 33\% (2.2 ± 0.1 g vs 1.4 ± 0.1 g) was observed after six days of no use of the soleus. The muscle that was not used but was casted had a major decrease in muscle mass, while muscles that were stretched had an increase in muscle mass. IGF-I measurements were gathered using situ hybridization sections. Specific data were not given for the IGF-1 mRNA level, but essentially the soleus was unchanged while the tibialis anterior increased five times greater than the unstretched leg (p < 0.001). After six days of immobilization in a stretched position, an increase in IGF-1 was seen, as well as an increase in muscle mass. After reviewing several other articles, Goldspink\textsuperscript{6} examined IGF-1 and concluded that IGF-1 is the main growth factor that is contributed to the repair and remodeling of tissue in several muscle types. Because this hormone is seen after long-term stretching, hypertrophy could be taking place.

Rather than focusing on one cause of stretch induced muscle hypertrophy, Sasai et al.\textsuperscript{42} combined several theories of stretch induced hypertrophy. Cell cultures of 13-day-old chicken embryos were used to analyze muscle growth. A stretch of 1/6 Hz was induced in the longitudinal axis for a period of 72 hours. An increase in diameter of 30 ± 1.91µm (micrometer)
after being stretched was significantly greater compared to the static culture with a diameter of
20 ± 0.66 µm (P < 0.01). The involvement of PI3K/Akt/TOR pathway was also analyzed in
stretch induced myotube hypertrophy. As IGF-1 stimulation decreased diameter, it was noted
that PI3K/Akt/TOR pathway could play an important role in stretch induced hypertrophy. This
pathway plays an important role due to its ability to accelerate protein synthesis as well as
prevent protein degradation. For this reason, IGF-1 is important in activating the PI3K/Akt/TOR
ultimately inducing hypertrophy in stretched muscles.

**Increase Muscle Length Theory.** The main goal of stretching has been believed to
decrease musculotendinous stiffness which in turn increases joint ROM. However, several
authors believe decreasing musculotendinous stiffness will increase strength as well. Some
authors have found a decrease in musculotendinous stiffness to be the main factor of stretch
induced muscle hypertrophy. Others believe an increase in strength is due to an increase in
ROM, as well as a decrease in musculotendinous stiffness. Guissard et al. looked at neural
and mechanical limits to ROM. Twelve subjects participated in 30 sessions of static stretching of
the plantar-flexor muscle. Passive stiffness was analyzed five separate times: before (1.13 ± 0.04
Nm/º), 10 sessions (0.96 ± 0.05 Nm/º), 20 sessions (0.89 ± 0.04 Nm/º), 30 sessions (0.76 ± 0.04
Nm/º), and retention (0.84 ± 0.04 Nm/º). A significant decrease was seen at 10 sessions and
within the last three groups (P < 0.001). Passive stiffness not only decreased after 10 days of
stretching but also continued to decrease during treatment and after treatment by 33%. Maximum
voluntary contraction was also noted to improve although this was not seen to be significant.
Data are as follows: before (93.7 ± 5.7 Nm), 10 sessions (94.5 ± 6.6 Nm/º), 20 sessions (96.4 ±
5.9 Nm/º), 30 sessions (98.8 ± 8.4 Nm/º), and retention (99.3 ± 4.8 Nm/º). Maximal voluntary
contraction of the plantar flexor muscles increased with time when a stretch was implemented.
The authors also concluded that an increase in ROM resulted in an increase in torque. As previously stated, increasing ROM could result in optimal muscle use. If the actin and myosin filaments can be fully used through the entire ROM, an increase in force produced will be seen. This increase in force production could in turn increase muscular strength. As of now, it is difficult to note if strength changes in humans are due to change in length or tension instead of a physiological change in muscle size.

**Summary**

Arguably, the easiest and safest method for stretching is static stretching. More specifically, constant–torque stretching is the most effective form of static stretching. Stretching should be held for 30 seconds to get the greatest gain in ROM. Acute effects of stretching, such as decrease muscle strength, have been found; however, the chronic effects of stretching have been shown to increase strength when added to a resistance training program. Chronic stretching alone has increased muscle strength in humans. Stretching during immobilization in animals has shown an increase in strength but has yet to be studied in humans. Based on current data, there could be several different factors that could explain the gains in strength through chronic stretching.
CHAPTER III. METHODS

Experimental Design

A 2 x 3 factorial pretest-posttest randomized control trial with a repeated measures design was used to examine the effects of two weeks of immobilization on the calf muscles strength, size, and dorsiflexion ROM after a chronic static stretching program was implemented. The independent variables are time (pre and post) and group (control, experimental, and experimental stretching). The control group (CG) completed the pre and post testing sessions. The experimental group (EG) completed a pre- and post-test and wore the immobilization boot called the Walker (Aircast FP Walker, DJO Inc., Vista, CA) for two weeks. The experimental stretching group (ESG) participated in the pre- and post-test, wore the Walker for two weeks, and participated in a stretching program twice daily. A total of 24 Walkers were donated by Don Joy. The dependent variables are calf strength, calf girth, and dorsiflexion ROM. Strength was measured by using the Cybex II Isokinetic Dynamometer (Cybex, Division of Lumex Inc., Ronkonkoma, NY) and muscle size was determined by using the Lufkin tape measure with a Gulick spring-loaded handle attachment (Lafayette Instruments, Laffayette, IN).

Subjects

Thirty-six female subjects enrolled at North Dakota State University participated in this study (C: n=12, EG: n=12, ESG: n=12). Subjects were excluded if they had a history of lower leg injuries or if they had lower extremity surgery in the past year. This study was approved by the North Dakota State University Institutional Review Board (Appendix A) and all subjects gave informed consent prior to participating in this study (Appendix B).
**Procedures**

Subjects were recruited through an email as seen in Appendix C. Subjects received compensation to participate in this study as shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Payments per Subject</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>Pre</td>
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<td>During</td>
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<td>Post</td>
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<td>Total</td>
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Total Amount $1,200.00

The study began by asking subjects to participate in a familiarization test. During this familiarization test, subjects practiced using the Cybex II Isokinetic Dynamometer. Additionally, each subject was informed regarding which group they were randomly selected to be in, C, EG, or ESG. For the pre-test, subjects warmed-up on the Monark 817 Stationary Exercise Bike for five minutes. Then, subjects performed measurement variables on both legs that include dorsiflexion ROM, calf girth, and calf strength. Dorsiflexion ROM was measured bilaterally while the subject was lying prone using a goniometer. Each subject started with their ankle in neutral then was asked to flex into complete dorsiflexion. Dorsiflexion ROM procedures followed that of the Starkey et al. book with the patient lying prone. However, the patient had the knee fully extended instead of flexed at 90°. Dorsiflexion ROM was measured three separate times and averaged. The procedure for girth measurements was the same as Ross et al. Girth was measured at the thickest part of each calf. This area was marked with a pen and measured from the distal aspect of the lateral malleolus to have the same distance for the post-test. Finally, the Cybex II Isokinetic Dynamometer test was done for both ankles. All of the data was recorded on a data sheet (Appendix D). Data recorded for the entire left leg can be seen in Appendix E.
Following the pre-test, subjects in EG and ESG were fitted for the Walker on the left leg.

The subjects were instructed to start wearing the Walker on day one of the experiment immediately upon waking the next morning. The subjects were allowed to remove the Walker when showering or when sleeping. They were given a log for self-recording of each time the subject’s took the Walker off (Appendix F). The EG was instructed to return on two separate occasions over the course of the experiment to be seen by one of the researchers. The schedule for EG and ESG is shown in Table 2.

**Table 2. Schedule**

<table>
<thead>
<tr>
<th>Familiarization Test/ Pre-Test</th>
<th>Familiarization with Cybex II Isokinetic Dynamometer Pre-test</th>
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</thead>
<tbody>
<tr>
<td>Fit left leg to Walker and receive Log</td>
<td>1. Record Demographics</td>
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<tr>
<td>2. Warm-up on Bike</td>
<td></td>
</tr>
<tr>
<td>3. GirthMeasurements</td>
<td></td>
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<tr>
<td>4. Dorsiflexion ROM with Goniometer</td>
<td></td>
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<tr>
<td>5. Cybex II</td>
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</tbody>
</table>

<table>
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<tr>
<th>Week One/Two: ESG</th>
<th>Day 1: Start wearing Walker in the morning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1-3, 7-10, 13-14:</td>
<td>1. Stretch with researcher</td>
</tr>
<tr>
<td>2. Stretch on your own</td>
<td></td>
</tr>
<tr>
<td>Day 4-6, 11-12:</td>
<td>1. Stretch on your own twice per day</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Week One/Two: EG</th>
<th>Day 1: Start wearing Walker in the morning</th>
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<tbody>
<tr>
<td>Day 3 and 10:</td>
<td>1. Meet with researcher</td>
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<table>
<thead>
<tr>
<th>Day 15 (All groups):</th>
<th>Post test</th>
</tr>
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<tbody>
<tr>
<td>1. Turn-in Walker and Log</td>
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<tr>
<td>2. Warm-up on Bike</td>
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<tr>
<td>3. Girth Measurements</td>
<td></td>
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<tr>
<td>4. Dorsiflexion ROM with Goniometer</td>
<td></td>
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<tr>
<td>5. Cybex II</td>
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<tr>
<td>Pay Subjects</td>
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</table>

When the EG and ESG returned to meet with the researcher, the researcher ensured that the Walker was being properly and safely worn at all times. The researcher also answered any questions and examined the physical appearance of the ankle to ensure safety throughout the
experiment. The ESG was also required to return during the weekdays to participate in a ten-minute stretching program of the immobilized leg. They were required to stretch for a total of 20 minutes a day (10 minutes with the researcher and 10 on their own).

The stretching program (see Table 3.) consisted of 30s periods of stretching on a pro-stretch (Prostretch original and wood, Medi-Dyne Health Care Productions, Colleyville, TX), which is a small manual therapy device used to assist with muscle stretching of the lower leg, with the knee bent at approximately 30º of knee flexion (followed by 30s of rest). The subjects stretched for 30s with a straight leg followed by another 30s of rest. This two-minute cycle of stretch (straight-leg)—relax—stretch (bent leg)—relax was repeated five times for a total time of 10 min. All stretching was done on the immobilized ankle.

<table>
<thead>
<tr>
<th>Table 3. Stretching Protocol</th>
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<tr>
<td>Stretch</td>
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<tr>
<td>Straight Leg</td>
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<td>Bent Knee</td>
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<tr>
<td>Straight Leg</td>
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<td>Bent Knee</td>
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</table>

The subjects were advised to stretch until discomfort was felt. At no time were the subjects asked to stretch to or beyond a level of pain. Throughout the remaining cycles of the stretching routine, subjects were encouraged to stretch their lower leg muscle group to a maximum tension without experiencing pain. No warm up was done prior to stretching.
A separate home-stretching program was given to the ESG to take home and complete every day. This stretching protocol was the same stretching routines that include wall stretching exercises (compared to using the pro-stretch) with a bent knee and straight leg for the same period as described previously. The ESG was told to stretch twice daily for two weeks, whether they are performing the stretching program with the researchers in the lab or performing the home-stretching program exercises.

Following two weeks of immobilization, each subject returned to the original familiarization location to participate in the post-tests. First, the subjects warmed up on the Monark 817 Stationary Exercise Bike for five minutes. Then, girth measurements were obtained and dorsiflexion ROM was measured bilaterally. Finally, the Cybex II Isokinetic Dynamometer test was administered. Identical testing procedures were used for both the pre- and post-tests. Following the post-test, each subject handed in their respective completed written logs and returned the Walker. Upon completion of the pre- and post-testing procedures, subjects were compensated for their services and were given a take home rehab program to regain muscle strength and obtain normal function (Appendix G). Subjects were debriefed and offered the opportunity to see the results of the study once the data was analyzed.

**Instrumentation**

The Cybex II Isokinetic Dynamometer was used to determine peak torque. To normalize this data, percent body weight (%BW) was used. The Cybex II Isokinetic Dynamometer was calibrated prior to testing. The ankle was tested as described in the Cybex II Isokinetic Dynamometer manual. Special attention was given to the proper placement of the joint to the axis. The speed was set at 30º/s for each subject. Verbal cues were given to motivate the subjects for maximum effort.
**Statistical Analysis**

An analysis of covariance (ANCOVA) was used to examine differences among groups with the covariant being pre-test scores. Post hoc analysis involved the use of least significant difference (LSD) post hoc t-test. The level of significance was set at $p<0.05$. The statistics are shown in Appendix H. The intraclass correlation coefficient for the pre-post left leg calf girth, calf strength, and dorsiflexion ROM were .992, .803, and .812 respectively. Statistical Analysis Software (SAS) was used for statistical analysis.
CHAPTER IV. MANUSCRIPT

Chronic stretching during two weeks of immobilization decreases loss of girth, strength, and Dorsiflexion ROM

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Formatted for submission to the Journal of Athletic Training®
Abstract

**Title:** Chronic stretching during two weeks of immobilization decreases loss of girth, strength, and Dorsiflexion ROM

**Context:** Chronic stretching is proposed to improve muscle performance. During immobilization, there is an extensive loss in muscle strength, muscle size, and joint ROM. Chronic static stretching may maintain muscle strength, muscle size, and dorsiflexion ROM during immobilization.

**Objective:** To investigate the effects of chronic static stretching on plantarflexor strength, calf girth, and dorsiflexion ROM after two weeks of immobilization.

**Design:** Randomized controlled clinical trial.

**Setting:** Athletic training room.

**Patients or Other Participants:** Thirty-six female college aged (19.81±2.48) students.

**Interventions:** Participants were randomly placed into one of three groups; control group (CG), experimental group (EG), and experimental stretching group (ESG). Each group participated in a familiarization period, a pre-test, and, two weeks later, a post-test. The EG and ESG wore the Walker for two weeks on the left leg. During this time, the ESG participated in a stretching program, which consisted of two 10-minute stretching procedures each day for the 14 days.

**Main Outcome Measures:** Three ANCOVA’s were used to determine differences in girth, strength, and dorsiflexion ROM between groups with an α level of < 0.05.

**Results:** A significant linear relationship between pre and post-test were found with girth (F_{1,31}=1158.3, p<0.0001), dorsiflexion ROM (F_{1,31}=89.02, p<0.0001), and strength (F_{1,31}=42.10, p<0.0001). Significant differences were found between groups for post-test measures of girth
(F₂,₃₁=6.50, p=0.0048), dorsiflexion ROM (F₂,₃₁=29.06, p<0.0001), and strength (F₂,₃₁=6.74, p=0.0041). Post hoc analysis showed that CG ultimately maintained girth, dorsiflexion ROM, and strength. Post hoc testing also showed that the EG lost more girth than the ESG, the EG decreased dorsiflexion ROM and the ESG increased in dorsiflexion ROM, and the EG decreased strength and the ESG increased in strength.

**Conclusion:** Chronic static stretching during two weeks of immobilization can decrease the loss of calf girth, calf strength, and dorsiflexion in the ankle.

**Keywords:** chronic stretching, immobilization

**Introduction**

Throughout the years, acute static stretching has been shown to decrease muscle performance.¹ However, chronic stretching has been shown to improve performances such as 1RM², flexibility, gait economy³, running speed⁴, peak torque⁵, and vertical jump⁶. Impressively, stretching has been shown to increase muscle strength and endurance over time when physical activity is limited.⁷ In an article by Kokkonen et al.⁷, 10 weeks of chronic stretching with limited activity resulted in an increase in 1RM knee flexion by 15.3%, 1RM extension by 32.4%, knee flexion endurance by 30.4%, and knee extension endurance by 28.5%. The researchers could only find two published studies that have not found significant increase in performance when stretching is implemented.⁸⁻⁹

Stretch induced hypertrophy has been found in several animal research studies with long periods of static stretching resulting in stretch induced hypertrophy in animals.¹⁰ Stretch induced hypertrophy is the promotion of tissue growth due to stretching. This promotion of tissue growth can be due to several aspects that result in hypertrophy including cellular, hormonal, or structural changes. Goldspink¹¹ found muscle protein synthesis was seen to have about 7% increase per
day when stretching was induced. Goldspink\textsuperscript{12} also found that immobilizing a rat’s hind legs in the muscles shortened position lead to a significant increase in atrophy, while immobilizing a rat’s hind leg in the muscles lengthened position stimulated protein synthesis and induced growth of the muscle. Furthermore, Leterme et al.\textsuperscript{13} immobilized rat hind legs during non-weight bearing activity and found additional sarcomeres added to both ends of the muscles. Pizza et al.\textsuperscript{14} found an increase in neutrophil and macrophage concentrations after three days of stretching rats. Holly et al.\textsuperscript{10} found muscle longitudinal and cross sectional area growth of chicken wings after stretching. Lastly, Coutinho et al.\textsuperscript{15} immobilized rat hind legs and then had some participate in 40 minutes of stretching daily mimicking something that could be done on humans. Slightly more muscle atrophy was found in the immobilized group compared to the immobilized stretching group. Much of the research performed on animals have had evidence of hypertrophic effects, however, we do not know the effect chronic static stretching has on immobilized human muscle. The lack of human research on chronic stretching during immobilization makes it difficult to be confident in the usefulness of stretching to increase or maintain muscle performance during immobilization.

Therefore, the purpose of our study was to investigate calf girth, dorsiflexion ROM, and calf strength when chronic static stretching was implemented during two weeks of immobilization. We hypothesize that the loss of strength, girth, and motion would be substantial in the EG but will be minor in the ESG.

\textit{Methods}

\textbf{Participants.} Thirty-six subjects participated in this study. Four subjects were removed from this study because they were suspected of not completing the protocol. The control group (CG) consisted of 12 women (age= 21.58 ± 3.29 years, height= 162.98 ± 4.94 cm, and mass=
64.11 ± 8.67 kg), the experimental group (EG) consisted of 10 women (age= 18.70 ± 0.82 years, height= 165.10 ± 5.49 cm, and mass= 62.73 ± 5.57 kg), and the experimental stretching group (ESG) consisted of 10 women (age= 18.80 ± 0.79 years, height= 164.59 ± 6.75 cm, and mass= 67.59 ± 18.81 kg). Subjects were excluded if they had a history of lower leg injuries or if they had lower extremity surgery in the past year. This study was approved by the Institutional Review Board and all subjects gave informed consent prior to participating in this study.

**Procedures.** One researcher completed all of the testing. For the first day, all subjects completed a familiarization test with the Cybex II Isokinetic Dynamometer (Cybex, Division of Lumex Inc., Ronkonkoma, NY). Then, for the pre-test, subjects warmed-up on a Monark 817 Stationary Exercise Bike for five minutes prior to collecting measurement variables that include dorsiflexion ROM, calf girth, and calf strength. Dorsiflexion ROM was measured with a goniometer with the patients lying prone as described in Starkey et al. This measure was taken three times with the average being used for the data analysis. The procedure for girth measurements followed the same procedure described in Ross et al. Girth was measured by using the Lufkin tape measure with a Gulick spring-loaded handle attachment (Lafayette Instruments, Laffayette, IN). Finally, the Cybex II Isokinetic Dynamometer was used to measure peak torque at a rate of 30°/s. Procedures for the Cybex II Isokinetic Dynamometer testing followed the Cybex II Instruction Manual.

Following the pre-test, subjects in EG and ESG were fitted for the Walker (Aircast FP Walker, DJO Inc, Vista, CA) on the left leg. The subjects were instructed to start wearing the Walker on day one of the experiment immediately upon waking the next morning. The subjects were allowed to remove the Walker when showering or when sleeping and were required to fill out a log for the amount of time the Walker was off and the reason why the Walker was off. The
EG was instructed to return on two separate occasions over the course of the experiment to be seen by a researcher to ensure the Walker was being worn and to examine any complaints. The ESG, however, saw the researcher on nine separate days to ensure the stretching program was completed and performed correctly. When the subjects were stretching with the researcher, the pro-stretch (Prostretch original and wood, Medi-Dyne Health Care Productions, Colleyville, TX) was used, however, when the subjects were stretching at home they used the wall stretch. The stretching program is shown in Table 1.

Following two weeks of immobilization, the post-tests were completed. Identical testing procedures were used for both the pre-tests and post-tests. Following the post-test, each subject handed in their respective completed written logs. All subjects recorded in their logs that they stretched every day twice a day. The subjects in the EG averaged 34.22 ± 12.50 min a day with the Walker off while the ESG had an average of 25.91 ± 16.49 min off. Means of girth, dorsiflexion, and strength are reported in Table 2.

**Statistical Analysis.** An analysis of covariance (ANCOVA) was used to examine differences among groups. The covariant was the pre-test scores. Post hoc analysis involved the use of least significant difference (LSD) post hoc t-test. The level of significance was set at $p<0.05$. The intraclass correlation coefficient for the pre-post left leg calf girth, calf strength, and dorsiflexion ROM were .992, .803, and .812 respectively. Statistical Analysis Software (SAS) was used for statistical analysis.

**Results.**

**Girth.** There was a significant linear relationship between pre-test and post-test girth scores ($F_{1,31} =1158.3$, $p<0.0001$), indicating there is a linear relationship between the two testing times. Furthermore, there was a significant difference of the post girth measure between the three
groups (F 2,31=6.50, p=0.0048). Post hoc analysis using LSD (least significant
difference=0.4132) and showed that the significance could be attributed to the CG maintaining
girth and the EG losing more girth than the ESG (Figure 1.).

**Range of Motion.** There was a significant linear relationship between pre-test and post-
test range of motion scores (F 1,31 =89.02, p<0.0001), indicating there is a linear relationship
between the two testing times. Furthermore, there was a significant difference of the post
dorsiflexion ROM measure between the three groups (F 2,31=29.06, p<0.0001). Post hoc analysis
used LSD (least significant difference=2.0316) and showed that the significance could be
attributed to the CG maintaining dorsiflexion ROM, the EG decreasing in dorsiflexion ROM and
the ESG increasing in dorsiflexion ROM (Figure 2.).

**Strength.** There was a significant linear relationship between the pre-test and post-test
strength scores (F 1,31 =42.10, p<0.0001), indicating there is a linear relationship between the two
testing times. Furthermore, there was a significant difference of the post strength measure
between the three groups (F 2,31=6.74, p=0.0041). Post hoc analysis using LSD (least significant
difference=4.3824) showed that the significance could be attributed to the CG maintaining
strength, the EG decreasing in strength and the ESG increasing in strength (Figure 3.).

**Discussion**

The most significant observation of our study was the response of the ESG on girth,
strength, and dorsiflexion ROM. As hypothesized, the ESG had a slightly smaller loss in calf
girth compared to the EG. Also, interestingly, the ESG increased in plantarflexor strength and
dorsiflexion ROM after the two weeks of immobilization when chronic static stretching was
implemented.
Muscle atrophy of 14.1% has been observed in the plantarflexor muscles of injured subjects in the first two weeks of immobilization. In our study, the EG lost approximately 0.58 cm girth after two weeks of immobilization compared to the ESG who lost approximately 0.34 cm. A study with a similar design as our study was done on rats with immobilized hind legs for three weeks and a 40 minute stretching program every three days showed a reduction in muscle atrophy compared to the group of rats that did not stretch during the three weeks of immobilization (22 ± 40 vs 37 ± 31%). Leterme et al. immobilized rat hind legs in the stretched position for 14 days and also saw that stretching prevented the loss of muscle mass. The group in the stretched position saw a 2% decrease in muscle wet weight (109.7 ± 7.5 mg), a 5% increase in muscle wet weight/body weight (0.44 ± 0.04 mg/g), and a 34% decrease in cross-sectional area (7.7 ± 0.9 mm²) when compared to control (111.5 ± 2.1mg, 0.42 ± 0.01mg/g, and 11.6 ± 0.4mm²). The rats who had their muscles immobilized in a shortened position had a 27% decrease in muscle wet weight (81.8 ± 4.7 mg), a 26% decrease in muscle wet weight/body weight (0.31 ± 0.02 mg/g), and a 56% decrease in muscle cross-sectional area (5.1 ± 0.3mm²) when compared to the control group. Although these are animal studies, our data is similar with current research in finding that stretching during immobilization can decrease the loss of girth.

Muscle ROM is usually increased with stretching. Our results show that dorsiflexion ROM increased in the ESG about 25% (14.28 ± 5.25 to 17.88 ± 5.48°) while the EG decreased about 28% (15.22 ± 3.74 to 10.88 ± 1.90°). Kokkonen et al. limited subject’s activity for 10 weeks while adding a 40-minute stretching program, 3 times per week and saw an increase in sit and reach of 18.1% (36.2 ± 5.5 to 42.6 ± 5.6 cm). Guissard et al. noted that 6 weeks of static stretching of the plantarflexor muscles for ten minutes five days a week increased ankle dorsiflexion 30.8% (24.6 ± 0.8 vs 32.2 ± 0.8°). Several animal studies also found an increase in
muscle length with different protocols. Coutinho et al.\textsuperscript{15} found an increase in muscle length in rat hind legs that were submitted to stretching every 3 days for 40 minutes for 3 weeks by 5 ± 2% (15 ± 0.7 vs 14 ± 0.9 mm). Holly et al.\textsuperscript{10} found that after stretching chicken wings for five weeks, longitudinal length was completed after one week.

In our study, strength increased in the ESG by about 11% (32.70 ± 7.20 vs 36.30 ± 8.50 %BW) while the EG decreased in strength by about 16% (37.10 ± 11.00 vs 31.20 ± 6.66 %BW). Kokkonen et al.\textsuperscript{7} found similar results when stretching inactive (<60min·d\textsuperscript{-1}) subjects for 10 weeks, 40 minutes per day, 3-d·wk\textsuperscript{-1}. Within the stretching group an increase in 1RM for knee flexion by 15.3% (51.0 ± 14.1kg vs 44.7 ± 14.5kg) and extension by 32.4% (82.0 ± 25.8 kg vs 63.8 ± 25.8 kg) was observed. Large increases in endurance were also noted in this study. Within the stretching group, an increase in knee flexion endurance by 30.4% (22.3 ± 4.7kg/17.2 ± 3.4kg) and extension by 28.5 % (23.7 ± 4.7 kg/ 18.5 ± 3.1 kg) were observed. Although activity was limited, the participants were still allowed to be active if it was less than 60 minutes a day for three days a week. Kokkonen et al.\textsuperscript{7} also found increases in sprint and jump performance, however, Bazett-Jones et al.\textsuperscript{8} did not find significant improvements in sprint and jump performances after 6 weeks of stretching.

Although this study does not examine why these results take place, it is important to know that the stretch-based muscle improvements may be due to changes such as the increase in protein synthesis, growth factor production, or neutrophil elevation. Goldspink\textsuperscript{11} found protein synthesis to be one of the main contributors to stretch induced hypertrophy. Rat muscle protein synthesis was seen to have about a 7% increase per day with stretching. This study concluded that the rapid growth in the rat soleus muscle was due to increased rates of protein synthesis, which induced the growth of muscle. Goldspink\textsuperscript{12} examined IGF-1 and concluded that IGF-1
was the main growth factor that was contributed to the repair and remodeling of tissue in several muscle types and was seen when chronic stretching was implemented. Lastly, Pizza et al. studied the effects of stretching and isometric contractions on neutrophil involvement. Rats induced to stretch saw an increase in neutrophil concentrations and macrophage concentrations most significantly after three days of a stretching program. There are several theories that range from neurological changes to the change in muscle length that have been observed to be the cause of stretch-induced hypertrophy but further research is needed for a better understanding of stretch-induced hypertrophy.

Chronic stretching should be considered in an athlete’s everyday training. However, one clinical implication of this study is that injured athletes who are currently limited in activity could stretch to see maintenance of performance while inactive. Although this study immobilized patients instead of limited the participant’s activity, others have found that stretching during limited activity will enhance strength. This study found that an increase in strength can happen if stretching is implemented to a muscle that is unused. These findings can also promote the implementation of stretching into the clinical setting to increase ROM. Although stretching has been used to increase ROM in active athletes for several years, this study shows that when immobilized, an increase in ROM can still be seen when stretching is implemented. Finally, atrophy happens immediately when immobilization takes place. Decreasing the amount of atrophy could improve the return to play progression. Our research found that less girth was lost during two weeks of immobilization when stretching was implemented. If stretching is an option for rehabilitation, according to this research, it should be used to possibly decrease the amount of girth loss.
One important limitation to this study was that there were no blinding actions taken for the subjects or researchers. To dismiss this limitation in the future, the researchers should not inform the subjects what the main outcome would be and blind the researcher who is doing the pre and post-testing. The second important limitation was that the subjects in this study were attempting to mimic the initial stages of an injury, but the participants were not actually injured. Also, during an immobilized injury it is unlikely an athlete would able to stretch as directed in this study, however, we wanted to isolate the calf to see how it would act independently when stretching was added during immobilization.

Future research should be done on stretching increasing strength in the contralateral muscle. Nelson et al.\textsuperscript{21} looked at this in humans and Coutinho\textsuperscript{15} found this in rats. More specifically, Nelson et al. stretched one calf 3 d·wk\textsuperscript{-1}. Nelson et al. examined uninjured patients and found in increase in 1RM of the stretched calf of 29\% and an increase in the non-stretched calf of 11\%. Many times, an athlete cannot move an injured body segment, so it would be beneficial to do research on the effects chronic static stretching the uninjured limb may have on the injured limb. It would also be important to look at the elderly who are inactive to see if stretching could help maintain strength. Also, more research should be done on different body segments to see if they act differently than the ankle. More research should also be done on mimicking the initial stages of rehab using injured patients instead of healthy ones. It is unknown if stretching during the initial stages of rehab in an injured athlete can reap benefits, however, this study suggests that stretching for 20 minutes a day for two weeks during immobilization of the ankle can decrease the loss of girth, strength, and ROM in healthy college aged students.
Conclusion

In conclusion, stretching during immobilization showed a decrease in the loss of strength, girth, and ROM. The physiological reasons as to why this happens still remains unclear so future research is needed to develop the reasons as to why the changes happen. This study provides a better base of research with stretching during immobilization and the effects on strength, girth, and ROM.

Acknowledgments

We thank NDSU’s Athletic Training Education Program, Department of Health, Nutrition, and Exercise Sciences, and College of Human Development. We also thank DonJoy for donating the FP Walkers.

References


### Tables

**Table 4. Stretching Protocol**

<table>
<thead>
<tr>
<th>Stretch</th>
<th>Stretch Time</th>
<th>Rest Time</th>
<th>Cumulative</th>
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<td>6:00</td>
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<tr>
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</table>

**Table 5. Girth, Dorsiflexion, and Strength (Mean ± SD)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Girth (cm)</th>
<th>ROM (º)</th>
<th>Strength (%BW)</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
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<tr>
<td>CG</td>
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<td>35.45 ± 2.72</td>
<td>15.00 ± 5.14</td>
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<tr>
<td>EG</td>
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<td>33.94 ± 1.63</td>
<td>15.22 ± 3.74</td>
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<tr>
<td>ESG</td>
<td>35.35 ± 4.30</td>
<td>35.01 ± 4.09</td>
<td>14.28 ± 5.25</td>
</tr>
</tbody>
</table>

CG= control group, EG= experimental group, ESG= experimental stretching group.
**Figures**

**Figure 1. Girth.** Mean ± SD calf girth measurements in cm at pre-test and two weeks after at the post-test. $^a=$CG\textsubscript{postgir} $>$ ESG\textsubscript{postgir} $>$ EG\textsubscript{postgir}. Significance accepted with $p < 0.05$.

**Figure 2. Dorsiflexion.** Mean ± SD dorsiflexion measurements at pre-test and post-test. $^a=$ESG\textsubscript{postrom} $>$ CG\textsubscript{postrom} $>$ EG\textsubscript{postrom}. Significance accepted with $p<0.05$
Figure 3. Plantarflexion. Mean ± SD Cybex II Isokinetic Dynamometer measurements at pre-test and post-test. a=ESG_{post} > EG_{post}. Significance accepted with p<0.05
CHAPTER V. DISCUSSION/CLINICAL APPLICATION

The research questions for this study were 1) does static stretching of the plantarflexor muscles during two weeks of immobilization result in a decrease in atrophy? 2) does static stretching of the plantarflexor muscles during two weeks of immobilization result in a reduction in loss of muscular strength? and 3) does static stretching of the plantarflexor muscles during two weeks of immobilization result in a reduction in loss of dorsiflexion ROM? The hypothesis was that static stretching during two weeks of immobilization of the plantarflexors would result in a decrease in atrophy, a reduction in loss of muscle strength, and a decrease in the loss of dorsiflexion ROM. After the statistical analysis, it was observed that there was a decrease in atrophy and there was a reduction in the loss of muscle strength. In fact, muscle strength increased after two weeks of stretching. One of the most significant observations in the study was the effect that stretching had on girth, strength, and dorsiflexion ROM. The ESG had a smaller decrease in girth measurements when compared to the EG. Additionally, the ESG increased in plantarflexor strength and dorsiflexion ROM after the two weeks of immobilization when chronic static stretching was implemented compared to the EG.

Stevens et al.\textsuperscript{7} found that muscle atrophy in the plantarflexor muscles of an injured subject was 14.1\% in the first two weeks of immobilization. This was over 50\% of the entire muscle atrophy that took place over a 7-week period (14.1\% \textit{vs} 24.4\%). In our study, the EG lost approximately 0.58 cm (34.52 ± 1.81 \textit{vs} 33.94 ± 1.63) girth after two weeks of immobilization compared to the ESG who lost approximately 0.34 cm (35.35 ± 4.30 \textit{vs} 35.01 ± 4.09). Our study closely mimicked the design of another study by Coutinho et al.,\textsuperscript{39} however, this study was an animal study. Coutinho et al.\textsuperscript{39} examined rats with immobilized hind legs for three weeks and a 40 minute stretching program every three days. The results showed a reduction in muscle
atrophy compared to the group of rats that did not stretch during the three weeks of immobilization (22 ± 40 vs 37 ± 31%).

In another study performed on animals, rat hind legs were held in the stretched position for 14 days and also saw that stretching prevented the loss of muscle mass. Leterme et al. found that the rats who had their muscles immobilized in a shortened position had a 27% decrease in muscle wet weight (81.8 ± 4.7 mg), a 26% decrease in muscle wet weight/body weight (0.31 ± 0.02 mg/g), and a 56% decrease in muscle cross-sectional area (5.1 ± 0.3 mm²) when compared to the control group (111.5 ± 2.1mg, 0.42 ± 0.01mg/g, and 11.6 ± 0.4mm²). However, the group that stretched during immobilization saw a 2% decrease in muscle wet weight (109.7 ± 7.5 mg), a 5% increase in muscle wet weight/body weight (0.44 ± 0.04 mg/g), and a 34% decrease in cross-sectional area (7.7 ± 0.9 mm²) when compared to the control group. Our data closely compares to these findings by other researchers. However, these studies are all animal studies which warrants further research supporting this study.

Range of motion is usually increased with stretching. Our results show that dorsiflexion ROM essentially stayed the same in the CG (15.00 ± 5.14 vs 14.10 ± 5.28), increased in the ESG about 25% (14.28 ± 5.25 vs 17.88 ± 5.48°), and decreased in the EG about 28% (15.22 ± 3.74 vs 10.88 ± 1.90°). Kokkonen et al. did not immobilize subjects but instead limited subject’s activity for 10 weeks while adding a 40-minute stretching program, 3 times per week. Stretching was observed to result in an increase in sit and reach scores of 18.1% (36.2 ± 5.5 vs 42.6 ± 5.6 cm). Additionally, Guissard et al. noted that 6 weeks of static stretching of the plantarflexor muscles for ten minutes five days a week increased ankle dorsiflexion 30.8% (24.6 ± 0.8 vs 32.2 ± 0.8°).
Several animal studies with different methods found an increase in muscle length after stretching was implemented. As previously stated, Coutinho et al.\textsuperscript{15} submitted rat hind legs to 40 minutes of stretching every three days for three weeks. This protocol was observed to increase muscle length by $5 \pm 2\%$ ($15 \pm 0.7$ vs $14 \pm 0.9$ mm). Holly et al.\textsuperscript{10} found an increase in longitudinal length after stretching chicken wings for five weeks. It was additionally noted that longitudinal length was complete after one week. These animal studies show that stretching for long periods during immobilization as well as immobilizing the muscle in a stretched position can increase muscular ROM. The human subject studies have shown that stretching can increase ROM but our study is the only one that has shown stretching during immobilization increased dorsiflexion ROM.

Kokkonen et al.\textsuperscript{7} stretched inactive (<60min·d$^{-1}$) subjects for 10 weeks, 40 minutes per day, 3-d·wk$^{-1}$ and examined the effects the stretching had on strength. Within the stretching group an increase in 1RM for knee flexion by 15.3% ($51.0 \pm 14.1$kg vs $44.7 \pm 14.5$kg) and extension by 32.4% ($82.0 \pm 25.8$ kg vs $63.8 \pm 25.8$ kg) was observed. Large increases in endurance were also noted in this study. Within the stretching group, an increase in knee flexion endurance by 30.4% ($22.3 \pm 4.7$kg/$17.2 \pm 3.4$kg) and extension by 28.5% ($23.7 \pm 4.7$ kg/$18.5 \pm 3.1$ kg) were observed. Kokkonen et al.\textsuperscript{7} also found increases in sprint and jump performance. However, Bazett-Jones et al.\textsuperscript{8} did not find significant improvements in sprint and jump performances after 6 weeks of stretching. Bazett-Jones et al.\textsuperscript{8} discussed the reason they did not experience an increase in performance may be due to the lack of ROM improvements shown in their study. Although activity was limited in the Kokkonen et al.\textsuperscript{7} study, the participants were still allowed to be active for less than 60 minutes a day for three days a week. In this study, however, activity was prohibited because the subjects were immobilized by the Walker. This
study found that after two weeks of immobilization, strength increased in the ESG by about 11% (32.70 ± 7.20 vs 36.30 ± 8.50 %BW) while the EG decreased in strength by about 16% (37.10 ± 11.00 vs 31.20 ± 6.66 %BW). The researchers could not find published research on human strength gains due to stretching during immobilization. Further research is needed to support the findings of this study.

It is important to examine why stretch-induced hypertrophy happens, even though this study did not examine the root cause. Many believe that stretch-induced hypertrophy is either a physiological or physical change. Protein synthesis is one of the processes that has been studied greatly and believed to be the main contributor to stretch-induced muscle hypertrophy. Rat muscle protein synthesis was found to have about a 7% increase per day when rat skeletal muscle was immobilized in a lengthened state. This study concluded that the rapid growth in the rat soleus muscle was due to increased rates of protein synthesis, which induced the growth of muscle.

Goldspink also examined IGF-1 and concluded that IGF-1 is the main growth factor that contributed to the repair and remodeling of tissue in several muscle types. Goldspink found this factor when chronic stretching was implemented. Yang et al. also looked at insulin-like growth hormone and found after six days, some increases in insulin-like growth hormone in the stretched muscle. Additionally, Holly et al. examined mitochondrial enzymes and noted proportions improved in chicken wings after stretching. Lastly, Pizza et al. studied the effects of stretching and isometric contractions on neutrophil involvement. Rats induced to stretch saw an increase in neutrophil concentrations and macrophage concentrations most significantly after three days of a stretching program. There are several theories that range from neurological changes to the change in muscle length that have been observed to be the cause of stretch-
induced hypertrophy. However, further research is needed for a better understanding of stretch-induced hypertrophy.

Chronic stretching should be implemented in an athlete’s everyday training when physically able. However, one clinical implication of this study is that injured athletes who are currently limited in activity could stretch to see maintenance of performance while inactive. Although this study immobilized patients instead of limiting the participants’ activity, atrophy occurs during inactivity and immobilization. This study found that an increase in strength can occur when stretching is implemented to a muscle that has limited activity. These findings can also promote the implementation of stretching into the clinical setting to increase ROM. Although stretching has been used to increase ROM in active athletes for many years, this study shows that when immobilized, an increase in dorsiflexion ROM can still happen when stretching is implemented. Finally, atrophy happens immediately when immobilization takes place. Decreasing the amount of atrophy could improve the return to play progression. This research found that less girth was lost during two weeks of immobilization when stretching was implemented. If stretching is an option for rehabilitation, according to this research, it could possibly be used to decrease the amount of girth loss. Further research is needed to confirm this assumption.

One important limitation to this study was that there was no blinding. To dismiss this limitation in the future, a double-blinded study should be implemented. The researchers should not inform the subjects what the main outcome would be, and the researcher who is doing the pre- and post-testing should be blinded as to who is in which group. This would especially be beneficial for the strength testing to ensure encouragement is given to all participants equally. The second important limitation is that this study was trying to mimic the initial stages of an
injury, but the participants were not injured. During an immobilized injury it is unlikely the
athlete is able to stretch as directed in this study, however, the calf was isolated to see how it
would act independently when stretching was added during immobilization. Some delimitations
in this study were the inclusion/exclusion criteria. Only healthy, college-aged, female students
with no history of chronic calf injury could participate. Also, another delimitation was the
selection of the stretching program. Finally, ANCOVA was chosen because some of the pre-test
measures varied between groups, even though subjects were randomly selected into groups.
Therefore, pre-test scores were used as the covariate.

Some assumptions made in this study were that the Cybex II Isokinetic Dynamometer is a
valid measurement of muscular strength and that the primary researcher was competent at taking
these measures. It was also assumed that each subject stretched the same based on the verbal
cues of the primary researcher. Additionally, whether the subjects actually stretched was based
on the findings in the logs and the interactions with the subjects, which was an
objective/subjective observation from the primary researcher.

Future research should be done on stretching increasing strength in the contralateral
muscle. Nelson et al.\textsuperscript{47} examined the effects stretching had on the contralateral muscle.
Participants stretched one calf 3 d·wk\textsuperscript{-1} and found in increase in 1RM of the stretched calf of
29\% and an increase in the non-stretched calf of 11\%. Often, moving the injured body segment is
unwarranted, so it would be beneficial to do research on the effects chronic static stretching the
uninjured limb may have on the injured limb. It would also be important to look at the elderly
who are inactive to see if stretching could help maintain strength. Also, more research should be
done on different body segments to see if they act differently than the calf muscle. More research
should also be done on mimicking the initial stages of rehab using injured patients instead of
healthy ones. It is unknown if stretching during the initial stages of rehab in an injured athlete can reap benefit, however, this study suggests that stretching for 20 minutes a day for two weeks during immobilization of the ankle can decrease the loss of girth, strength, and dorsiflexion ROM in healthy college aged students.

In conclusion, stretching during immobilization showed a decrease in the loss of strength, girth, and dorsiflexion ROM. The physiological reasons as to why this happens still remain unclear, so future research is needed to develop a reason as to why it happens. This study provides a better base of research with stretching during immobilization and the effects on strength, girth, and dorsiflexion ROM.
REFERENCES


APPENDIX A. IRB APPROVAL LETTER

NORTH DAKOTA STATE UNIVERSITY

Institutional Review Board
Office of the Vice President for Research, Creative Activities and Technology Transfer
NDSU Dept. 4000
1735 NDSU Research Park Drive
Research 1, R.O. Box 6050
Fargo, ND 58108-6050

September 24, 2012

Bryan Christensen
Department of Health, Nutrition & Exercise Sciences
1BBFH

IRB Approval of Protocol #HE13017, “Effects on plantaflexor strength and calf girth when static stretching is implemented during two weeks of immobilization”
Co-Investigator(s) and research team: Samantha Narveson, Kara Gange


Research site(s): NDSU Funding agency: n/a
Review Type: Full Board, meeting date – 8/10/2012
Risk Level: No more than minimal risk
IRB approval is based on original submission, with revised: protocol (received 9/19/2012) and consent (received 9/24/2012).

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

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

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

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

Sincerely,

Kristy Shirley, CIP
Research Compliance Administrator

Last printed 9/24/2012 11:49:00 AM
APPENDIX B. INFORMED CONSENT

NDSU  North Dakota State University
Department of Health, Nutrition, and Exercise Sciences
PO Box 6050
Fargo, ND 58108-6050
507-951-3128

Title of Research Study: Effects on plantarflexor strength and calf girth when static stretching is implemented during two weeks of immobilization.

This study is being conducted by: Bryan Christensen, PhD, CSCS
Samantha J. Narveson, ATC, LAT, CPT

Why am I being asked to take part in this research study? You are being asked to participate in this study because you: 1) are a healthy, college aged person, 2) have no history of calf injuries, and 3) have no history of lower extremity surgery in the past year.

What is the reason for doing the study? The purpose of this study is to 1) determine if static stretching of the calf muscles during two weeks of immobilization decreases atrophy and 2) decreases the loss of muscular strength.

What will I be asked to do? You will be placed into one of three groups (control, experimental group, and experimental stretching group). The control group will participate in the pre and post tests, the experimental group will participate in the pre and post tests and wear the Walker (ankle immobilizer) for two weeks, and the experimental stretching group will stretch during the two weeks they are immobilized.

All Groups:
You will participate in a familiarization week where you will come in on two separate days to perform a pre-test. On day one, you will get up a time to meet in the BSA Athletic Training Room and will participate in a familiarization session of the Cybex II (muscle testing equipment). After day one, you must wait 48 hours to complete the second day of testing. Day two will consist of test measurements for calf girth (circumference), strength, and flexibility. The post-test will occur after two weeks. If you are in the experimental or experimental stretching group, you will do the posttest within 48 hours of the last day of immobilization.

Experimental Group and Experimental Stretching Groups:
During the two weeks between the pre and post tests, you will wear a Walker on your non-dominant leg. You will be fitted into the Walker during the familiarization week and will be given a log to document the amount of time the Walker is not being worn. You may take the Walker off when sleeping, showering, and stretching. If you are in the experimental group, you will come into the BBF on three separate occasions (day 2, 5, and 10) for a check up on the log and to answer any questions. If you are in the experimental stretching group, you will come in on weekdays to the BBF for a ten minute stretching period and will also be instructed to stretch on your own each day. You will be asked to stretch twice daily: once with the researcher and once on your own.

Where is the study going to take place, and how long will it take? The study will take place in the Bison Sports Arena (Athletic Training Room) for the pre-test and post-test and at the Benson Bunker Fieldhouse (Athletic Training Room) for the stretching protocol with the researcher. The pre-test and

Institutional Review Board
North Dakota State University

PROTOCOL #: 1101-248
APPROVED: 9/12/12
EXPIRES: 9/12/13

Revised September 2012
post-test are anticipated to take half an hour to complete while the stretching program will take a total of ten minutes two times per day for 14 days. The total time that will be required to complete the study is as follows: Control Group: 1 hour; Experimental Group: 1.5 hours of testing, plus 2 weeks of immobilization; Stretching Group: 2.7 hours of testing/stretching, plus 2 weeks of immobilization.

**What are the risks and discomforts?** A common consequence of immobilization is a decrease in muscle strength and muscle size. Muscle strength and size can be regained following the completion of the study. This risk may have a negative effect on ankle function but will be monitored closely. Secondary risks include muscle soreness and weakness on your leg without the brace and irregular gait. It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known risks to the participant. If new findings develop during the course of this research which may change your willingness to participate, we will tell you about these findings.

**What are the benefits to me?** You are not expected to get any benefit from being in this research study. However, if you are a student, you may gain some benefit by seeing how experimental research is performed.

**What are the benefits to other people?** This research can potentially help many gain knowledge on the benefits of stretching during immobilization.

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

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

**Who will see the information that I give?** Your name will be documented in a password protected computer that only the researchers will be able to access. When you are selected for a group, you will be given an identification number that you will be referred to as for the length of the study. We will keep private all research records that identify you. Your information will be combined with information from other people taking part in the study. When we write about the study, we will write about the combined information that we have gathered. We may publish the results of the study; however, we will keep your name and other identifying information private. We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock.

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

**Will I receive any compensation for taking part in this study?** Each subject will receive 10 to 45 dollars to participate in this study based on which group you are randomly placed in.

- Control Group: $10
- Experimental Group: $45
- Experimental Stretching Group: $45

Revised September 2012
If you choose to discontinue the study you will be paid for the time you have put in. ($5 pre-test, $5 post-test, and $2.50 daily for the experimental and experimental stretching group)

What happens if I am injured because of this research? If you receive an injury in the course of taking part in the research, you should contact Dr. Margret Fitzgerald, head of the department of Health, Nutrition, and Exercise Sciences, at the following phone number (701) 231-5590. Treatment for the injury will be available including first aid, emergency treatment and follow-up care as needed. Payment for this treatment must be provided by you and your third party payer (such as health insurance or Medicare). This does not mean that you are releasing or waiving any legal right you might have against the researcher or NDSU as a result of your participation in this research.

What if I have questions?
Before you decide whether to accept this invitation to take part in the research study, please ask any questions that might come to mind now. Later, if you have any questions about the study, you can contact the researcher, Samantha Narveson at 507-951-3128.

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

- Telephone: 701.231.8908
- Email: ndsu.irm@ndsu.edu
- Mail: NDSU HRPP Office, NDSU Dept. 4000, PO Box 6050, Fargo, ND 58108-6050.

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

Documentation of Informed Consent:
You are freely making a decision whether to be in this research study. Signing this form means that
1. you have read and understood this consent form
2. you have had your questions answered, and
3. you have decided to be in the study.

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

_________________________________________________________  __________________________
Your signature                                            Date

_________________________________________________________
Your printed name

_________________________________________________________  __________________________
Signature of researcher explaining study                   Date

Institutional Review Board
North Dakota State University

PROTOCOL #: HB617
APPROVED: 12/10/12
EXPIRES: 05/13/13

Revised September 2012
Printed name of researcher explaining study
APPENDIX C. PARTICIPANT RECRUITMENT EMAIL

Email Heading: EARN MONEY BY PARTICIPATING IN A RESEARCH STUDY

NOTICE TO PARTICIPATE IN A STUDY:

Dr. Bryan Christensen PhD and graduate student Samantha Narveson ATC are conducting a study on the effects of stretching during two weeks of immobilization. Subjects will receive $10 to $45 compensation based on what group they are randomly placed in.

Control Group
· Complete pretest (30 min)
· Come back two weeks later to complete post test (30 min)
· Compensation is $10.00

Experimental Group
· Complete pretest (30 min)
· Wear ankle brace for two weeks
· Come in to researchers on 3 occasions for examination
· Complete posttest (30 min)
· Compensation is $45.00

Stretching Group
· Complete Pretest (30 min)
· Wear ankle brace for two weeks
· Stretch for 20 min a day (weekdays stretch with researcher for 10 min)
· Complete posttest (30 min)
· Compensation is $45.00

*The pre and posttests consist of tests for calf strength, flexibility, and size.

The purpose of this study is to determine if stretching during immobilization can decrease in the loss of muscle size and strength.

If you are interested in participating in this study or have any questions, please contact Samantha Narveson at 507-951-3128 or samantha.narveson@ndsu.edu or Dr. Bryan Christensen at bryan.christensen.1@ndsu.edu. Thank you for your time!

Samantha Narveson, ATC, LAT, CPT
Graduate Assistant Athletic Trainer
North Dakota State University Volleyball
Phone: 701-793-4746
Email: Samantha.Narveson@ndsu.edu
## APPENDIX D. DATA SHEET

**Pre-Test/Post-Test**

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# APPENDIX E. RECORDED DATA

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<th>Group #</th>
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<th>ROM</th>
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### APPENDIX F. TIME LOG

Name: ___________________________  Group (circle one): Stretching/ NON-Stretching

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thirteen</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Fourteen</td>
<td></td>
<td></td>
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</tbody>
</table>
### APPENDIX G. REHABILITATION PROGRAM

<table>
<thead>
<tr>
<th>Range of motion</th>
<th>Strengthening</th>
<th>Balance/Proprioception</th>
<th>Functional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing ProStretch 3 x 30 sec</td>
<td>Theraband 3 x 15 each leg  - Inversion  - Eversion  - dorsiflexion</td>
<td>Windmills 3 x 45 sec each leg</td>
<td>Line Jumps 3 x 30 sec  - forward/back  - side/side</td>
</tr>
<tr>
<td>Wall Stretch 3x30 seconds  - Bent Knee  - Straight Leg</td>
<td>Calf Raisers 2x25 each leg</td>
<td>Ant Stomps 3 x 10 each leg</td>
<td>Heisman’s 3 x 30 sec</td>
</tr>
<tr>
<td>Walking 20 yards each  - Toes  - Inversion  - Eversion</td>
<td>Single Leg Ball Toss 2 x 15 each leg</td>
<td>Box Jumps 3x1 minute</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX H. STATISTICS

Ankle Strength Study -- Samantha Narveson
ANCOVA for Girth

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>262.4242391</td>
<td>87.4747464</td>
<td>405.99</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>28</td>
<td>6.0329484</td>
<td>0.2154624</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>31</td>
<td>268.4571875</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>PostGirL Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.977527</td>
<td>1.332293</td>
<td>0.464179</td>
<td>34.84063</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>2</td>
<td>12.8541875</td>
<td>6.4270937</td>
<td>29.83</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>PreGirL</td>
<td>1</td>
<td>249.5700516</td>
<td>249.5700516</td>
<td>1158.30</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>2</td>
<td>2.8009586</td>
<td>1.4004793</td>
<td>6.50</td>
<td>0.0048</td>
</tr>
<tr>
<td>PreGirL</td>
<td>1</td>
<td>249.5700516</td>
<td>249.5700516</td>
<td>1158.30</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**t Tests (LSD) for PostGirL**

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| Alpha                           | 0.05 |
| Error Degrees of Freedom       | 28   |
| Error Mean Square              | 0.215462 |
| Critical Value of t            | 2.04841 |
| Least Significant Difference   | 0.4132 |
| Harmonic Mean of Cell Sizes    | 10.58824 |

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>t Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35.4500</td>
<td>12</td>
<td>CG</td>
</tr>
<tr>
<td>B</td>
<td>35.0100</td>
<td>10</td>
<td>ESG</td>
</tr>
<tr>
<td>C</td>
<td>33.9400</td>
<td>10</td>
<td>EG</td>
</tr>
</tbody>
</table>
Ankle Strength Study -- Samantha Narveson

ANCOVA for Range of Motion

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>708.4780323</td>
<td>236.1593441</td>
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<td>&lt;.0001</td>
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<tr>
<td>Error</td>
<td>28</td>
<td>145.8166552</td>
<td>5.2077377</td>
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<tr>
<td>Corrected Total</td>
<td>31</td>
<td>854.2946875</td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>PostRomL Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.829313</td>
<td>15.98282</td>
<td>2.282047</td>
<td>14.27813</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
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<td>244.9096875</td>
<td>122.4548437</td>
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<tr>
<td>PreRomL</td>
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<td>463.5683448</td>
<td>89.02</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
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<td>302.6438445</td>
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<td>PreRomL</td>
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<td>463.5683448</td>
<td>463.5683448</td>
<td>89.02</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

**t Tests (LSD) for PostRomL**

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

<table>
<thead>
<tr>
<th>Alpha</th>
<th>0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Degrees of Freedom</td>
<td>28</td>
</tr>
<tr>
<td>Error Mean Square</td>
<td>5.207738</td>
</tr>
<tr>
<td>Critical Value of t</td>
<td>2.04841</td>
</tr>
<tr>
<td>Least Significant Difference</td>
<td>2.0316</td>
</tr>
<tr>
<td>Harmonic Mean of Cell Sizes</td>
<td>10.58824</td>
</tr>
</tbody>
</table>

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>t Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17.8800</td>
<td>10</td>
<td>ESG</td>
</tr>
<tr>
<td>B</td>
<td>14.1000</td>
<td>12</td>
<td>CG</td>
</tr>
<tr>
<td>C</td>
<td>10.8900</td>
<td>10</td>
<td>EG</td>
</tr>
</tbody>
</table>
Ankle Strength Study -- Samantha Narveson

ANCOVA for Cybex

### Sum of Squares

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>1155.389772</td>
<td>385.129924</td>
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</tr>
<tr>
<td>Error</td>
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<td>678.485228</td>
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<tr>
<td>Corrected Total</td>
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<td>1833.875000</td>
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### R-Square

<table>
<thead>
<tr>
<th>Source</th>
<th>R-Square</th>
<th>Coeff Var</th>
<th>Root MSE</th>
<th>PostCybexL Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.630026</td>
<td>14.72168</td>
<td>4.922562</td>
<td>33.43750</td>
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</table>

### Source Table (Type I)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
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<td>135.258333</td>
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<td>2.79</td>
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<td>1020.131438</td>
<td>42.10</td>
<td>&lt;.0001</td>
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</tbody>
</table>

### Source Table (Type III)

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<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>2</td>
<td>326.430640</td>
<td>163.215320</td>
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<td>0.0041</td>
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<td>PreCybexL</td>
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<td>1020.131438</td>
<td>1020.131438</td>
<td>42.10</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

### t Tests (LSD) for PostCybexL

**NOTE:** This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

<table>
<thead>
<tr>
<th>Alpha</th>
<th>Error Degrees of Freedom</th>
<th>Error Mean Square</th>
<th>Critical Value of t</th>
<th>Least Significant Difference</th>
<th>Harmonic Mean of Cell Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>28</td>
<td>24.23162</td>
<td>2.04841</td>
<td>4.3824</td>
<td>10.58824</td>
</tr>
</tbody>
</table>

**NOTE:** Cell sizes are not equal.

Means with the same letter are not significantly different.

### t Grouping

<table>
<thead>
<tr>
<th>t Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>36.300</td>
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<td>ESG</td>
</tr>
<tr>
<td>B</td>
<td>32.917</td>
<td>12</td>
<td>CG</td>
</tr>
<tr>
<td>B</td>
<td>31.200</td>
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<td>EG</td>
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73