Ice Application Facilitates Soleus Motoneuron Pool Excitability in Subjects with Functional Ankle Instability

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Jeffrey R. Doeringer

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by
JEFFREY R. DOERINGER

has been approved for
the School of Recreation and Sport Sciences
and the College of Health and Human Services by

Andrew Krause
Assistant Professor of Recreation and Sport Sciences

Gary S. Neiman
Dean, College of Health and Human Services
ABSTRACT

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Evidence suggest cooling the ankle joint facilitates soleus involuntary peak motoneuron excitability in healthy subjects has been reported. Our objective is to identify the effects of ankle joint cooling on the soleus, peroneus longus, and tibialis anterior Hoffmann reflex and isokinetic eversion and inversion torque. A repeated measures design was used to analyze Hoffmann reflex in subjects with functional ankle instability (FAI) compared to healthy, stable subjects. Baseline Hoffmann reflexes, eversion and inversion torques were collected. Following these pretest measurements a 1.5L cubed ice bag was applied to the dorsum of the foot and ankle. Hoffmann reflexes, eversion and inversion torque were collected following the ice bag application. Pre- and post-cooling data were repeated during a rest session on a subsequent day. The soleus and peroneus longus H:M_{max} were facilitated following ice application in FAI subjects and healthy subjects. No statistically significant differences were observed for the tibialis anterior H:M_{max} and eversion or inversion torque between the FAI group and healthy subjects.

Approved: _____________________________________________________________

Andrew Krause

Assistant Professor of Recreation and Sport Sciences
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CHAPTER 1: INTRODUCTION

Ankle injuries are the most common musculoskeletal injuries of physically active people (Hootman et al., 2002). Lateral ankle sprains range from 33-73% of sport-related injuries. In 38 countries, lateral ankle sprains are the most common injury in 34% of sports (Fong, Hong, Chan, Yung, & Chan, 2007). The most frequently reported orthopedic injury in epidemiology reports from the National Basketball Association (NBA) and the Women’s National Basketball Association (WNBA) are lateral ankle sprains. Specifically, NBA reports show lateral ankle sprains range from 9.4-14.3% of the total injuries sustained. The WNBA reports indicate 12.7% of all injuries are lateral ankle sprains (Deitch, Starkey, Walters, & Moseley, 2006; Starkey, 2000).

The predisposition for recurrent sprains to the same ankle increases following a single injury (Hertel, 2000, 2002). Recurrent ankle sprains lead to mechanical or functional instability of the ankle (Hertel, 2000, 2002). Mechanical instability is a product of anatomical changes in the ankle following a sprain, leading to pathologic laxity, impaired arthrokinematics, synovial changes, and development of degenerative joint disease. Functional ankle instability (FAI) is a neuromuscular deficiency described by the feeling of the joint “giving way” following a lateral sprain and causing recurrent ankle instability (Hertel, 2000, 2002; McKnight & Armstrong, 1997; McVey, Palmieri, Docherty, Zinder, & Ingersoll, 2005; Tropp, 2002). FAI initially caused by a single ankle sprain results in the impairment of neuromuscular recruitment, postural control, and strength deficits in ankle musculature (Hertel, 2002; McVey et al., 2005; Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004; Tropp, 2002). Soleus and peroneus longus
muscle inhibition develops following recurrent ankle sprain and remains present in individuals with FAI (McVey et al., 2005). Individuals with functional ankle instability have damaged ankle joint structures. This induces the reflex mechanisms that limit the ability of the muscles to stabilize the joint. This phenomenon is known as arthrogenic muscle response (AMR).

AMR is an ongoing reflex response to musculature following damage to or distension of structures surrounding the joint (Hopkins et al., 2006). This response is either inhibitory or facilitory (Hopkins & Ingersoll, 2000; Hopkins, Ingersoll, Edwards, & Klootwyk, 2002; Hopkins & Stencil, 2002; Krause, 2004; Krause, Hopkins, Ingersoll, Cordova, & Edwards, 2000; McVey, Palmieri, Docherty, Zinder, & Ingersoll, 2005; Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004). Interrupted feedback from damaged joint receptors causes decreased neuromuscular recruitment. The inhibitory response, known as arthrogenic muscle inhibition (AMI), may limit progress in rehabilitation and may increase the chance of recurrent injury (Hopkins & Ingersoll, 2000; McVey et al., 2005). During ankle trauma, capsuloligamentous mechanoreceptors become damaged and affect the soleus, peroneus longus, and tibialis anterior muscle spindle activity (McVey et al., 2005; Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004). Trauma to the ankle may cause lower leg musculature initially to become facilitated as a protective mechanism (Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004) and eventually to become inhibited during chronic stages of an inversion ankle sprain. The soleus, peroneus longus, and tibialis anterior are key muscles that control ankle movements (McVey et al., 2005).
Recurrent injury is likely if appropriate therapeutic interventions are not implemented. Treatments such as cryotherapy or transcutaneous electrical neuromuscular stimulation (TENS) to the joint area have been observed to minimize AMI (Hopkins & Ingersoll, 2000; Hopkins et al., 2002). Cold application to the ankle joint facilitates leg musculature to overcome AMI, thereby allowing an aggressive rehabilitation and a quicker return to activity. Cooling the joint alters spinal inhibitory interneurons. In subjects with AMI who have undergone joint cooling, the afferent signals are altered which allows the muscle to reach or exceed its peak potential motoneuron pool recruitment (Hopkins & Ingersoll, 2000). Investigators observed facilitation by cooling in both healthy ankles and ankles that had been injected with saline to mimic ankle injury. Research suggests that muscle facilitation continues well after the cooling modality is removed from the joint (Hopkins et al., 2002; Hopkins & Stencil, 2002; Krause, 2004; Krause et al., 2000). Thus, increased muscle activation could be the key mechanism in the positive effects of cryotherapy observed clinically during musculoskeletal rehabilitation.

The Hoffmann reflex (H-reflex) is an estimate of alpha motoneuron activity in the target motoneuron pool (Hoffman et al., 2003). This electrically induced spinal reflex has been used to show motoneuron pool facilitation in the soleus (Hopkins & Stencil, 2002) and peroneus longus (Krause, 2004) following a 30-minute ice bag application. These facilitory effects of joint cooling provide evidence of the positive therapeutic effects of cryokinetics—a treatment combining cold application and active exercise (Hopkins et al.,
2002; Hopkins & Stencil, 2002; Krause et al., 2000; Krause, 2004). H-reflex has also been used to demonstrate inhibition in individuals with FAI (McVey et al., 2005).

**Statement of the Problem**

Recent literature has not investigated the effects of ankle joint cooling on leg muscle motoneuron pool recruitment in subjects with FAI.

**Purpose of the Study**

The purpose of this study was to identify the effects of ankle joint cooling on the soleus, peroneus longus, and tibialis anterior Hoffmann reflex, and isokinetic eversion and inversion torque, in individuals who have functional ankle instability.

**Research Questions**

Prior to and following cold application the Hoffmann reflex, and eversion and inversion isokinetic torque, were measured to answer the following research questions:

1. Does cooling the ankle joint affect the soleus, peroneus longus, and tibialis anterior Hoffmann reflex?
2. Does cooling the ankle joint change the eversion and inversion isokinetic torque?
3. Do the soleus, peroneus longus, and tibialis anterior Hoffmann reflex differ between functionally unstable and healthy subjects following a 30-minute ankle joint cooling?
4. Do eversion and inversion isokinetic torque differ between functionally unstable and healthy subjects following a 30-minute ankle joint cooling?
Delimitations

The study was conducted with the following delimitations:

1. College-aged females and males between 18 and 26 years old were used in this study.
2. Participants were 8 males and 16 females.
3. Participants had FAI (n = 12) or had healthy ankles (n = 12).
4. Individuals who had FAI were compared to individuals who had no history of an ankle sprain.
5. The maximum Hoffmann reflex to maximum muscle response ratio (H:M$_{max}$) was used as the measure of motoneuron recruitment.

Limitations

The following limitations were observed during this study:

1. Functional ankle instability status was determined subjectively.
2. The Hoffmann reflex is sensitive to electrical noise.
3. Only the soleus, peroneus longus and tibialis anterior Hoffmann reflexes were measured.
4. Only eversion and inversion peak torque were measured.

Assumptions

The following assumptions were made during this study:

1. The Hoffmann reflex accurately measured the soleus, peroneus longus, and tibialis anterior motoneuron pool recruitment.
2. The BIODEX accurately measured the eversion and inversion torque in these subjects.

3. The ankle instability instrument sets guidelines to determine whether an individual has FAI.

4. Subjects answered questions truthfully on the ankle instability instrument.

5. Subjects did not participate in moderate physical activity and did not drink alcohol or highly caffeinated drinks (coffee, tea, etc.) within 24 hours of data collection.

6. The cubed ice bag cooled the ankle joint enough to determine accurate effects of arthrogenic muscle response on subjects.

7. One-minute rest between sets on the BIODEX machine reduced fatigue in each subject’s leg musculature.

**Definition of Terms**

*Arthrogenic muscle inhibition (AMI):* An inhibitory response that decreases progression in rehabilitation, including any factors that may predispose individuals to recurrent injury (Hopkins & Ingersoll, 2000; McVey, Palmieri, Docherty, Zinder, & Ingersoll, 2005).

*Arthrogenic muscle response (AMR):* An ongoing reflex response to musculature following damage to or distension of structures surrounding the joint (Hopkins & Ingersoll, 2000; Hopkins, Ingersoll, Edwards, & Klootwyk, 2002; Hopkins & Stencil, 2002; Krause, Hopkins, Ingersoll, Cordova, & Edwards, 2000; McVey, Palmieri, Docherty, Zinder, & Ingersoll, 2005; Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004).
**Biodex System III Pro Multijoint Dynamometer (BIODEX):** An isokinetic machine used to measure peak torque of the peroneus longus and tibialis anterior muscle at 60 deg/sec.

*Chronic ankle instability (CAI):* Is driven by mechanical and functional instability following a lateral sprain and recurrent ankle instability (Hertel, 2002).

*Functional ankle instability (FAI):* A neuromuscular deficiency described by the feeling of the joint “giving way” following a lateral sprain and recurrent ankle instability (Hertel, 2000, 2002; McKnight & Armstrong, 1997; McVey et al., 2005; Tropp, 2002).

\( H:M_{\text{max}} \): The ratio between the maximum Hoffmann reflex and the maximum muscle response and is a measure of involuntary muscle activation (Hoffman, Palmieri, & Ingersoll, 2003).

*Healthy ankle subjects:* Individuals with no past ankle injury/pathology and no ankle instability (McVey et al., 2005).

*Hoffmann reflex (H-reflex):* An estimate of alpha motoneuron activity in the target motoneuron pool; used to measure the maximum Hoffmann reflex to maximum muscle response ratio \( H:M_{\text{max}} \) in the soleus, peroneus longus, and tibialis anterior (Hoffman, Palmieri, & Ingersoll, 2003).

*Muscle response:* The maximum motoneuron activity in the target motoneuron pool (Hoffman, Palmieri, & Ingersoll, 2003).
CHAPTER 2: LITERATURE REVIEW

Epidemiology of Musculoskeletal Injuries

Hootman et al. (2002) conducted an epidemiology report over a 16-year (1970-1986) span on physically active individuals. During the first 12 years (1970-1982) of the study, baseline clinical examinations were used to determine potential subjects’ health status and injuries incurred. Individuals’ ages ranged between 20-85 years. They were affiliated with the Cooper Clinic in Dallas, Texas and were enrolled in the Aerobic Center Longitudinal study on health effects. All participants received a follow-up survey in 1986 to determine their orthopedic injuries, physical activity levels, and exercise habits during the previous 12-month period. Out of 11,972 surveyed, only 1,601 responses were used. Of the 1,601 subjects, 1,290 were male and 311 were female. The surveys revealed that 7.5% of males and 8.7% of females suffered ankle injuries from moderate physical activity. The investigators also found 82% of males and 84% of females injuries had orthopedic injuries related to physical activity (Hootman et al., 2002).

Starkey (2000) conducted an epidemiology study on the National Basketball Association (NBA) over a 10-year (1988-1998) span. Of the NBA teams, 29 (86%) were compliant with the epidemiology reports. Over 10 years, 1,094 individuals had 3,843 injuries or illnesses. About 78% of the injuries were orthopedic in nature. Lateral ankle sprain was the most common orthopedic injury reported. Of the injuries sustained during the 10 years, 10.7% were ankle injuries and 9.4% were sprains. Injuries were reported only if a physician’s referral was made, a practice or game was missed, or emergency care was rendered (Starkey, 2000).
Deitch et al. (2006) conducted an epidemiology report comparing the injury rate between the NBA and Women’s National Basketball Association (WNBA). The NBA had 702 individuals and the WNBA had 443 individuals on record for reporting injuries and illnesses over 6 years. The NBA athletic trainers reported 16.9% and the WNBA athletic trainers reported 15% of injuries involved the ankle. As reported by the athletic trainers, 14.3% of NBA injuries and 12.7% of WNBA injuries were lateral ankle sprains. Lateral ankle sprains were the most common orthopaedic injury and diagnosis in both leagues (Deitch et al., 2006).

Fong et al. (2007) completed a systematic review on epidemiology reports that focused on ankle injuries in sports, including data collected between 1977 to 2005. The data represented 70 different sports from 38 different countries. This report reviewed 201,600 patients with ankle injuries. Ankle sprains represented 33-73% of all sport injuries and were the most common injury in 34% (24 out of 70) of the sports. Ankle injuries were the highest reported injury in 77% (33 out of 43) of the sports included in the review. As a result, a high number of ankle injuries have been reported in court games and team sports (Fong et al., 2007).

Ankle Instability

Tropp (2002) ascribed chronic ankle instability (CAI) to both mechanical and neuromuscular factors. Ankle injuries occur when the foot receives an external load forcing movement of the subtalar joint into supination. When the force is excessive, the peroneal muscles are not able to react to the external load and the subtalar-joint shifts 3-4 cm into excessive inversion. Damage to the peroneals creates instability and can lead to
future ankle instability. Imbalances between inversion and eversion strength increase the risk of future injuries to the unstable ankle (Tropp, 2002).

Hertel (2002) observed that CAI is driven by mechanical instability and functional instability (see Figure 1). Mechanical instability is a product of anatomical changes in the ankle following a sprain, leading to changes in pathologic laxity, impaired arthrokinematics, synovial changes, and development of degenerative joint disease. Pathologic laxity arises from damage to ligaments. The subsequent elongation of tissues can be problematic when the joint is in a vulnerable position, leading to a recurring injury. Impaired arthrokinematics is common in a tibiofibular joint where the fibula is displaced and the anterior talofibular ligament is lax, leading to repetitive ankle sprains. Mechanical instability of the joint can also be related to synovial and degenerative changes. These changes include synovial hypertrophy, impingement, inflammation, and joint lesions (Hertel, 2002). Hertel (2002) reported several cases of synovial and degenerative changes contributing to numerous cases of ankle instability.
The other ankle insufficiency is functional instability. Functional instability is reduced ankle support due to sensorimotor system deficits. Functional instability is categorized as impaired or decreased: proprioception, cutaneous sensation, neuromuscular response time, strength, and postural control. Deficits in proprioception and cutaneous sensation cause alterations in muscle-spindle activity in the peroneal muscles and mechanoreceptor response following ankle injuries. Impaired neuromuscular-firing patterns exist when the peroneus longus muscle attempts to respond to the ankle “giving way” or subtalar joint rolls into excessive inversion. A final theory to
functional instability is impaired postural control, which is a muscle’s inability to maintain a center of gravity while bearing weight (Hertel, 2002).

Konradsen (2002) investigated kinesthesia and joint position senses in subjects with CAI. Mechanoreceptors of the lateral ligaments transmit proprioception information following an inversion injury. Trauma to the area may cause misinterpreted sensory information. As a result, the patient will lose sense of joint position, leading to the ankle being unstable. Lack of kinesthesia is a problem that may occur during acute or CAI. This renders a person with a diminished awareness of foot movement and placement, which creates errors in joint position during walking and/or activity. Experiencing joint position error during walking causes the lateral border of the foot to drop 5 mm immediately before the foot makes contact with the ground. Altered kinesthesia and joint position can lead to a recurring injury in an unstable ankle (Konradsen, 2002).

Konradsen and Ravn (1991) addressed the peroneal reaction time during an unexpected 30° inversion drop of the ankle. The investigators used EMG readings to determine how long the peroneus longus and brevis took to respond to the sudden inversion drop. Subjects were tested bilaterally and on average the stable side responded to an unexpected drop about 13 msec faster than the unstable side. The stable leg responded in 72 msec and the unstable leg responded in 85 msec. The altered reaction time in individuals with FAI most likely is triggered by the damaged mechanoreceptors in the joint capsule, lateral ligaments, and/or peroneal muscles (Konradsen & Ravn, 1991).
In contrast, Vaes et al. (2002) found no difference between unstable ankles and healthy ankles following six continuous unanticipated inversion drops at 50° on a controlled foot platform. The investigators categorized the different variables being tested or measured, which included: total inversion time; timing of the first deceleration; timing of the second deceleration; latency of the peroneal muscles; electromechanical delay; electromyography from beginning activity in the foot to move completely into eversion in peroneal muscles; and motor response in the peroneal muscles. The first deceleration was the only element showing a difference between the two groups, since the unstable ankle group may have less control over the sudden drop. The peroneal muscles reactions are important but when considering subjects with CAI or FAI, many different aspects need careful observation. Until this time, there remained few objective ways to quantify FAI clinically (Vaes et al., 2002).

Docherty et al. (2006) developed a reliable instrument to determine FAI (see Appendix C). The investigators of the study compared three factors including severity of initial ankle sprain, history of ankle instability, and instability during activities of daily life. The Ankle Instability Instrument (AII) reliability was .95 for intraclass correlation coefficient. This test was consistent on 139 ankles measured with the AII questionnaire. A compilation of 12 questions made up this AII. Scoring of this instrument involved analyzing the population on what determines ankle instability (Docherty et al., 2006). McVey et al. (2005) used the AII to determine FAI. In this study, answering five or more questions with “yes” classified having a functionally unstable ankle (McVey et al., 2005).
Hertel (2000) assembled a literature review of the occurrences of FAI following lateral ankles sprains. He found following recurrent lateral sprains, either mechanical or functional instability occurred. In few cases, both occurred simultaneously following a recurrent lateral sprain. Balance deficits showed a significant impact on the limb of someone with ankle instability and recurrent sprains compared to the person’s uninjured/uninvolved leg. Balance tests completed used subjective and objective methods. The Romberg test can clinically assess differences between the patients involved and uninjured ankle. Other areas of instability are seen because of delayed reaction time, altered nerve function, and strength deficits in the peroneals (Hertel, 2000). Deficits are mostly seen in agonist evertors of the ankle; however some studies show deficits in inverter muscles (Hartsell & Spaulding, 1999). Decreased dorsiflexion is another possible element in patients with instability. Decreased dorsiflexion increases the possibility the individual will invert the foot secondary to the internal rotation of the subtalar joint. Determining the patient’s specific deficits will help the clinician properly rehabilitate the problem, aiding in an accelerated return to activity and/or decrease the regular occurrences of recurrent sprains or sensation of the ankle giving away (Hertel, 2000).

Ankle Instability Strength Deficits

One accepted theory is that individuals with recurrent ankle problems show a deficit in eversion muscle strength (Hartsell & Spaulding, 1999; Hertel, 2000, 2002; Hopkins & Ingersoll, 2000; Kaminski, Perrin, & Gansneder, 1999; Konradsen & Ravn, 1991; McVey et al., 2005; Vaes et al., 2002; Willems, Witvrouw, Verstuyft, Vaes, & De
Clercq, 2002). Willem et al. (2002) investigated deficits in individuals with CAI by measuring their strength and proprioceptive differences. Subjects were divided into four groups: control group with no injuries, instability group with recurrent sprains/instability, sprained ankle group with several sprains but no instability for 2 years prior to study, and sprained ankle group with several sprains but no instability for 3-5 years prior to study. The investigators used a Biodex System III Pro dynamometer to measure eversion and inversion during concentric and eccentric strength for each movement. Each subject performed eversion and inversion for three repetitions at 30 deg/sec and five repetitions at 120 deg/sec for both legs. Each subject performed four tests for each leg. Peak torque and peak torque/body-weight values were measured. Significant differences were found between the control and instability groups for concentric and eccentric eversion at 30 deg/sec was found. The instability group had lower eversion strength values for peak torque/body-weight than both of the sprained ankle groups and the control group. There were no significant differences between the control group and the two sprained ankle groups (Willems et al., 2002).

Hartsell and Spaulding (1999) investigated eccentric/concentric ratios during inversion and eversion movements in individuals with ankle instability. Both the control group and the CAI group performed five maximal eccentric and concentric contractions for both inversion and eversion at 60 deg/sec, 120 deg/sec, 180 deg/sec, and 240 deg/sec. Evidence showed that subjects with CAI were significantly weaker during eccentric/concentric inversion and eversion movements than stable ankle subjects. A correlation between chronic instability and muscle weakness during both concentric and
eccentric movements was discovered. This weakness suggested high risk of instability leading to recurrent injury (Hartsell & Spaulding, 1999).

Munn, Beard, Refshauge, and Lee (2003) used the Biodex dynamometer to determine if subjects with FAI had deficits in inversion and eversion comparing concentric and eccentric strength. Speeds of 60 deg/sec and 120 deg/sec were used during five maximal contractions for each condition. Subjects who sprained their ankle 1-12 months prior to the study, and had a history of at least two sprains to the lateral ligaments of the tested ankle, determined ankle instability. A significant difference was observed in eccentric inversion weakness for individuals with ankle instability compared to individuals with stable ankles. Investigators theorized that this weakness might explain the dynamic stabilization role of individuals who have recurrent ankle sprains, i.e., individuals with FAI may be unable to control lateral postural sway during stability weight bearing activities (Munn, Beard, Refshauge, & Lee, 2003).

McKnight and Armstrong (1997) tested subjects with FAI and strength for all plane movements of the ankle. They examined three groups: no history of ankle problems, FAI with no rehabilitation, and FAI with rehabilitation following injury. The Biodex System III dynamometer was used to measure concentric contraction for each ankle motion. Each subject performed three repetitions at 30 deg/sec and fifteen repetitions at 240 deg/sec for each direction of motion. There was no significant difference among groups for each of the strength measurements (McKnight & Armstrong, 1997).
Kaminski et al. (1999) found no significant difference in eccentric, concentric, and isometric eversion strength between individuals with stable ankles and unstable ankles. Investigators paired the stable group with an unstable group during eversion at several velocities. Three maximal concentric and eccentric contractions were performed for each of the velocities (30, 60, 90, 120, 150, and 180 deg/sec). During isometric contraction, each individual held the contraction for 5 seconds and rested for 30 seconds. There were no significant differences between the two groups for peak torque or peak torque/body weight (Kaminski et al., 1999).

**Arthrogenic Muscle Response**

Arthrogenic muscle response (AMR) is a reflex response of joint musculature following distension of or damage to structures of the joint (Hopkins, 2006). Hopkins and Ingersoll investigated the effects of therapeutic intervention on arthrogenic muscle inhibition (AMI). They studied methods of measuring AMI, identifying its clinical signs, and identifying therapeutic approaches to reduce it. They found that AMI would reduce the ability of muscles to activate following joint tissue damage (see Appendix A and Appendix B). This inhibitory effect arises from damaged mechanoreceptors in and around the joint. Joint musculature motoneuron pool recruitment is inhibited at the interneuron synapses, due to deficits in afferent and proprioceptive input during AMI. This alteration results in decreased joint musculature activation. AMI has a direct and negative effect on the process of tissue healing following distention or damage. Hopkins and Ingersoll described this as a “hard-wired” inhibitory reflex that decreases muscle recovery and return to activity (Hopkins & Ingersoll, 2000).
In the joint, mechanoreceptors consist of Ruffini endings, Golgi-like endings, and Pacinian corpuscles. Ruffini endings respond to slight movement but are slow to respond to a low threshold. Golgi-like endings fire rapidly in response to changes in movement. Pacinian corpuscles adapt quickly to changes in joint movement, acceleration/deceleration, joint position sense, and environment changes. In addition, free nerve endings in joints respond to joint movement by releasing a pain message during distention of or damage to the joint or other overlying structures. The portion of the damaged joint will affect those receptors. As a result, the receptors will send inhibitory messages through afferent neurons (Hopkins & Ingersoll, 2000).

Presynaptic and postsynaptic inhibitions are two types of inhibitory changes that may occur following trauma to a joint. The inhibition occurs either where neurotransmitters cross the synapse between neurons or in the membrane. The exchanges of neurotransmitters between neurons relay signals of inhibition or facilitation. Postsynaptic inhibition occurs when inhibitory neurotransmitters cause ion channels to open, causing a change in their polarization. Hyperpolarization decreases the chances of a contraction if a neurotransmitter does not bind to an adjacent neuron. The inhibitory neurotransmitter is defined as \( \gamma \)-aminobutyrate (GABA). Inhibited postsynaptic activity also affects presynaptic activity. Presynaptic inhibition causes calcium to flood the terminal synapse leading to decreased binding. Presynaptic inhibition only affects one synapse whereas postsynaptic inhibition affects the whole neuron. However, once inhibition occurs, whether it is postsynaptic or presynaptic, both become affected and work together to cause an inhibitory effect (Hopkins & Ingersoll, 2000).
Muscle response inhibition may cause gait deficiencies and/or adversely affect the rehabilitation process. Decreasing the muscle’s ability to fully contract will diminish its aptitude to strengthen and fully hypertrophy. When a muscle is facilitated, the rehabilitation process may be enhanced in the following ways: decreased healing time, increased strength, reduced stiffness of ligaments, increased collagen synthesis in tendons, increased proteoglycan content in articular cartilage, and periosteal expansion of bone tissue. The use of cold therapy has shown to effect motoneuron pool recruitment on overall muscle movement (Hopkins & Ingersoll, 2000). Hopkins (2006) found evidence that the application of cryotherapy to an experimentally induced knee showed no decrease in the quadriceps activation. In comparison, an effusion control group displayed decreased peak torque output during recumbent stepping motion. Showing recruitment improved peak power following cryotherapy treatment (Hopkins, 2006).

**Knee Arthrogenic Muscle Response**

Hopkins et al. (2002) investigated AMR of the vastus medialis during experimentally induced knee effusion with different therapeutic interventions, including cold application and transcutaneous electric nerve stimulation (TENS). The investigators measured H-reflex and motoneuron excitability every 15 minutes over a span of 60 minutes. The participants were all healthy neurologically sound individuals. The cryotherapy and TENS treatments were applied for 30 minutes beginning immediately following injection of 60 mL of saline solution. Results showed a facilitation of the vastus medialis in the cryotherapy group during the entire 60 minutes of induced knee effusion. The TENS group demonstrated an increase in quadriceps muscle activation
while the electrical current was delivered, but when the current was stopped, quadriceps inhibition returned. The control group showed an initial increase during postinjection but a drastic decrease continuing throughout, noting the presence of quadriceps inhibition. The study concluded that without a therapeutic intervention, cold application, or TENS, AMI occurs in the vastus medialis during joint effusion (Hopkins et al., 2002).

Hopkins et al. (2001) examined the effects of knee joint effusion on quadriceps (vastus medialis) and soleus muscle. This study examined what kind of AMR occurred following simulated trauma or damage to the knee joint by inducing fluid. H-reflex peak was taken prior to 30mL induced effusion knee, 30-min, 90-min, 150-min, and 210-min post over a 4-hour period. The vastus medialis muscle was stimulated in the femoral triangle first. Second, the posterior tibial nerve was stimulated for the soleus measurement. H-reflex increased in the soleus and decreased in the vastus medialis. This provides evidence the soleus is facilitated in response to joint effusion, while the vastus medialis is inhibited (Hopkins, Ingersoll, Krause, Edwards, & Cordova, 2001). In addition to the vastus medialis being inhibited, Palmieri, Ingersoll, Edwards, et al. (2003) studied the contralateral side to the inhibition from injected knee infusion. Sixteen subjects were divided into control and knee-effused groups. During this study, investigators induced the knee with 60 mL of saline in the effused-knee group while the control group rested for 8 minutes. H:M_max data were collected before injection and at 10, 20, and 30 minutes post intervention. Evidence indicated a significant decrease in the H-reflex max and H:M_max of the effused knee compared to the contralateral side. In the
control group, no differences were documented (Palmieri, Ingersoll, Edwards, et al., 2003).

Palmieri, Tom, et al. (2004) investigated the pre- and post- synaptic spinal mechanism of soleus AMR. During this study, investigators induced joint effusion in the knee to determine both immediate effects and up to 45 minutes post effects. Saline was injected to simulate knee trauma. Ten subjects participated in two sessions. During one session, the subject’s knee was injected with 60 mL saline to simulate joint effusion. The second session was a control. The measurements used during the study were paired reflex depression and recurrent inhibition. Paired reflex depression was measured from 25% of maximum muscle response and was set for a fixed stimulus during H-reflex measurement. Subjects were measured eight times at 80 ms intervals. During recurrent inhibition, the first set was the same as paired reflex depression but instead of 80 ms apart, the measurement was taken at 10 ms intervals. Measurements for both effusion and control sessions were taken at baseline, post needle stick, post Xylidocaine, and both 25 minutes and 45 minutes post effusion. The control session was the same as the effusion session except for the injection of Xylidocaine and effusion. Investigators observed a significant increase in the post 25- and 45-minute measurements in unconditioned reflex amplitude for recurrent inhibition and reflex activation. Results showed that a soleus AMR following effusion was mediated by pre- and post- synaptic control mechanisms. Results indicated that the soleus muscle is facilitated as a protective mechanism when the knee joint simulates damage (Palmieri, Tom, et al., 2004).
Palmieri, Weltman, et al. (2005) furthered their research by investigating presynaptic modulation of quadriceps (vastus medialis) AMI. During this study, they induced joint effusion to the knee to determine the immediate and up to 45 minutes post effects. They injected the effusion to simulate posttraumatic swelling. Eight subjects were used in two sessions. In one session, the investigators injected subjects’ knees with saline to simulate joint effusion; the other session was a control. The measurements used during the study were H-reflex, muscle response, reflex activation history, plasma epinephrine, and norepinephrine. Measurements for both effusion and control sessions were taken at baseline, post needle stick, post lidocaine, and both 25- and 45-minute post effusion. The control session was the same except for the actual injection of lidocaine and fluids. Investigators observed a significant decrease in the post 25- and 45-minute measurements in maximum H-reflex, but no difference in maximum muscle response during any measurements were noted. The results showed a quadriceps AMI following effusion was at least mediated by a presynaptic mechanism (Palmieri, Weltman, et al., 2005).

Palmieri, Ingersoll, Cordova, et al., (2003) investigated postural control in induced-knee effusion of health subjects. Twenty subjects were split into two groups: effused-knee group and control group. Subjects in the effused-knee group were injected with 60 mL of saline and subjects in the control group rested for 8 minutes. Investigators measured postural control on a Kistler piezoelectric force platform to measure the center of pressure and mean power frequency. Measurements were taken prior to and post injection of saline, and during rest. Researchers found a decrease in center of pressure from pre- to post-injection and observed no differences in the control group. The mean
power frequency did not change for either group from baseline to post measurements (Palmieri, Ingersoll, Cordova, et al., 2003).

**Ankle Arthrogenic Muscle Response**

Hopkins and Palmieri (2004) investigated the effects of induced ankle joint effusion on lower leg function. They examined the soleus, peroneus longus, and tibialis anterior on ankle joint peak torque, peak power, and root mean square power during a closed kinetic chain exercise following the ankle induced with effusion. Twenty subjects were divided into two groups. The effused group had measurements taken prior to effusion, immediately post, and 30 minutes post. An Omnikinetic closed chain dynamometer was used to collect ankle function by performing six repetitions at 35% of one max repetition. Decreases in ankle plantar flexion torque and plantar flexion EMG indicated a neuromuscular deficit existing in the presence of edema could increase the susceptibility for further ankle injury. Decreases were also seen in the peroneus longus EMG immediately following and 30 minutes post injection (Hopkins & Palmieri, 2004).

Hall, Nyland, Nitz, Pinerola, and Johnson (1999) investigated the relationship between acute swelling in the ankle to flexor digitorum longus and peroneus longus H-reflex latency. Fifteen subjects with an acute inversion ankle sprain within three days of the injury were examined. Researchers measured swelling by using a tape measurer in a figure eight technique. They collected the H-reflex latency and compared the involved leg to the contralateral leg. No significant differences in H-reflex amplitude and peroneus longus latency between ankles were recorded. Researchers found that in grade I or II inversion sprains and the related swelling appeared to delay ankle flexor digitorum
longus latency to a greater extent than peroneus longus latency. With the information provided the researchers recommended focusing on ankle invertors during rehabilitation (Hall, Nyland, Nitz, Pinerola, & Johnson, 1999).

McVey et al. (2005) conducted a study investigating the AMR in individuals who had unilateral FAI compared to control healthy subjects. They performed an H:M$_{\text{max}}$ on bilateral limbs by testing the soleus, peroneus longus, and tibialis anterior muscles in both FAI subjects and healthy subjects. The Ankle Instability Instrument (AII) was used to determine the FAI. Subjects with unilateral FAI had lower H:M$_{\text{max}}$ in their unstable ankles than in their contralateral legs. The healthy group had minimal differences between the bilateral comparison. Individuals with unilateral FAI displayed AMI in the limb, with a sensation of the ankle giving way (McVey et al., 2005).

Sedory, McVey, Cross, Ingersoll, and Hertel (2007) further investigated CAI to determine the AMR in the quadriceps and hamstrings. Two groups in this study received measurements bilaterally: a control group with no ankle instability or sprain, and a group with CAI. Each control subject limb was matched with a CAI subject’s limb. The matched pair between groups compared limbs on the same side of the body with an unstable limb (Sedory, McVey, Cross, Ingersoll, & Hertel, 2007). In contrast to the McVey et al. (2005) study, the investigators used a center activation ratio (CAR), a ratio of the force exerted in a maximal voluntary isometric contraction (MVIC) divided by the force exerted when an MVIC is performed concurrently with a superimposed burst of electric stimulation (Sedory et al., 2007). Each subject was placed into a BIODEX chair where they performed a 3-second MVIC for both extension and flexion of the knee.
During the MVIC a superimposed burst was administered. Subjects performed three trials of each movement with a 3-minute rest between each set. Results showed a significantly higher quadriceps CAR in the limb of CAI subjects relative to the contralateral side of the control subjects who exhibited no significant difference bilaterally. In contrast, the hamstring CAR was significantly lower in the involved limb than in the contralateral side of individuals with unilateral ankle instability compared to the control group, who did not show a difference bilaterally. This provided evidence that both lower extremities need attention when rehabilitating an individual with ankle instability or ankle pathology (Sedory et al., 2007).

Cryotherapy applied to a joint allows inhibited muscle recruitment patterns to return to previous baseline measurements prior to pre-exposed trauma. Hopkins and Stencil (2002) found facilitation in the soleus following ankle cryotherapy. The investigators recruited a healthy ankle population for the study and split the subjects into a cryotherapy and control group. A BIOPAC system was used to collect soleus H:M_{max} and plantar flexion peak torque on a Cybex NORM dynamometer to attain the measurements. Baseline measurements were collected prior to the intervention of 1.5 L crushed ice bag applied over the anteriolateral portion of the ankle joint for subjects in the cryotherapy group and a 30 minutes control rest session for the control subjects. Measurement was taken immediately following cooling the ankle joint (30-minutes following baseline), and 30 minutes, and 60 minutes post intervention. Evidence found that cooling the skin over the ankle joint rather than the muscle increased facilitation of motoneuron pool recruitment. An increase in planter flexion torque in the cryotherapy
group following the 30-minute ice intervention was also found. The investigators agreed that cryotherapy applied prior to rehabilitation and activity has positive effects (Hopkins & Stencil, 2002). Palmieri, Ingersoll, Hoffman, Cordova, et al. (2004) discovered that simulating joint effusion in the ankle causing an AMR facilitated the musculature around the joint. The muscles tested were the soleus, peroneus longus, and tibialis anterior. Following artificial effusion, muscles became facilitated and the motoneuron recruitment increased from prior baseline measurements. Ankle mechanoreceptors responded to effusion as if it was an injury, causing facilitation of muscles around the joint to protect it from further trauma (Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004).

Krause (2004) investigated peroneus longus motoneuron activity and eversion peak torque following cold application to the ankle joint. During this study, the use of H-reflex was used to collect peak H-reflex and muscle response and H:M_max. Isokinetic exercises of peak eversion torque were performed 60°/sec for an average of five trials. Data was collected prior to the intervention, immediately following the intervention, and 30 minutes post a 30-minute ice bag to the dorsum of the ankle and foot. The researcher found significant increases in peroneus longus H:M_max and peak eversion torque immediately following and 30-minutes post cold application. This study provided evidence that facilitated peroneus longus and increased eversion torque would enhance ankle joint rehabilitation (Krause, 2004).

**Cryotherapy**

Cryotherapy is one of the most popular therapeutic interventions for the treatment of musculoskeletal injury. Evans, Ingersoll, Knight, and Worrell (1995) used three agility
tests to compare the effects of cryotherapy on performance: co-contraction test, carioca test, and shuttle run. The dominant leg was immersed to 8 cm above the lateral malleolus in 1°C ice water for 20 minutes. The immersion did not directly affect the muscles. Data identified changes in the rate of performing activities. However, no significant difference was demonstrated between groups (Evans, Ingersoll, Knight, & Worrell, 1995). In contrast, Cross et al. (1996) found functional performance was hindered when the subject’s dominant leg was immersed to the fibular head in 13°C ice water for 20 minutes. Three functional tasks were performed: single leg vertical jump, shuttle run, and 6 m hop test. The shuttle run times were slower and vertical jumps decreased following cryotherapy. Investigators did not detect any changes in the 6 m hop test following cryotherapy. Cryotherapy has been shown to impede the performance of muscle spindles and the myotatic reflex in muscles directly affected by the treatment (Cross et al., 1996).

Cryotherapy was used when testing the effects on isokinetic eccentric plantar flexion peak torque at 30 deg/sec and 120 deg/sec. No significant difference was found between the cryotherapy group and the control group. The cryotherapy group immersed their limb to mid-thigh in ice water at 10°C for 30 minutes. Testing at 120 deg/sec of peak torque showed total work increased during an eccentric contraction (Kimura, Gulick, & Thompson, 1997). Cross et al. (1996) found no changes in eccentric peak torque when the immersion covered the entire muscle mass (Cross et al., 1996). Hatzel and Kaminski (2000) studied effects of cryotherapy on concentric and eccentric peak torque of ankle muscle performance. The movements studied included ankle plantar flexion, dorsiflexion, inversion, and eversion. Subjects were immersed in ice water up to
their tibia plateau, then measured isokinetic muscle activity at 60 deg/sec, and 120 deg/sec. Concentric dorsiflexion measured immediately following ice immersion was negatively affected by the treatment. Measurements of eccentric peak torque were higher than concentric measurements before and after ice immersion. Testing in multiple planes of the ankle joint provided evidence that cryotherapy does not hinder performance of the musculature, but showed no increase in muscle activity post treatment (Hatzel & Kaminski, 2000).

**Hoffmann Reflex**

H-reflex is an estimate of alpha motoneuron activity in the target motoneuron pool (Hoffman et al., 2003). H-reflex is an electrically-induced stimulation that bypasses the muscle spindle to assess modulation of spinal monosynaptic reflex activity. Eliciting H-reflex, a stimulus of short-duration (1 ms) and relatively low intensity (10-100 mV) is induced above the muscle spindle of the corresponding muscle. As the stimulus intensity increased a reflex potential is observed using EMG. Depolarization results in action potentials traveling up the afferent Ia pathways to the spinal cord. Action potential is adequate when depolarization is seen in a presynaptic cleft, resulting in a release of neurotransmitters to stimulate excitatory postsynaptic potentials (EPSPs). Depolarization will occur in the alpha motoneuron axon, causing an action potential to descend towards the muscle. The volley of efferent action potential is recorded on the EMG as H-reflex. As the stimulus intensity is increased further (100-200 mV) a muscle response is recorded and represents the direct depolarization of efferent alpha motoneurons (see Figure 2). This depolarization will lead to an action potential and will cause acetylcholine
release from the neuromuscular junction (Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004).

When H-reflex reaches its maximum amplitude it will disappear off the EMG reading as the stimulus intensity is increased to elicit the muscle response. H-reflex disappears as the result of antidromic collision. This collision occurs when the volley of electric activity travels in the wrong direction on the efferent motor nerve and collides with reflexive orthodromic which is the volley going in the correct direction. On EMG, the H-reflex reading starts to decline due to the size of antidromic activity. If the two are equal in size or antidromic is larger than orthodromic, the H-reflex trace on the EMG will disappear (Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004).

![Figure 2. Soleus muscle H-reflex curve.](image)

This test of motoneuron recruitment has its own limitations. Subjects are tested using H-reflex have to be completely relaxed including their resting heart rate, no distractions, all limbs supported, placed in prone or supine position, palms open, blinking
controlled, and head supported. All of these components are necessary to keep the measurements consistent and reliable (Palmieri, Ingersoll, & Hoffman, 2004; Zehr, 2002).

Evidence showed that stimulating the sciatic nerve while simultaneously measuring the soleus, peroneal, and tibialis anterior is a reliable test. Palmieri, Hoffman, et al. (2002) measured peak to peak maximum H-reflex, maximum muscle response, and H:M ratio. Stimulation of the sciatic nerve occurs above where the nerve bifurcates into the posterior tibial nerve and the common peroneal nerve. Typically, stimulation of this nerve is found in the superior/medial portion of the popliteal fossa. Two EMG electrodes were placed on each muscle following skin preparation in the distal third of the lower leg on the midline of the muscle belly of the soleus, 2 to 3 cm distal to the fibula head in the center of the peroneal muscle belly, and in the midpoint of the muscle belly of the tibialis anterior. Strong intraclass correlations for all measurements, except the H:M ratio for tibialis anterior was shown. The soleus intraclass correlation for H-reflex was .99, muscle response was .95, and H:M ratio was .97. The peroneal intraclass correlation for H-reflex was .99, muscle response was .99, and H:M ratio was .96. The tibialis anterior intraclass correlation for H-reflex was .85, muscle response was .99, and H:M ratio was .78. These data showed that using the H-reflex on the soleus, peroneal, and tibialis anterior is highly reliable during stimulation of the sciatic nerve (Palmieri, Hoffman, et al., 2002).

The H-reflex is used to determine the effects of induced effusion and cryotherapy on specific motoneuron pool recruitment (Hopkins & Stencil, 2002; Krause et al., 2000; Krause, 2004; Palmieri, Ingersoll, Cordova, et al., 2003; Palmieri, Ingersoll, Hoffman,
Cordova, et al., 2004; Palmieri, Tom, et al., 2004; Palmieri, Weltman, et al., 2005).

Studying the effect of knee joint effusion on muscle activity found cryotherapy application brought motoneuron recruitment measurements to baseline or above. In this particular study, the soleus was facilitated following ankle joint cryotherapy, resulting in increased motoneuron activity (Hopkins & Stencil, 2002). Palmieri, Ingersoll, Hoffman, Cordova, et al. (2004) reported similar findings during facilitation of muscles around the ankle during an artificial joint effusion model. H-reflex measurements demonstrate evidence of increased recruitment due to the protective mechanism of the ankle associated with trauma (Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004). Krause et al. (2000) suggest that a change in temperature will affect motoneuron activity of a muscle. Cooling the joint rather than muscle tissue is necessary to alter H-reflex measurements. Positive alterations detected during and following cooling could be beneficial during sessions of cryokinetics to enhance faster injury recovery (Krause et al., 2000).

**Isokinetic Dynamometer**

Biodex System III Pro dynamometer is a widely used instrument to measure muscular strength. It is used for controlled motion and reliable accurate measurements. Aydog et al. (2004) provided evidence the Biodex System III Pro dynamometer is reliable with the foot in neutral position during inversion and eversion strength measurements. They analyzed intratester and intertester reliability at 60 deg/sec and 180 deg/sec during seven different trials of ankle motion. Intratester coefficient correlation for ankle inversion and eversion ranged .81 to .96 during the 60 deg/sec and 180 deg/sec
conditions. Intertester coefficient correlation was .92 to .95 during the set up of the Biodex for the subject performing 60 deg/sec and 180 deg/sec by two different physicians (Aydog et al., 2004).

Amaral De Noronha and Borges (2004) investigated the reliability of isokinetic testing for inversion and eversion strength on subjects with recurrent ankle sprains. The criteria for recruitment were: one ankle with recurrent sprain, occurring at least 4 months prior but no longer than 12 months prior to the study. Each individual performed a practice cycle, then progressed to perform two test cycles of five maximal contractions at 30 deg/sec and 120 deg/sec. The subject’s contralateral limb served as a control. The intraclass coefficient correlations (ICC) for inversion and eversion test were .71 to .92 for the injured ankle and .90 to .95 for the noninjured ankle. The ICC .71 represented the evertors during the 30 deg/sec cycle of the study but there was a .89 ICC during the 120 deg/sec cycle (Amaral De Noronha & Borges, 2004). An individual with recurrent ankle trauma may show weakness in the peroneals (McVey et al., 2005).

Lund, Sondergaard, Zachariassen, Christensen, Bulow, and Henriksen (2005) investigated learning effects during trials of isokinetic measurements. Flexion and extension for both knee and elbow were examined to determine reliability and possible learning during these two measurements on a Biodex System III Pro dynamometer to a Lido Multijoint II system. Five trials of three repetitions performed on the Biodex system at 60 deg/sec were tested. Subjects had a 20-minute rest between each set of measurement. No significant differences were found on learning over time during five different trials on the Biodex System III Pro dynamometer. The ICC reliability were: .94
for knee flexion; .92 for knee extension; .95 for elbow extension; and .96 for elbow flexion during the third, fourth, and fifth measurement of the Biodex System. The researchers found ICC to be highly reliable one week after the last set of isokinetic testing. The ICC reliability were: .91 for knee flexion; .89 for knee extension; .93 for elbow extension; and .97 for elbow flexion. Evidence showed high reliability using the Biodex System III Pro dynamometer compared to Lido Multijoint II system (Lund, Sondergaard, Zachariassen, Christensen, Bulow, & Henriksen., 2005).

Porter & Kaminski (2004) investigated different knee angles during peak torque concentric eversion-inversion on a Kin Com 125 AP isokinetic dynamometer. During this investigation, there were concerns of gastrocnemius and plantaris muscles activating movements at the talocrural joint with the knee in near extension position. Activation of movement at two joints in the ankle will produce supination. Supination occurs when the ankle-foot complex performs inversion, adduction, and plantar flexion. Researchers investigated the knee at 15°, 30°, and 45° of knee flexion while the foot was in 10° plantar flexion while performing concentric eversion-inversion movements on the isokinetic dynamometer. The contralateral leg served as a control group. Eversion-inversion was performed for three repetitions at 30 deg/sec and 120 deg/sec. Results showed no significant differences between knee angles during either velocity. Eversion concentric and inversion concentric strength ratios during the 30 deg/sec ranged between .87-1.02, and during 120 deg/sec between .86-1.00. Different knee angles during eversion-inversion isokinetic exercises have no substantial influence in changes of torque outcome (Porter & Kaminski, 2004).
CHAPTER 3: METHODS

Research Design

A 2 x 2 x 2 repeated measures factorial design was used in this study. The dependent variables were the H:M_{max}, isokinetic eversion and inversion torque. Independent variables were the 1) condition (FAI compared to healthy subjects), 2) intervention (30-minute ice bag application compared to a 30-minute rest session), 3) time (data collected before and after the intervention). Conditions were assigned randomly to control for learning effects (see Table 1).

Table 1

\textit{Research Design Timeline}

\begin{tabular}{lll}
Baseline & Intervention (30 Minutes) & Posttest \\
H:M_{max} & Ice Bag Application & H:M_{max} \\
\textit{OR} & & \\
Eversion and Inversion Torque & Rest & Eversion and Inversion Torque \\
1 & 2 & 3
\end{tabular}
Null Hypothesis

H$_{01}$: Cooling has no significant effect on the soleus, peroneus longus, and tibialis anterior H-reflex.

H$_{02}$: Cooling has no significant effect on eversion and inversion isokinetic torque.

H$_{03}$: There will be no significant difference in the soleus, peroneus longus, and tibialis anterior H-reflex between the functionally unstable and healthy subjects following ankle joint cooling.

H$_{04}$: There will be no significant difference in the eversion and inversion isokinetic torque between the functionally unstable and healthy subjects following ankle joint cooling.

Operational Definitions

Ankle Instability Instrument (AII): Is a reliable instrument to classify FAI. The AII has nine questions, two questions were the minimum qualifier for FAI: have you ever sprained an ankle; have you ever experienced a sensation of your ankle “giving way” (Docherty, Gansneder, Arnold, & Hurwitz, 2006).

Eversion torque: Angular velocity for eversion foot motion at 60 deg/sec taken following a bout of three sets of five repetitions (Aydog, Aydog, Cakci, & Doral, 2004).

Ice bag application: A cubed ice bag applied to the anteriolateral portion of the ankle joint (Hopkins & Stencil, 2002; Hopkins et al., 2002; Krause et al., 2000).

Inversion torque: Angular velocity for inversion foot motion at 60 deg/sec taken following a bout of three sets of five repetitions (Aydog, Aydog, Cakci, & Doral, 2004).
Peak torque: The maximum angular velocity for eversion and inversion at 60 deg/sec taken following a bout of three sets of five repetitions (Aydog, Aydog, Cakci, & Doral, 2004).

Rest session: A 30-minute control rest session where the subject will remain seated in the BIODEX chair (Hopkins & Stencil, 2002).

Subjects

Twenty-four male and female college aged subjects volunteered. The FAI group had three males and nine females with a mean age of 23 (SD = 1.1) years, mean body mass of 73.4 (SD = 20.0) kg, mean height of 168.7 (SD = 9.8) cm. The healthy subject group had five males and seven females with a mean age of 23 (SD = 1.1) years, mean mass of 77.9 (SD = 14.9) kg, mean height of 171.7 (SD = 7.0) cm. Volunteers were neurologically sound, physically active Ohio University students. The focus for recruitment of subjects was individuals with a chronic sensation of their ankle giving way. AII determined if subjects had FAI (see Appendix C). The AII has nine questions; two questions were the minimum qualifier for FAI: have you ever sprained an ankle; have you ever experienced a sensation of your ankle “giving way.” The AII has been observed recently to be a reliable measure of FAI with an intraclass correlation coefficient of .95 (Docherty et al., 2006). Healthy subjects were recruited based on no history previous ankle injury. Each subject signed an IRB approved informed consent prior to participation. This study was approved by the Ohio University Committee on Human Subjects Research.
**Instrumentation**

*H-reflex.* Instruments used to elicit and record the H:M\textsubscript{max} included electromyography (MP150, BIOPAC Systems, Inc., Santa Barbara, CA) and Acqknowledge 3.8 software (Palo Alto, CA). The stimulator module (STM 100C, BIOPAC System Inc., Santa Barbara, CA) with a 200-V maximum stimulus isolation adaptor applied a 1 ms stimulus to the sciatic nerve behind the knee. The stimulus was delivered through an isolation adaptor (STMISOC, BIOPAC System, Inc., Santa Barbara, CA) and a shielded bar electrode (EL503, BIOPAC System, Inc., Santa Barbara, CA). The EMG electrodes were pre-gelled single Ag-AgCl electrodes (see Figure 3).

![Figure 3. Computer and the BIOPAC.](image)

*Isokinetic peak torque.* The Biodex System III Pro (Biodex Systems, Inc., Shirley, NY) was used to measure eversion torque and inversion torque of the peroneus longus and tibialis anterior muscles (see Figure 6).
**Cryotherapy.** Cold treatment included one 1.5 L crushed ice bag. Research has provided evidence a 30-minute treatment is safe and appropriate (Hopkins, 2006; Hopkins & Stencil, 2002; Hopkins et al., 2002). Cold application is becoming a recommended treatment to facilitate musculature to promote functional capabilities in musculoskeletal rehabilitation (Hopkins & Stencil, 2002; Hopkins et al., 2002; Krause et al., 2000).

**Ankle Instability Instrument (AII).** The AII questionnaire was used to determine inclusion of subjects with FAI (Docherty et al., 2006). If subjects answered yes to question 1 and 4 of the AII they were included in the FAI group (see Appendix C).

**Procedures**

Subjects completed an ankle instability instrument, physical activity readiness questionnaire, and a health questionnaire to document their past history (see Appendix C, E, & F). An orientation session in the laboratory briefed subjects on the study’s methods and allowed them to read, understand, and provide their informed consent. Data collected from healthy subjects came from the individual’s dominant leg, determined by the leg used to kick a ball.

Each subject’s skin was prepared at the site of electrode placement. Preparation of the skin consisted of shaving, abrading with fine sandpaper, and cleaning with alcohol prep pads. Soleus EMG electrodes were placed 2 cm below the medial gastrocnemius and in the midline of the muscle belly. Peroneus longus electrodes were placed 2 cm distal to the fibula head and located in the center of the width of the muscle belly. Tibialis anterior electrodes were placed on the center midpoint of the muscle belly. Two electrodes for
each muscle followed the line of muscle fibers and had an interelectrode distance of 2 cm. A reference electrode was affixed to the tibial tuberosity. A bipolar, bar stimulating electrode was placed in superior-medial popliteal space over the sciatic nerve (see Figure 4).

![Figure 4. Hoffmann reflex electrode placement.](image)

Subjects were positioned on the BIODEX during the collection of H:M\text{max} data. The BIODEX setup involved the chair adjusted to 125°, with the subjects secured to the chair at the chest, waist, and leg not tested. To prevent muscle substitution during the peak torque production the lower leg was supported just superior to the knee joint at a 60° angle and the foot stabilized in a neutral position strapped to the footplate. Subjects relaxed with their eyes open while listening to wave sounds through headphones. Adhesive tape held each electrode and stimulating electrode in place (see Figure 5). The investigators found the peak-to-peak H-reflex and muscle response amplitude by trial and error. The 1 ms stimulus was increased in increments of .2 - .5 mA starting with 1 mA to
identify the peak-peak H-reflex amplitude and the peak-peak muscle response amplitude used to compute the $H:M_{\text{max}}$. Once maximum peak-to-peak amplitudes were observed three trials were recorded for the maximum H-reflex and three trials for the maximum muscle response.

Figure 5. Subject setup in the BIODEX.

The BIODEX measured the eversion torque, and inversion torque of the ankle musculature. Subjects remained positioned in a supported position on the BIODEX. The investigator recorded start and stop angles of eversion-inversion full range of motion. Subjects performed five warm-ups at a submaximal level of 120 deg/sec before three sets of five repetitions were collected at 60 deg/sec. Subjects received a one-minute rest between each set to reduce muscle fatigue. Subjects only received instructions prior to isokinetic dynamometer use. Verbal encouragement was not used during the study.
The intervention following the baseline $H:M_{\text{max}}$, eversion torque, and inversion torque measurements included the application of a 1.5 L cubed ice bag over the anteriolateral ankle joint of the tested leg (see Figure 6). Thirty-minutes of cold application was applied before post measurements of $H:M_{\text{max}}$, eversion torque, and inversion torque in both groups. During the control session, subjects rested for 30 minutes in the BIODEX chair. Immediately following cold application or control session, subjects were tested for post $H:M_{\text{max}}$, eversion torque, and inversion torque measurements.

*Figure 6.* Cubed ice bag was applied to the limb being tested for 30 minutes.

**Statistical Analysis**

Soleus, peroneus longus, and tibialis anterior H-reflexes were analyzed as a percent change from the baseline, pre-treatment to post-treatment measurements. Eversion and inversion isokinetic torques were analyzed as a percent change from the
baseline, pre-treatment to post-treatment measurements. Research question 1 was analyzed using a multivariate analysis of variance (MANOVA) comparing the effects of cooling (treatment) on the percent change from baseline in the soleus, peroneus longus, tibialis anterior H:M_max. Research question 2 was analyzed using a MANOVA comparing the effects of cooling (treatment) on the percent change in eversion and inversion torque. In research question 3, a two-way MANOVA was used to compare the effects of cooling on the percent change from baseline soleus, peroneus longus, tibialis anterior H:M_max in subjects with FAI. In research question 4, a two-way MANOVA was used to compare the effects of cooling on the percent change from baseline eversion and inversion torques in subjects with FAI. The SPSS 15.0 (SPSS Inc., Chicago, IL) was used to analyze the data in this study. A probability level of $p < .05$ was established *a priori*. 
CHAPTER 4: RESULTS

The means and standard deviations of the raw H-reflex and isokinetic torque data are presented in Table 2. For statistical analysis, the H-reflexes and isokinetic torques are represented as the percent change in those variables between the baseline, pre-treatment and post-treatment measurements.

**Research Question 1**

Does cooling the ankle joint affect the soleus, peroneus longus, and tibialis anterior H-reflex? The one-way MANOVA showed a significant percentage change from baseline to posttreatment in ankle cooling ($F(3,44) = 3.34, p = .03, 1-\beta = .72, \eta^2 = .19$).

The univariate ANOVA revealed a significant increase in the soleus H: $M_{max}$ ($F(1,46) = 7.11, p = .01, 1-\beta = .23, \eta^2 = .95$) and peroneus longus H: $M_{max}$ ($F(1,46) = 4.97, p = .03, 1-\beta = .13, \eta^2 = .74$) from baseline following ankle cooling. However, the tibialis anterior H: $M_{max}$ ($F(1,46) = 1.91, p = .17, 1-\beta = .09, \eta^2 = .55$) showed no significant change from baseline following ankle cooling (see Figure 7).

![Figure 7](image)

*Figure 7.* Soleus, peroneus longus, and tibialis anterior H: $M_{max}$ percentage changes from baseline between treatments.
Research Question 2

Does cooling the ankle joint change the eversion and inversion isokinetic torque?

The one-way MANOVA showed a significant percentage change from baseline to posttreatment in ankle cooling (F(2,45) = 3.60, \( p = .04, 1-\beta = .14, \eta^2 = .64 \)). The univariate ANOVA revealed a significant decrease in the eversion torque (F(1,46) = 5.56, \( p = .02, 1-\beta = .11, \eta^2 = .64 \)) from baseline following ankle cooling. However, inversion torque (F(1,46) = .11, \( p = .74, 1-\beta = .00, \eta^2 = .06 \)) showed no significant change from baseline following ankle cooling (see Figure 8).

Figure 8. Eversion and inversion torque percentage changes from baseline between treatments.
Research Question 3

Do the soleus, peroneus longus, and tibialis anterior H-reflex differ between functionally unstable and healthy subjects following a 30-minute ankle joint cooling? The two-way MANOVA showed no statistically significant percentage change in the H-reflex between the FAI and healthy groups following ankle cooling (F(3,42) = 1.16, p = .34, 1-β = .08, η² = .29). This indicates there is no interaction effect between the cold application treatment and ankle instability on the soleus, peroneus longus, and tibialis anterior H-reflexes (see Figure 9).

Figure 9. Soleus, peroneus longus, and tibialis anterior H:M_max percentage changes from baseline following ankle cooling between groups.
Research Question 4

Do eversion and inversion isokinetic torque differ between functionally unstable and healthy subjects following a 30-minute ankle joint cooling? A two-way MANOVA showed no statistically significant percentage change from baseline to post-treatment in ankle cooling between groups ($F(2,43) = .55$, $p = .58$, $1-\beta = .03$, $\eta^2 = .14$). This indicates there is no interaction effect between the cold application treatment and ankle instability on eversion and inversion isokinetic torque (see Figure 10).

Figure 10. Eversion and inversion torque percentage changes from baseline following ankle cooling between groups.
### Table 2

**Raw Means and Standard Deviations**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ice Bag Application Session</th>
<th>Rest Session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
</tr>
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<td>.22</td>
</tr>
<tr>
<td>Healthy</td>
<td>.67</td>
<td>.23</td>
</tr>
<tr>
<td>Both</td>
<td>.64</td>
<td>.22</td>
</tr>
<tr>
<td>Peroneus Longus</td>
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<td></td>
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<tr>
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</tr>
<tr>
<td>Healthy</td>
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<td>.10</td>
</tr>
<tr>
<td>Both</td>
<td>.21</td>
<td>.10</td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAI</td>
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<td>.23</td>
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<td>Healthy</td>
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<td>.17</td>
</tr>
<tr>
<td>Both</td>
<td>.24</td>
<td>.20</td>
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<tr>
<td>Peak Eversion Torque</td>
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<td></td>
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<tr>
<td>FAI</td>
<td>13.94</td>
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<td>6.84</td>
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<tr>
<td>Both</td>
<td>16.81</td>
<td>6.53</td>
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<tr>
<td>Peak Inversion Torque</td>
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<td></td>
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<tr>
<td>FAI</td>
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</tr>
<tr>
<td>Healthy</td>
<td>16.98</td>
<td>5.25</td>
</tr>
<tr>
<td>Both</td>
<td>15.87</td>
<td>5.25</td>
</tr>
</tbody>
</table>

*Note. M = Mean, SD = Standard Deviation, FAI = functional ankle instability, Healthy = healthy ankles, *p < .05.*
CHAPTER 5: DISCUSSION

This thesis attempted to investigate the effects of cooling the motoneuron pool recruitment of leg muscles in a pathologic population. Few studies have investigated neuromuscular changes of focal joint cooling in injured subjects. The results showed that the soleus $H:M_{\text{max}}$ ($p < .05$) and peroneus longus ($p < .05$) were facilitated following ice application when FAI and healthy ankles were included as one group. This evidence supports the concept that the soleus and peroneus longus have a heightened motoneuron pool activation following ankle joint ice bag application in subjects with FAI and normal healthy ankles. These data confirm a previous observation that cooling the ankle joint for 30 minutes facilitates the soleus muscle (Hopkins and Stencil, 2002). The motoneuron pool changes observed over a 90-minute period showed that the $H:M_{\text{max}}$ in healthy subjects increased following cooling compared to a control session (Hopkins & Stencil, 2002). The current data indicate that the change in the peroneus longus $H:M_{\text{max}}$ was statistically significant following a 30-minute ice bag application ($p < .05$) when FAI and healthy ankle were collapsed as one sample group. These data agree with a previous observation that cooling the ankle joint for 30 minutes facilitates the peroneus longus muscle (Krause, 2004). The peroneus longus and eversion torque both increased immediately after and 30 minutes following a 30-minute ankle ice bag application (Krause, 2004). This mounting evidence indicates that motoneuron pool activation increases following ankle joint cooling. These data may explain increases in functional abilities following cryokinetics, a common musculoskeletal rehabilitation intervention that combines cold application with active exercise.
AMR is an ongoing reflex response of musculature following damage to or distension of structures that surround a joint (Hopkins & Ingersoll, 2000; Hopkins & Stencil, 2002; Hopkins et al., 2002; McVey et al., 2005; Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004). This phenomenon has been observed in experimentally induced joint effusion. When effusion is present, motoneuron pool recruitment may be facilitated or inhibited (Hopkins & Palmieri, 2004; Palmieri, Ingersoll, Edwards, et al., 2003; Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004; Palmieri, Weltman, et al., 2005).

Following experimentally induced ankle joint effusion, the soleus, peroneus longus, and tibialis anterior were facilitated following the injection. This experimentally induced effusion simulates joint swelling seen in the acute stages of a lateral ankle sprain (Palmieri, Ingersoll, Hoffman, Cordova, et al., 2004). A single ankle sprain may lead to inhibition of the lower leg muscles and may cause recurrent ankle sprains. McVey et al. (2005) observed inhibition of the soleus and peroneus longus muscle in functional unstable subjects (McVey et al., 2005). Recurrent ankle sprains occurred because of an ankle instability deficit as a result of ankle joint ligament laxity, neuromuscular responses, or a combination of both. In this thesis, the effects of ice bag application on individuals with FAI were measured. An increase in motoneuron pool recruitment was observed in the soleus and peroneus longus after cooling the ankle for 30 minutes in individuals with FAI.

**Ankle Instability**

When investigating injuries or functional instability of the ankle, inhibition or deficits to the peroneus longus muscle is expected (Hartsell & Spaulding, 1999; Hertel,
2000, 2002; Kaminski, 1999; Konradsen & Ravn, 1991; McKnight & Armstrong, 1997; McVey et al., 2005; Vaes et al., 2002; Willems et al., 2002). When clinicians evaluate and develop the prognosis of ankle pathologies, the soleus muscle is not the focus. Researchers have established that ankle instability is either mechanical, functional, or a combination of both. Mechanical instability is a change in pathologic laxity, impaired arthrokinematics, synovial changes, or development of degenerative joint disease seen in an individual with one or more injuries to the ankle (Hertel, 2002). This thesis investigated individuals who may have functional instability. In functional instability, an individual may have impaired or decreased: proprioception, cutaneous sensation, neuromuscular response time, strength, and/or postural control deficits. The peroneus longus and brevis muscles are expected to have impaired neuromuscular firing patterns leading to a delayed response when the ankle excessively inverts (Hertel, 2000, 2002).

There are limited studies comparing FAI to normal healthy ankle subjects measuring motoneuron pool recruitment. One study to date observed inhibition of the soleus, peroneus longus, and tibialis anterior compared to the contralateral limb. In the unstable ankles the soleus and peroneus longus muscles were inhibited (McVey et al., 2005). This thesis did not compare the H:M_{max} bilaterally to determine whether the individuals had neuromuscular deficits, which was a limitation. This thesis investigated how focal cooling the ankle joint affected the motoneuron recruitment in musculature around the ankle joint, and how it would differ between FAI and normal healthy ankle subjects. The results showed a significant increase in the soleus (p < .05) and peroneus
longus H:\M_{\text{max}} (p < .05) in response to ankle cooling, but no difference was observed between the FAI and healthy groups ($p > .05$).

**Eversion and Inversion Torque**

The eversion ($p > .05$) and inversion torque ($p > .05$) did not change between groups following ankle cooling during my study. McKnight and Armstrong (1997) tested the isokinetic torque of subjects with FAI. The authors did not observe differences in isokinetic torque between FAI and healthy subjects (McKnight & Armstrong, 1997). Kaminski et al. (1999) found no significant difference in eccentric, concentric, and isometric eversion torque between individuals with stable and unstable ankles (Kaminski et al., 1999).

Other investigators found significant differences between groups with different ankle torque movements (Amaral De Noronha & Borges, 2004; Hartsell & Spaulding, 1999; Munn et al., 2003; Willem et al., 2002). Amaral De Noronha and Borges (2004) investigated the reliability of subjects with recurrent ankle sprains having a deficit in eversion and inversion torque. Their subjects performed two sets of five maximal contractions at 30 deg/sec and 120 deg/sec with the contralateral limb serving as a control. The intraclass correlation coefficients for eversion and inversion were between .71 to .92 for the injured ankle and .90 to .95 for the non-injured ankle (Amaral De Noronha & Borges, 2004). Munn et al. (2003) used isokinetic dynamometry to determine whether subjects with FAI had deficits in eversion and inversion torque. This demonstrates an inhibition of the dynamic ankle stabilizers, decreasing the ability to control lateral postural sway during unstable weight bearing activities (Munn et al.,
Hartsell and Spaulding (1999) investigated eccentric/concentric ratios of individuals with ankle instability. Subjects with CAI were significantly weaker during eccentric/concentric eversion and inversion compared to stable ankle subjects (Hartsell & Spaulding, 1999). In contrast, my data did not detect significant weakness in eversion or inversion torque. Eversion and inversion torque may not be the limiting factor in individuals with FAI. Decreased eccentric plantar flexion torque has been observed in individuals with FAI, suggesting there may be a link between individuals having unstable ankles and weakness or decreased control with responses in the plantar flexors (Fox, Docherty, Schrader, & Applegate, 2008).

This thesis data demonstrate a significant decrease in the eversion torque ($p < .05$) when data were collapsed across FAI and normal healthy ankle subjects following cooling. Hopkins & Stencil (2002) observed increased plantar flexion torque up to 60 minutes after ice application (Hopkins and Stencil, 2002). Kimura et al. (1997) used cold application to test the effects on isokinetic eccentric plantar flexion torque at 30 deg/sec and 120 deg/sec. The cryotherapy group immersed their limb to mid-thigh in 10°C ice water for 30 minutes. Evidence showed an increase in plantar flexion torque during an eccentric contraction at 120 deg/sec (Kimura et al, 1997). Hatzel and Kaminski (2000) studied effects of ice water immersion up to the tibia plateau on ankle plantar flexion, dorsiflexion, inversion, and eversion concentric and eccentric peak torque. Concentric dorsiflexion decreased immediately following ice immersion. Measurements of eccentric peak torque were higher than concentric measurements pre and post ice immersion. Testing the ankle in multiple directions provided evidence that directly cooling the joint
does not hinder performance of dynamic musculature, but that no increase muscle activity occurs post treatment (Hatzel & Kaminski, 2000).

**Relationship between Motoneuron Pool Recruitment and Voluntary Excitability**

A previous study observed a weak correlation ($r = .38, r^2 = .15$) between the soleus H:M ratio and plantar flexion torque (Hopkins & Stencil, 2002). In this thesis, a weak correlation in FAI subjects between peroneus longus H:M$_{\text{max}}$ and eversion torque ($r = .16, r^2 = .03$) following ankle joint cooling and a rest session. There was also a weak correlation in both groups following ankle cooling ($r = .19, r^2 = .44$). In addition, a weak correlation in FAI subjects between the tibialis anterior H:M$_{\text{max}}$ and inversion torque ($r = -.36, r^2 = .60$) following cooling the ankle joint and the rest intervention. There was also a weak correlation in both groups following ankle cooling ($r = -.27, r^2 = .52$). This thesis data support previous reports that the H-reflex and isokinetic torque are not correlated. The H-reflex does not predict muscle force production; rather it estimates a muscle’s potential to maximally recruit motoneurons (Palmieri, Ingersoll, & Hoffmann, 2004).

Investigators have theorized that interventions increasing the H:M$_{\text{max}}$ would overcome AMI during rehabilitation or activity (Hopkins & Stencil, 2002). Testing the muscle in a controlled isokinetic movement only approximates functional tasks or speeds that a physically active individual might produce. Future investigation of how cryokinetics affect involuntary motoneuron recruitment is warranted.

**Limitations**

A limitation of this study is that the soleus, peroneus longus and tibialis anterior H-reflex were recorded simultaneously. The H-reflex is a particularly sensitive
measurement. The exact peak of each muscle might not have been found during the maximum H-reflex (H max) and maximum muscle response (M max) collection. Another unavoidable limitation was the Biodex System III Pro location during data collection. The Sports Medicine facility in which it is located produced electrical noise, which may have interfered with the BIOPAC System during H-reflex measurements. The unwanted 60 Hz electrical noise included, but not limited to: the ventilation unit, ice machine, Biodex System III Pro, fluorescent lighting and various therapeutic equipment. All appropriate measures were taken to limit this electrical noise.

The electrode placements were crucial when testing the peroneus longus and tibialis anterior muscles. The possibility of cross talk (electromyography signals from adjacent muscles) occurred between the muscles in the anterior, lateral and superficial posterior compartments of the leg. The peroneus longus and tibialis anterior, both small muscles, are more difficult to capture a clean H-reflex than the soleus. This potential for greater variability in the peroneus longus and tibialis anterior likely contributed to the smaller observed power and effect size for these two muscles.

When recruiting volunteers, the All was used to determine if they had FAI or not. Decisions were based on the subjective information subjects provided. No physical examination was used to determine FAI status. The assumption was that subjects who volunteered and participated in the FAI group were truthful in their answers and were in fact functionally unstable.
Recommendations

Further research is warranted to investigate if the soleus may have a more substantial role in preventing lateral ankle sprains or protecting the ankle from recurrent ankle sprains. In artificially induced ankle effusion, the soleus, tibialis anterior and the peroneus longus muscles are facilitated. This indicates a protective mechanism of these muscles initially following ankle injury (Palmieri, Ingersoll, Hoffman, Cordova, et al. 2004). In subjects with demonstrated ankle instability due to chronic, recurrent injury, these muscles are inhibited (McVey et al., 2005). A better understanding of the neuromuscular adaptations that occur between an initial ankle injury and the development of ankle instability would allow clinicians to make better clinical decisions in the management of acute and chronic ankle injury. Typically, the peroneus longus is targeted when investigating the effects of subjects with ankle pathology. The peroneus longus muscle responds to excessive inversion and ankle instability to protect the joint from further damage. However, the soleus plays a crucial role in posture and maintaining balance (Hertel, 2000, 2002). Further investigation is needed to study the role of the soleus during sudden, excessive inversion and what are the long-term disabilities to the patient.

In this thesis, the effects of cold application on controlled ankle movements. A similar study observed increases in plantar flexion isokinetic torque (Hopkins & Stencil, 2002). Soleus muscle facilitation has been observed up to 60 minutes after ankle joint cold application (Hopkins & Stencil, 2002). Future studies should focus attention on the changes in functional task completion following ankle joint cold application. This will
assist the sports medicine community understand the effects of overcoming inhibition during more clinical situations.

The results showed facilitation to the ankle musculature motoneuron pool recruitment following a single ice application to the ankle joint (Hopkins & Stencil, 2002; Hopkins et al., 2002; Krause et al., 2000; Krause, 2004). Further exploration is needed to discover what changes occur to the musculature in the lower limb following therapeutic intervention, including focal cold application. By experimentally observing the effects of different therapeutic interventions, health care professions will make evidence-based decisions and will improve patient care.

An additional suggestion for future research is to identify different therapeutic interventions that will increase the motoneuron pool recruitment. So far, ice bag application to the ankle joint is the most significant intervention that has increased motoneuron pool recruitment (Hopkins & Stencil, 2002; Krause et al., 2000; Krause, 2004). Transcutaneous Electric Neuromuscular Stimulation (TENS) around the knee joint brought the inhibition induced effused knee joint back to the motoneuron excitability level obtained prior to the effusion but did not facilitate the musculature (Hopkins et al., 2002). More therapeutic interventions would increase the options available to health care professionals who treat athletes.

The clinical relevance of this study is the use of cold application on individuals who have functionally unstable ankles, which will aid in the disinhibiting of the soleus motoneuron pool recruitment. Disinhibiting the lower leg muscles will aid in more efficient rehabilitation in the clinical setting. This provides further evidence that the use
of cryokinetics will enhance rehabilitation for an athlete. In addition, the use of cryotherapy prior to activity is safe and may elicit better performance from the muscles, especially the soleus.

As clinicians we seek to better the health of our patient whether it is an acute or a chronic injury. We see inhibitions within the adjacent muscles around the joint. The muscles may either have a decreased feedback response or a strength deficit (Hopkins & Ingersoll, 2000). Knowing if the patient has a chronic instability, a reflexive disability, or a strength deficit will allow the clinicians to choose treatment options more efficiently. Knowing both the treatment and necessary rehabilitation for the musculature around the joint will enhance our profession as health care providers.

**Conclusions**

The use of cryotherapy applied over the joint prior to rehabilitation or activity is appropriate and can enhance the performance of the muscles around the particular joint. This facilitation is observed both in healthy and pathological populations (Hopkins & Stencil, 2002; Hopkins et al., 2002; Krause et al., 2000; Krause, 2004; McVey et al., 2005). The results showed in this thesis a facilitation in the soleus and peroneus longus H:M_max following ankle ice application in subjects with FAI. This supports the growing body of literature that has reported increases in motoneuron pool excitability following focal joint cooling (Hopkins et al., 2000; Hopkins & Stencil, 2002; Krause et al., 2000, Krause, 2004). Additional research should focus on what exercises in conjunction with cold application to the joint will further disinhibit the musculature. A single therapeutic modality applied to the joint will not disinhibit the musculature over time. In addition,
individuals with a recurrent ankle sprain or sensation of their ankle giving way may have a mechanical or functional instability. The functional instability may not only involve the peroneus longus muscle but also the soleus muscle. Soleus facilitation, in lieu of increases in peroneus longus or tibialis anterior excitability or force production, may indicate that the soleus plays a greater role in dynamic ankle stability than previously reported.
REFERENCES


## APPENDIX A: KNEE ARTHROGENIC MUSCLE INHIBITION

<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
<th>Year</th>
<th>Purpose of Investigation</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hopkins</td>
<td>Knee joint effusion and cryotherapy alter lower chain kinetics and muscle activity</td>
<td>2006</td>
<td>To quantify muscle recruitment changes and knee joint function following joint effusion and subsequent joint cryotherapy.</td>
<td>Joint cryotherapy negated movement deficiencies represented by knee peak torque and power decreases due to facilitated vastus lateralis activation relative to other groups.</td>
</tr>
<tr>
<td>Palmieri et al.</td>
<td>Pre-synaptic modulation of quadriceps arthrogenic muscle inhibition</td>
<td>2005</td>
<td>To determine if quadriceps AMI is mediated by a presynaptic regulatory mechanism</td>
<td>The percent of the unconditioned reflex amplitude for reflex activation history and the maximum Hoffmann reflex were decreased at 25 and 45 min post effusion. Quadriceps AMI is, at least in part, mediated by a presynaptic mechanism.</td>
</tr>
<tr>
<td>Palmieri et al.</td>
<td>Arthrogenic muscle response induced by an experimental knee joint effusion is mediated by pre- and post-synaptic spinal mechanisms</td>
<td>2004</td>
<td>Determine if soleus arthrogenic muscle response is regulated by pre- or post-synaptic spinal mechanisms.</td>
<td>Reflex activation history significantly increased from baseline at 25 and 45 min post-effusion. Soleus arthrogenic muscle response seen following knee joint effusion is mediated by both pre- and post-synaptic mechanisms.</td>
</tr>
<tr>
<td>Palmieri et al.</td>
<td>Arthrogenic muscle inhibition is not present in the limb contralateral to a simulated knee joint effusion</td>
<td>2003</td>
<td>Estimate bilateral neuromuscular activity in the vastus medialis on induction of a unilateral knee joint effusion.</td>
<td>Knee joint effusion results in ipsilateral but not contralateral impairment of quadriceps function.</td>
</tr>
<tr>
<td>Palmieri et al.</td>
<td>The effect of a simulated knee joint effusion on postural control in healthy subjects</td>
<td>2003</td>
<td>To determine the effects of a simulated knee joint effusion on center of pressure path and mean power frequency during standing.</td>
<td>Knee effusion resulted in increased postural control. Possible explanations for the improved postural control following the effusion include additional somatosensory feedback, an augmented neural drive to the soleus, and/or increased capsular tension</td>
</tr>
<tr>
<td>Hopkins et al.</td>
<td>Cryotherapy and transcutaneous electric neuromuscular stimulation decrease arthrogenic muscle inhibition of the vastus medialis following knee joint effusion</td>
<td>2002</td>
<td>To verify the vastus medialis is inhibited using the knee effusion model and investigate the effects of cryotherapy and TENS on AMI.</td>
<td>Cryotherapy and TENS both disinhibit the quadriceps following knee joint effusion, and cryotherapy further facilitates the quadriceps motoneuron pool. Cryotherapy treatment resulted in facilitation of the VM motoneuron pool during the post-treatment phase. The TENS treatment failed to disinhibit the VM motoneuron pool by 30 minutes postinjection.</td>
</tr>
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## APPENDIX B: ANKLE ARTHROGENIC MUSCLE RESPONSE

<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
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<th>Purpose of Investigation</th>
<th>Conclusion</th>
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<tr>
<td>McVey et al.</td>
<td>Arthrogenic muscle inhibition in the leg muscles of subjects exhibiting functional ankle instability</td>
<td>2005</td>
<td>To determine if arthrogenic muscle inhibition is present in the ankle joint musculature of patients exhibiting unilateral functional ankle instability.</td>
<td>Depressed H:M ratios in the injured limb suggest arthrogenic muscle inhibition is present in the ankle musculature of patients exhibiting functional ankle instability.</td>
</tr>
<tr>
<td>Hopkins et al.</td>
<td>Effects of ankle joint effusion on lower leg function</td>
<td>2004</td>
<td>To quantify muscle activation in the peroneal, tibialis anterior, and soleus musculature as well as to determine ankle joint peak torque, peak power, and root mean square power during a closed kinetic chain activity following artificial ankle effusion.</td>
<td>Decreases in ankle plantar flexion torque and plantar flexion EMG indicate a neuromuscular deficit exists in the presence of edema could increase the susceptibility for further ankle injury.</td>
</tr>
<tr>
<td>Palmieri et al.</td>
<td>Arthrogenic muscle response to a simulated ankle joint effusion</td>
<td>2004</td>
<td>To determine if AMI is present in the soleus, peroneus longus, and tibialis anterior musculature following a simulated ankle joint effusion.</td>
<td>Simulated ankle joint effusion results in facilitation of the soleus, peroneus longus, and tibialis anterior motoneuron pools.</td>
</tr>
<tr>
<td>Krause et al.</td>
<td>Ankle cryotherapy facilitates peroneus longus motoneuron activity</td>
<td>2004</td>
<td>Examine the effects of ankle cryotherapy on resting motor function and eversion torque in the peroneus longus muscle.</td>
<td>Hoffmann reflexes and eversion peak torque were higher immediately and 30 minutes post cryotherapy relative to baseline measurements.</td>
</tr>
<tr>
<td>Hopkins et al.</td>
<td>Ankle cryotherapy facilitates soleus function</td>
<td>2002</td>
<td>To determine the effects of ankle cryotherapy on voluntary and resting motor function of the soleus over a 60-minute period. To determine if a relationship exists between changes in torque production and Hoffmann reflex following ankle joint cryotherapy treatment.</td>
<td>Both peak Hoffmann reflex and plantar flexion torque at 30, 60, and 90 minutes increased relative to baseline measurements. Each measurement was also greater than the corresponding control at 30, 60, and 90 minutes. The soleus motoneuron pool is facilitated following a 30-minute crushed ice application to the ankle and over a 60-minute post-cooling period.</td>
</tr>
<tr>
<td>Hopkins et al.</td>
<td>Effect of knee joint effusion on quadriceps and soleus motoneuron pool excitability</td>
<td>2001</td>
<td>Examine changes in quadriceps and soleus MN pool activity resulting from knee joint effusion over a 4-h period and assess the relationship between the muscles.</td>
<td>Facilitation of the soleus during knee effusion may be a compensatory response to quadriceps inhibition, possibly to facilitate ambulation. Furthermore, a moderate inverse correlation exists between these two muscles following knee effusion.</td>
</tr>
<tr>
<td>Hall et al.</td>
<td>Relationship between ankle invertors Hoffmann reflexes and acute swelling induced by inversion ankle sprain</td>
<td>1999</td>
<td>To determine relationships between ankle swelling and flexor digitorum longus and peroneus longus Hoffmann reflex amplitude and latency. The relationship between ankle swelling and invertor or evertor Hoffmann reflexes has not been reported.</td>
<td>There were no significant differences in Hoffmann reflex amplitude and peroneus longus latency between ankles. Grade I or II inversion sprains and the related swelling appear to delay involved ankle flexor digitorum longus latency to a greater extent than peroneus longus latency.</td>
</tr>
</tbody>
</table>
## APPENDIX C: ANKLE INSTABILITY INSTRUMENT (AII)

<table>
<thead>
<tr>
<th>Ankle Instability Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instructions</td>
</tr>
<tr>
<td>This form will be used to categorize your ankle instability. A separate form should be used for the right and left ankles. Please fill out the form completely. If you have any questions, please ask the administrator of the survey. Thank you for your participation.</td>
</tr>
<tr>
<td>1. Have you ever sprained an ankle? □ Yes □ No</td>
</tr>
<tr>
<td>2. Have you ever seen a doctor for an ankle sprain? □ Yes □ No</td>
</tr>
<tr>
<td>If yes, 2a. How did the doctor categorize your most serious ankle sprain? □ Mild (grade 1) □ Moderate (grade 2) □ Severe (grade 3)</td>
</tr>
<tr>
<td>3. Did you ever use a device (such as crutches) because you could not bear weight due to an ankle sprain? □ Yes □ No</td>
</tr>
<tr>
<td>If yes, 3a. In the most serious case, how long did you need to use the device? □ 1–3 days □ 4–7 days □ 1–2 weeks □ 2–3 weeks □ &gt;3 weeks</td>
</tr>
<tr>
<td>4. Have you ever experienced a sensation of your ankle “giving way”? □ Yes □ No</td>
</tr>
<tr>
<td>If yes, 4a. When was the last time your ankle “gave way”? □ &lt;1 month □ 1–8 months ago □ 6–12 months ago □ 1–2 years ago □ &gt;2 years</td>
</tr>
<tr>
<td>5. Does your ankle ever feel unstable while walking on a flat surface? □ Yes □ No</td>
</tr>
<tr>
<td>6. Does your ankle ever feel unstable while walking on uneven ground? □ Yes □ No</td>
</tr>
<tr>
<td>7. Does your ankle ever feel unstable during recreational or sport activity? □ Yes □ No □ N/A</td>
</tr>
<tr>
<td>8. Does your ankle ever feel unstable while going up stairs? □ Yes □ No</td>
</tr>
<tr>
<td>9. Does your ankle ever feel unstable while going down stairs? □ Yes □ No</td>
</tr>
</tbody>
</table>

APPENDIX D: PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

Physical Activity Readiness Questionnaire
PAR-Q

For most people physical activity should not pose any problem or hazard. PAR-Q has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable for them.

Common sense is your best guide in answering these few questions. Please read them carefully and check the yes or no opposite the question if it applies to you.

Yes No
1. __ __ Has your doctor ever said you have heart trouble?
2. __ __ Do you frequently have pains in your heart and chest?
3. __ __ Do you often feel faint or have spells of severe dizziness?
4. __ __ Has a doctor ever said your blood pressure was too high?
5. __ __ Has your doctor ever told you that you have a bone or joint problem such as arthritis that has been aggravated by exercise, or might be made worse with exercise?
6. __ __ Is there a good physical reason not mentioned here why you should not follow an activity program even if you wanted to?
7. __ __ Are you over age 65 and not accustomed to vigorous exercise?

If you answered YES to one or more questions...

if you have not recently done so, consult with your personal physician by telephone or in person before increasing your physical activity and/or taking a fitness test.

If you answered NO to all questions...

If you answered PAR-Q accurately, you have reasonable assurance of your present suitability for an exercise test.
APPENDIX E: PARTICIPANT HEALTH QUESTIONNAIRE

Participant Health Questionnaire

Number: ___

Age: ________

Height: _______

Weight: _______

Have you had any acute injury (ankle sprain, fracture to the lower leg below the knee, in the ankle, or in the foot, and/or surgery to the lower leg below the knee, in the ankle, or in the foot) to the lower extremity (Leg) within the last 6 months? If yes, please explain.

Have you had any surgery to the lower extremity (Leg) within the last 6 months? If yes, please explain.

Are you sensitive to cold or have any cold related allergy?

Sensitive to Cold - (cold irritates you, you do not handle having cold on your skin well, or would not be able to deal with having a cold ice bag on your ankle for up to 30 minutes)

Cold related Allergy - (cold related allergy consist of hives or feel the sensation of itching in the area of the cold application or have a Neurovascular disorder called Raynaud’s phenomenon which is decreased supply of oxygenated blood to the region of cold application)

If Yes please explain.
APPENDIX F: IRB INITIAL APPROVAL

The following research study has been approved by the Institutional Review Board at Ohio University for the period listed below.

Project: The Effects of Cold Application on Subjects with Functional Ankle Instability

Researcher(s): Jeffrey Doeringer
                Jeffrey Seegmiller
                Matthew Hoch

Advisor: Andrew Krause

Department: Recreation and Sport Sciences

Jacqueline Legg, M.B.A., Chair
Institutional Review Board

Approval Date 11/1/06
Expiration Date 11/11/07

This approval is valid until expiration date listed above. If you wish to continue beyond expiration date, you must submit a periodic review application and obtain approval prior to continuation.

The approval remains in effect provided the study is conducted exactly as described in your application for review. Any additions or modifications to the project must be approved by the IRB (as an amendment) prior to implementation.

Adverse events must be reported to the IRB promptly, within 5 working days of the occurrence.
APPENDIX G: IRB APPROVED AMENDMENT I

The amendment, detailed below, and submitted for the following research study has been approved by the Institutional Review Board at Ohio University. Approval date of this amendment does not affect the expiration date of the original approval.

Amendment: Title change. Testing site change. Strength testing change to 3 sets of 5 reps with one min. recovery between sets. Eight change in number of data collection trials. Subject limitations added to revised ICF.

Project: The Effects of Cold Application on Subjects with Functional Ankle Instability

Project Director: Jeffrey Doeringer
Jeffrey Seegmiller
Matthew Hoch

Advisor: Andrew Krause

Department: Recreation and Sport Sciences

Jacqueline Legg, M.B.A., Chair
Institutional Review Board

5/14/07 Date
APPENDIX H: IRB APPROVED CONSENT FORM

Appendix A: Consent Form

Title of Research: The Effects of Cold Application on Individuals with Functional Ankle Instability

Principal Investigator: Jeffrey R. Doeringer, LAT
Advisor: Dr. Andrew Krause, LAT
Co-Investigator: Dr. Jeffrey Seegmiller, LAT and Matthew C. Hoch, LAT
Department: Graduate Athletic Training Program

Federal and university regulations require signed consent for participation in research involving human subjects. After reading the statements below, please indicate your consent by signing this form.

Explanation of Study

The purpose of this study is to identify the effects of cold application (cryotherapy) on neuromuscular recruitment, known as the Hoffman reflex (H-reflex). The H-reflex sends an electrical stimulus to the nerve which innervates the muscle being tested. In this investigation we are testing the H-reflex of the muscles that stabilize the ankle. This will be comparing subjects who have sensation of the ankle giving away (functional ankle instability) to subjects without the sensation of the ankle giving away after a 30 minute cold application vs. 30 minutes of rest. This study will also determine the effects from cold application on peak torque (strength).

Procedures to be followed:

1. You will attend an orientation session held in the Athletic Training Research Laboratory (Grover Center E207) where the methods, risks, and benefits will be presented to you. You will read and complete the Ankle Instability Instrument and general health questionnaire. You will then read the informed consent and have the opportunity to ask questions to the investigators.
2. At a subsequent time, if you are to be included in the study, you will return to Athletic Training Research Lab, reread the informed consent and if you agree to participate, sign the consent form.
3. You are asked to refrain from physical activity 24 hours previous, refrain from caffeine intake the day(s) of the data collection, not apply skin lotion to the leg being tested, and are asked to wear shorts and a t-shirt on the day you partake in the study.
4. Your skin will be prepared over the site of electrodes for the Hoffman reflex. Preparation of the skin consists of shaving, cleaning with alcohol, and abrading (to gently rub the skin from top to bottom with fine sandpaper). This increases the signal by decreasing resistance from the skin. There will

Please refer to Guidelines for assistance in completing the form.

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be seven electrodes applied to your lower leg. Three electrodes will be placed near your shin, two to the outside of the lower leg, and two below your calf. The stimulator will be placed on the back of your knee.

5. You will be seated on a Biodex Multijoint System III dynamometer. This device tests muscle function by allowing you to move through a full range of motion at predetermined speeds (typically set at between 30–280 degrees per second). You will be secured into the chair of the Biodex. You will have your hands to the side with palms open. You will focus on a spot on the wall and will be required to listen to wave sounds through headphones throughout data collections. Each electrode and the stimulator held in place by adhesive tape.

6. The stimulating electrode will deliver a therapeutic accepted stimulus (one millisecond pulse) to a nerve behind the knee. This produces a physiologic response (the Hoffmann reflex) to the muscles which is recorded by electrodes placed over these muscles. Baseline measurements for the h-reflex will be recorded for all subjects.

7. After baseline H-reflex measurements are taken, whether you are Functional Ankle Instability (FAI) group or a control group you will complete strengthening exercises of the aforementioned leg muscles. Prior to the start of the exercises, your beginning and end range of motion will be found. The Biodex exercise system is a controlled machine which does not allow any unwanted movement and prevents you from getting hurt. During the exercises, the motions used will be ankle pushing side to side. You will complete three set of five repetitions at a speed of 60 degrees/second with a 1 minute rest in between to reduce the chance of fatigue.

8. Then after pretests you will either receive one of two randomly assigned treatments. One treatment is a 30 minute ankle ice bag application. The second you will remain at rest sitting in the Biodex chair.

9. Immediately after the cold application or the rest period you will have a first posttest of H-reflex and a first posttest strength exercise of the aforementioned leg muscles for three set of five repetitions at a speed of 60 degrees/second will be measured with a 1 minute rest in between to reduce the chance of fatigue.

10. After the first strength exercise is measured, a second posttest H-reflex and then a second posttest strength exercise for three sets of five repetitions at a speed of 60 degrees/second will be measured with a 1 minute rest in between to reduce the chance of fatigue.

11. You will come back on another day (no sooner than 72hrs.) to perform the other method of treatment whether it is the cold application or the rest period sitting in the Biodex. Steps 3–10 will be repeated.

Duration of subject's participation
This study involves two sessions. One session you are a part of the cold application (cryotherapy) group and the other you are in the control group. Each session will take about two hours.

Risks and Discomforts
During the cold application, you will be exposed to a minimal risk for pain and discomfort and cold related allergy. If you experience pain, discomfort or an allergic
Please refer to Guidelines for assistance in completing the form.
reaction to the treatment, your participation will be stopped (Cold related allergy consist of hives or feel the sensation of itching in the area of the cold application. A disorder called Raynaud’s phenomenon may develop, which manifests by a decreased supply of oxygenated blood to the area with cold application.

Delayed Onset Muscle Soreness (DOMS) may develop after the strength exercise, soreness usually occurs between 24-72 hours after exercise. You will not be tested for your second trial any sooner than 72 hours.

During the Hoffmann reflex you will be given a quick therapeutically accepted electrical stimulus. This brief quick ‘shock’ is delivered to the nerve behind the knee. This stimulus may cause mild discomfort. If you experience pain or discomfort the treatment will be stopped. During the strengthen exercises on the Biodex subjects will perform 3 set of 5 repetitions with a 1 minute rest in between to reduce the chance of fatigue. If any pain, discomfort, or if subject is unable to finish the set then exercise will be stopped.

There is a minimal risk of irritation or mild abrasion the skin during the preparation of electrodes with shaving and the use of sandpaper. This is used to exfoliate the skin allowing for increased signal capture of the EMG. This procedure will be stopped if any of the preceding occurs.

Benefits
You will receive no benefit during this study. The sports medicine community will benefit from the knowledge gained in this research study. The understanding of ankle giving away (functional ankle instability) and the cold treatment effects on the ability to do rehabilitation or activity for individuals with the sensation of his/her ankle giving away (functional ankle instability).

Confidentiality and Records
Confidentiality will be maintained at all times. Data collected in this study will be coded so that your identity is not known when analyzing the data. All files containing your ID code will be maintained in a locked cabinet in the faculty advisor’s office (Dr. Andrew Krause). Dr. Krause and I will have access to the key of the ID codes and they will be held until all the data has been analyzed and the study is complete. The information will be shredded after the data information is not needed anymore.

Compensation
You will receive no compensation during this study.

Contact Information
If you have any questions regarding this study, please contact

Principal Investigator: Jeffrey Doering, LAT at JD304506@ohio.edu (570)362-3572
Advisor: Dr. Andrew Krause, LAT at krausea@ohio.edu (740)593-4648

If you have any questions regarding your rights as a research participant, please contact Jo Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.

Please refer to Guidelines for assistance in completing the form.

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I certify that I have read and understand this consent form and agree to participate as a subject in the research described. I agree that known risks to me have been explained to my satisfaction and I understand that no compensation is available from Ohio University and its employees for any injury resulting from my participation in this research. I certify that I am 18 years of age or older. My participation in this research is given voluntarily. I understand that I may discontinue participation at any time without penalty or loss of any benefits to which I may otherwise be entitled. I certify that I have been given a copy of this consent form to take with me.

Signature_________________________________________ Date________________
Printed Name________________________________________

Please refer to Guidelines for assistance in completing the form.

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APPENDIX I: IRB APPROVED PERIODIC REVIEW

The following research study has been approved by the institutional Review Board at Ohio University for the period listed below.

Project: The Effects of Cold Application on Individuals with Functional Ankle Instability

Researcher(s): Jeffrey Doeringer
Jeffrey Seegmiller
Matthew Hcch

Advisor: Andrew Krause

Department: Recreation and Sport Sciences

Jacqueline Legg, M.B.A., Chair
Institutional Review Board

Approval Date: 10/2/08
Expiration Date: 10/2/08

This approval is valid until expiration date listed above. If you wish to continue beyond expiration date, you must submit a periodic review application and obtain approval prior to continuation.

The approval remains in effect provided the study is conducted exactly as described in your application for review. Any additions or modifications to the project must be approved by the IRB (as an amendment) prior to implementation.

Adverse events must be reported to the IRB promptly, within 5 working days of the occurrence.
APPENDIX J: IRB APPROVED AMENDMENT II

The amendment, detailed below, and submitted for the following research study has been approved by the Institutional Review Board at Ohio University. Approval date of this amendment does not affect the expiration date of the original approval.

Amendment: Remove Jeff Seegmiller as co-investigator. Add Chad Starkey, Kristy White, and Dave Domingues as co-investigators.

Project: The Effects of Cold Application on Individuals with Functional Ankle Instability

Project Director: Jeffrey Doeringer
Matthew Hoch
Chad Starkey
Kristy White
Dave Domingues

Advisor: Andrew Krause

Department: Recreation and Sport Sciences

Jacqueline Legg, M. B. A., Chair
Institutional Review Board

Date