Focal Wrist Cooling Does Not Alter Indices of Spinal Excitability in the Flexor Carpi Radialis Muscle

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Master of Science

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This thesis titled
Focal Wrist Cooling Does Not Alter Indices of Spinal Excitability in the Flexor Carpi Radialis Muscle

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ABSTRACT

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Focal Wrist Cooling Does Not Alter Indices of Spinal Excitability in the Flexor Carpi Radialis Muscle

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Focal joint cooling has been shown to increase the Hoffmann reflex (H-reflex), an index of spinal excitability, in the lower extremity. Unfortunately, the H-reflex is influenced by many segmental physiological factors making it difficult to delineate the mechanistic underpinnings of this previously observed phenomenon. Accordingly, this thesis sought to determine if cryotherapy alters motoneuron excitability by using an innovative technique that permits a more direct assessment of α-motoneuron excitability. To approach this aim, we investigated the effects of focal wrist joint cooling on the flexor carpi radialis muscle’s H-reflex response as well as cervicomedullary evoked potentials (CMEPs) in 10 healthy male participants. A 2 x 2 repeated measures ANOVA was used to compare the H-reflex and CMEP measurements following a cryotherapy or sham intervention. No time by treatment interactions were observed for any outcome variables. In summary, focal joint cooling does not alter H-reflex or CMEP amplitude measurements immediately posttreatment.

Approved: _____________________________________________________________

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CHAPTER 1: INTRODUCTION

Within the field of Athletic Training, cryotherapy—the therapeutic use of cold—is the most frequently used modality in managing musculoskeletal injuries. Due to its well established clinical efficacy during rehabilitation, cryotherapy is known to decrease residual pain, cell metabolism, and acutely enhance muscular performance (e.g., increase voluntary muscle strength). The mechanism explaining the enhanced muscular performance has previously been postulated to be an acute facilitation in \( \alpha \)-motoneuron excitability. However, to date the specific segmental effects of focal joint cryotherapy on \( \alpha \)-motoneuron excitability have not been determined largely due to the lack of experimental techniques to assess \( \alpha \)-motoneuron excitability.

The Hoffmann reflex (H-reflex) is a spinal reflex response that results from submaximal electrical stimulation of sensory nerve fibers that project back on and excite \( \alpha \)-motoneurons. Understanding adaptations in spinal cord function is difficult without concomitant measurements of other outcome variables due to the numerous influences on the H-reflex. Thus, the specific adaptations in spinal excitability to focal joint cooling are not fully understood, with regard to changes in the excitability of the \( \alpha \)-motoneurons. For example, there are limitations to relying solely on the H-reflex technique in assessing spinal adaptations. The H-reflex only provides a global measure of spinal excitability, as it can be modulated by a number of potential factors including: (1) presynaptic inhibition, (2) variation in the amount of neurotransmitter released by the Ia terminal, (3) fluctuations in the membrane potential arising, and (4) alterations in the intrinsic properties in the motoneurons. Conversely, over the last couple of decades,
technological innovations have now allowed for *in vivo* examination of the human neuromuscular system. Magnetic stimulation at the level of the cervicomedullary junction evokes single descending volleys which activate α-motoneuron axons primarily through a monosynaptic connection, and can be used to more directly assess α-motoneuron excitability without the confounds inherent in the H-reflex.\textsuperscript{17} While this technique is still in its early stages, it shows great potential for longitudinally assessing changes in α-motoneuron excitability.\textsuperscript{16,17} Combining H-reflex and CMEP techniques will result in a greater understanding of the regulation of spinal cord excitability.

There are two novelties about this thesis. One specific aim was to identify the effects of focal wrist joint cooling on upper extremity motoneuron excitability. The hypothesis was that flexor carpi radialis muscle H-reflexes would increase following a 15-minute wrist joint ice bag application. The second focus of this thesis was to measure CMEPs as a more direct estimation of motoneuron excitability following cryotherapy. Examining these two outcome measures of motoneuron excitability will provide a more thorough understanding of changes in spinal neuronal properties following cryotherapy and give credence to cryokinetics during the management of musculoskeletal and orthopedic pathologies.

**Statement of Problem**

Scientific research varies regarding the effects of cryotherapy modulating neurophysiological pathways, motoneuron excitability, and neuromuscular facilitation; the effects of cryotherapy have not been studied extensively in the upper extremity. This thesis specifically examines the following research questions:
1. Does a 15-minute focal wrist cooling intervention facilitate the flexor carpi radialis Hoffmann reflex?

2. Does a 15-minute focal wrist cooling intervention facilitate the flexor carpi radialis CMEP?

Purpose of the Study

This thesis compares changes in flexor carpi radialis H-reflex and CMEP measures after a 15-minute focal wrist cryotherapy treatment. The experimental design allowed comparisons of the flexor carpi radialis H-reflex and CMEP measurements across pretreatment, posttreatment, and 15-minute posttreatment intervals.

Null Hypothesis

H\textsubscript{01}: There is no difference in facilitation of the flexor carpi radialis H-reflex following a 15-minute focal wrist cooling intervention.

H\textsubscript{02}: There is no difference in facilitation of the flexor carpi radialis CMEP following a 15-minute focal wrist cooling intervention.

Delimitations

This study was conducted with the following delimitations.

1. Only college-aged males from Ohio University participated in this study.

2. H-reflex data were collected and analyzed for the flexor carpi radialis using surface electromyography.

3. CMEP data were collected and analyzed for the flexor carpi radialis using surface electromyography.
Limitations

The following limitations were observed during this research study.

1. H-reflex data were only recorded for the flexor carpi radialis muscle.
2. CMEP data were only recorded for the flexor carpi radialis muscle.
3. A small sample size was used during the investigation.

Assumptions

The following assumptions were made regarding this research.

1. Subjects were honest and precise when reporting their medical health history.
2. All instruments were performing properly.
3. Measurements were taken accurately.

Definition of Terms

*Cervicomedullary motor evoked potentials (CMEP).* Magnetic or electrical stimulation at the cervicomedullary junction that can evoke single descending volleys that produce muscular responses in the upper extremity.¹⁷

*Central activation ratio (CAR).* Measurement of voluntary motoneuron excitability.⁵⁰

*Corticospinal tract.* Collection of axons that travel between the cerebral cortex of the brain and the spinal cord.

*Disinhibition.* Return of some type of recruitment measure to baseline levels.⁶

*Electromyography (EMG).* A method for registering compound action potentials generated by muscle fibers.³⁹
**Flexor carpi radialis (FCR).** Muscle of the forearm that flexes the wrist and abducts the hand.

**Hoffmann reflex (H-reflex).** Spinal reflex response that results from submaximal electrical stimulation of sensory nerve fibers that project back on and excite the α-motoneurons.46

**Inhibition.** Decreased availability of neurons within a motoneuron pool.5,8

**Maximum voluntary isometric contraction (MVIC).** Greatest force a muscle can produce as it contracts while pulling against a stationary object.
CHAPTER 2: REVIEW OF LITERATURE

Cryotherapy

Cryotherapy is a universal method for treating musculoskeletal injuries.\textsuperscript{18-20} It is defined as the therapeutic application of cold agents to the body with a temperature range between 0°C and 18.3°C that results in removal of heat from the body, causing a decrease in tissue temperature.\textsuperscript{1,3} The possible advantageous effects of cold application include reduction of cellular metabolism, pain, inflammation, and muscle spasm. Sports medicine professionals use cryotherapy as a therapeutic modality during the acute stages of injury to decrease cell metabolism in order to prevent secondary hypoxic injury and tissue damage.\textsuperscript{1-4} From a rehabilitation perspective, cryotherapy is used to alleviate pain prior to performing therapeutic exercises because it promotes early and progressive function, which allows injured athletes to work on restoring normal motor patterns.\textsuperscript{11} Some examples of cryotherapy techniques include cold packs, ice massage, and ice immersion.\textsuperscript{3} Frequent sensations involved with cold application include cold, burning, aching, analgesia, and numbness, which are a result of engaging sensory afferents and decreased nerve conduction velocity\textsuperscript{21}

\textit{Neural Effects of Cold}

Cooling a muscle causes a reduction in nerve impulse firing rates and an increase in depolarization. Nerve conduction velocity and depolarization do not occur at the same time because soft tissues do not cool at the same rates.\textsuperscript{22} Neural changes begin to take place in the body when cold application causes skin temperature to decrease around \textminus12.7°C. During a \textminus10.4°C decrease in skin temperature, motor nerve conduction velocity
reduces to 14% and sensory afferent nerve conduction velocity reduces to 33%.

Reduced synaptic transmission and increased time required for a nerve to depolarize cause decreased nerve conduction velocity.\textsuperscript{22}

Afferent information is transported to the spinal cord from different nerve endings by the same spinal pathway. This mechanism is unknown but there is some speculation that there is possibility that a decrease in action potential leads to a reduction in nerve conduction velocity and an increase in pressure needed to stimulate a muscle contraction following cold application.\textsuperscript{24} In addition, activation of motor units may possibly be modified due to nerve conduction velocity initiating changes in the muscular force.\textsuperscript{25}

Assuming that there is a large sensory afferent signal at rest, decreased nerve conduction velocity and reduced rate of mechanoreceptors result in less amount of information being sent to the spinal cord, which leads to a decline in inhibition.\textsuperscript{7}

\textit{Cutaneous Receptors}

Cryotherapy stimulates cutaneous receptors, including thermoreceptors, which are sensitive to cold and warm temperatures, and mechanoreceptors, which are sensitive to pressure. Cold sensitivity seems to be due to inhibitory synaptic input from nearby warm-sensitive neurons. Cold neurons in the skin originate directly underneath the epidermis and convey signals by thin myelinated A\textsuperscript{\text{\textdelta}} fibers. Warm neurons are much deeper in the dermis and travel by means of C fibers Cold and warm sensors are very active during temperature changes and adaptable during steady state temperatures.\textsuperscript{56} Mechanoreceptors play a significant role in tactile discrimination, because there are many different types that are found in the hand. For instance, Meissner corpuscles and Merkel disks are
located most closely to the skin’s surface on the perimeter of the epidermis and dermis. Ruffini endings are deep down in the dermis and Pacinian corpuscles are even deeper down in subcutaneous tissue. On the skin’s surface, afferent axons innervate Merkel disks, which react to vertical pressure, and Meissner corpuscles, which respond to quick changes in pressure. Ruffini endings are activated by stimuli approximately 5 cm deep and respond to skin deformation. Pacinian corpuscles, which are the largest mechanoreceptors, react to changes in mechanical deformation. Quickly adapting mechanoreceptors excite the Ia interneurons, resulting in motoneuron excitability. It is possible that large amounts of information reaching the spinal cord from several different sensory receptors creates an environment mediated by supraspinal centers.

Physiological Effects on Muscle Function

Cryotherapy has been shown to produce various effects on muscle function and strength. The physiologic effects of cryotherapy on the nervous and muscular systems are controversial in terms of muscle activation during force production and functional performance. Previous research focusing on the effects of cryotherapy on muscular function have found reductions in a maximal voluntary isometric contraction, decreases in isokinetic strength, no effects on functional performance, and no alterations on agility and balance. Recent clinical observations have shown that disinhibiting a muscle prior to performing therapeutic exercise can facilitate neural activation and produce enhanced motor patterns. Motoneuron excitability has also been observed to increase during focal cooling and continues to increase post-treatment. McGowan examined the effect of ice immersion for approximately 5 minutes on
maximal isometric contractions and found increases in force. These results identify the potential value of a short focal cooling procedure on maximal isometric contractions.

**Focal Joint Cooling**

There has been a series of literature investigating the effects of muscle activity after focally cooling a joint.\textsuperscript{6,7,10,51} Krause et al\textsuperscript{10} observed increases in motoneuron excitability before and after focally cooling the ankle joint. In healthy subjects with experimentally effused knee joints, both transcutaneous electrical nerve stimulation (TENS) and focal cooling have each been shown to increase motor neuron excitability in the quadriceps.\textsuperscript{7,51} Focal cooling has also been observed to increase torque output following 20 minutes of application\textsuperscript{6} and motoneuron excitability for up to 40 minutes after cold application removal.\textsuperscript{7} It has been theorized that TENS and cryotherapy may aid in superseding the inhibitory signals arising from an injury because of increased excitatory afferent stimuli sent to the spinal cord.\textsuperscript{7} Evidence suggests that motoneuron facilitation after focal cooling could lead to enhanced functional capabilities as a result of cryokinetics.\textsuperscript{10}

**Cold Pressor Test**

The Cold Pressor Test (CPT) was originally used as a method to increase blood pressure in hypertension studies.\textsuperscript{33} The procedure entailed immersion of one of the extremities in ice cold water for 1–2 minutes and recording of variables such as heart rate, arterial blood pressure, and muscle or skin sympathetic nerve activity.\textsuperscript{34,35} The CPT requires participants to immerse their hand in flowing ice water at a temperature range of 10°C–1°C for approximately 2 minutes.\textsuperscript{36} Ice immersion is the favored method for
cooling distal extremities such as the foot, ankle, and hand. The most advantageous
temperature for ice immersion is not truly specified, but ranges anywhere from 2°C–4°C
to 10°C–15°C. Nonetheless, lower temperatures are better because hypalgesia occurs in
an earlier fashion. Depending on cold intensity, the CPT can increase heart rate, cardiac
contractility, and vascular resistance through sympathetic activation.

The CPT has been widely used in research by clinicians as an intervention
because it is simple and easy to perform. It has commonly been used as a method to
measure the function of neural control on the cardiovascular system. There has not been
any significant evidence produced concerning the neural effects of the procedure on
motoneuron facilitation. A better understanding of the mechanisms that drive muscle
activation following cold immersion using the CPT will help elucidate neural control on
muscle activation.

Muscle Activation

The spinal cord is made up of a complex system of channels sending electronic
information throughout the human body. The central and peripheral nervous systems
collaborate together to coordinate movement by gathering, transmitting, and processing
information from several different neurophysiological systems. The majority of receptors
are specialized endings to sensory nerve fibers. Stimulation of a receptor causes a change
in cell membrane potential, leading to depolarization and the creation of an action
potential. The action potential courses its way along the dendrite until it reaches the body
of the cell. The cell body then moves through the dorsal horn of the spinal cord, where it
makes connections with different types of neurons.
A muscle contains motor units, which consist of a motor neuron and all the muscle fibers that are innervated by it. Motor neuron activation will cause force production in all of the fibers contained in one motor unit. A single motoneuron will elicit a single force response, which is known as a twitch. The amount of muscle force depends upon the number of motor units recruited, the frequency of stimulation, and the size of motor units activated. Motor units are recruited in a set order throughout most types of contraction. During a graded increase in an isometric force, motor units are believed to be recruited according to the size principle. According to the size principle of motor unit recruitment, small motor units are recruited initially in a graded muscular contraction. As force demands increase, larger motor units are recruited and the previously recruited motor units increase their firing rates. The size principle demonstrates that muscular force can be regulated by increasing the number and firing frequency of motor units. In skeletal muscles of the arms and legs it is understood that both of these mechanisms work for isometric forces up to 60% of the maximal force. At about 60% of the maximal isometric force, the majority of motor units are recruited, but the remaining 40% of force production needed to reach maximal force has to be achieved by increasing motor unit recruitment.

Signals from the central nervous system are sent to muscles by neural cells called α-motoneurons that travel from the spinal cord through the ventral roots. The sum of α-motoneurons that innervate a single muscle are called a motoneuronal. Axons of α-motoneurons synapse on specialized interneurons called Renshaw cells. The axons of these cells project back on the α motoneurons, creating inhibitory synapses. Another
group of inhibitory interneurons are made up of Ia interneurons that receive signals from Ia afferent spindles and send their axons to α motoneurons. A decrease of availability of neurons within a motoneuron pool is classified as inhibition. Two inhibitory mechanisms within the central nervous system are known as postsynaptic and presynaptic inhibition. Postsynaptic inhibition decreases neuron sensitivity and any incoming excitatory signal and presynaptic inhibition makes synapses into the neuron less effective. For disinhibition to occur, Renshaw cells have to slow down Ia interneurons and then decrease the interneurons inhibitory effects on α motoneurons.

Electromyography

The two fundamental methods available for recording muscle activity are needle, or intramuscular EMG and surface, or interferential EMG. During intramuscular EMG, a small needle with a very thin wire is inserted into a muscle and the difference between potentials at the tip of the wire and needle is amplified and recorded. Surface EMG is used most often in studies that focus on voluntary movements. The procedure totals up the activity of as many motor units as possible across a muscle and the difference of the potential is amplified. The typical setup for surface EMG involves placing a pair of electrodes directly over the muscle belly. Research studies that focus on smaller muscles, such as the flexor carpi radialis should use smaller electrodes, so that activity is not picked up from surrounding muscles in the forearm. The diameter of electrode size for surface EMG ranges anywhere from 1 to 20mm and the distance between electrodes varies from 5 to 55mm. Appropriate placement of electrodes provides the best overall results.
Hoffmann Reflex

The Hoffmann reflex (H-reflex) is an electrically induced impulse that measures spinal monosynaptic reflex activity. The H-reflex measures synaptic transmission as the impulse travels in type Ia afferent fibers to the motoneuron pool of the corresponding muscle to the efferent fibers. The afferent portion of the H-reflex starts once electrical stimulation is applied and stops when the afferent fibers synapse on $\alpha$-motoneurons. The efferent H-reflex pathway starts from action potentials, generated by $\alpha$-motoneurons, and stops when the efferent fibers meet the neuromuscular junction. The product from the synchronized contraction is a twitch response measured by EMG. The efferent arc produced on the EMG is known as the M-wave.\textsuperscript{15}

The H-reflex is possibly the most widely examined reflex in neurophysiology. This is due mainly because it can be elicited in a variety of muscles.\textsuperscript{43} In both standing and supine positions, the H-reflex has a high reliability measure (92-93\%) of motoneuron excitability.\textsuperscript{44,45} H-reflexes have been evoked in many different muscles in both the upper and lower extremities. The most commonly used muscle in the lower extremity is the soleus; and in the upper extremity, the flexor carpi radialis.\textsuperscript{46} EMG electrode placement is very important for accurate measurements. For the flexor carpi radialis electrode placement is one-third distal on a line from the medial epicondyle of the humerus to the radial styloid.\textsuperscript{46}

The process of eliciting the H-reflex in the flexor carpi radialis involves applying stimulation to the median nerve (C6 and C7). During low levels of stimulation, type Ia afferent nerve fibers are stimulated. As intensity increases, recruitment of more Ia fibers
leads to activation of $\alpha$ motoneurons. When stimulation is increased an M-wave will appear in the EMG with the H-reflex. Maximal reflex activation of the H-reflex is referred to as $(H_{\text{max}})$ and complete activation of the motoneuron pool is referred to as $(M_{\text{max}})$. If stimulus intensity is slowly increased from zero to an intensity that would elicit $M_{\text{max}}$, a recruitment curve will be represented.\textsuperscript{15}

Extended stimulus intensity will ultimately cause the H-reflex to reach its maximum peak and disappear from the EMG, while the M-wave remains steady.\textsuperscript{15} What causes the H-reflex to disappear from the EMG is known as antidromic collision.\textsuperscript{47} Essentially, antidromic collision causes a volley of electrical activity to travel backwards up the motor axon and toward the spinal cord, causing a collision with the afferent reflex volley. Therefore, when the H-reflex starts to diminish, no signal proceeds to the muscle due to the size of the backwards volley being equal to or larger than the afferent reflex volley.\textsuperscript{15}

As a valuable measurement tool, the H-reflex can be used to study the response of the nervous system to application of thermal therapeutic modalities, exercise training, and performance of motor tasks.\textsuperscript{15,46} H-reflex measurements can assess the nervous system’s response to clinical applications in controlled laboratory settings.\textsuperscript{15} While the H-reflex literature investigating joint cooling is limited, previous investigators\textsuperscript{6,7,9,10,12} have examined the H-reflex after applying cryotherapy to a healthy joint and have found increases in strength. The improved function that athletes experience in conjunction with cryotherapy has primarily been thought to result in pain reduction. Based on this
information, the use of cryotherapy can be used to enhance motor output to a muscle, thereby increasing muscular facilitation.\textsuperscript{15}

**Central Activation Ratio**

Precise evaluations of central neuromuscular measures are essential for examining voluntary muscle activation.\textsuperscript{13} Early assessment of neural activation was performed by using electrical stimulation to apply a superimposed burst to a muscle during a voluntary contraction.\textsuperscript{48} A term for the degree of neural activation of a muscle can be expressed as “central activation ratio” (CAR).\textsuperscript{49} After a voluntary contraction, motor units that are not fully activated are stimulated. An estimation of muscle activation can be inferred from the CAR, which is essentially a measurement of voluntary motoneuron pool excitability.\textsuperscript{50} The ratio is computed by dividing the maximal voluntary contraction force over the sum of the superimposed burst force and the maximal voluntary contraction force.\textsuperscript{49-51} CAR measurements of 1.0 signify total central activation, whereas any measurement less than 1.0 results in incomplete muscle activation.\textsuperscript{14,52} The CAR has been shown to increase compared to baseline levels following cold intervention.\textsuperscript{7,51}

**Cervicomedullary Motor Evoked Potentials**

In order to investigate motor control in humans, it is imperative to understand the contraction of muscle fibers that are controlled by the rapid release of motoneurons to the spinal cord.\textsuperscript{17} Spinal tracts can be stimulated noninvasively in human subjects by electrical and magnetic stimulation.\textsuperscript{16} CMEPs can stimulate descending tracts at the cervicomedullary junction (see Figure 1) and evoke a short latency excitatory response in muscle, which can be used to examine the corticospinal pathway and motoneuron
excitability in the upper extremity.\textsuperscript{16,17} CMEPs can be used to examine motoneuron excitability during voluntary, fatiguing, and contralateral contractions through the corticospinal pathway. Analyzing CMEPs during and following a maximal voluntary isometric contraction reveals changes in motoneuron excitability and also suggests activity changes from the corticospinal tract.\textsuperscript{17} CMEPs reflect alterations at the spinal motoneuron pool itself based on the idea that the corticospinal tract is free from presynaptic control.\textsuperscript{53,54} Compared to the H-reflex, CMEPs are believed to be free of presynaptic inhibition and work well during maximal voluntary contractions. Therefore, CMEPs are a direct measure of activity at the cortical versus spinal level during muscle activation.\textsuperscript{16}

Stimulation at the cervicomedullary junction using a double-cone magnetic coil can be used in laboratory settings to test activation of spinal tracts and evoked motor responses with the same latency as electrical stimulation. Magnetic stimulation is not as painful compared to electrical stimulation because skin has a low current density. Responses in a relaxed muscle are often small, but, during a voluntary contraction, responses can be attained because of increased excitability within the motoneuron pool of the muscle. During the CMEP procedure, accurate placement of the double-cone coil is required for stimulation. Placement of the coil is recommended to start over the subject’s inion but can be placed within 1–2 cm from the inion to attain the best stimulation site.\textsuperscript{16}
CHAPTER 3: METHODS

Design

This investigation used an experimental 2 x 2 repeated measures crossover design. Dependent variables were the flexor carpi radialis Hoffmann reflex, represented as the ratio between the maximum H-reflex and the maximum muscle response (H_{max}:M_{max}) and CMEP. Independent variables included intervention (ice and sham) and time (pre, post, and post-15). Individuals participated in two testing sessions. In one session, the intervention was application of a crushed ice bag to the dorsum of the wrist joint for 15 minutes, and, in the other session, application of a sham ice bag for 15 minutes. Interventions were counterbalance using random assignment.

Participants

Ten apparently healthy and neurologically sound male adults volunteered with an age (M = 23.6 ± .94) years, mass (M = 96.8 ± 15.8) kg, and height (M = 182.3 ± 5.1) cm. See Appendix D for a complete demographic analysis. To be included in the study participants had to be healthy, physically active, adult males between the ages of 18–27. Physically active was defined as engaging in exercise at least 30 minutes a day, 5 days a week, with vigorous exercise on at least three of the days. Participants had no history of upper extremity injury or surgery within the past year and passed a magnetic stimulation screening. Exclusion criteria included not passing the magnetic stimulation screen, and history of an upper extremity musculoskeletal injury or sensorimotor deficits. If participants experienced loss of sensation in the upper extremity during data collection, they were removed from further testing. Participants provided their informed consent and
completed a medical history questionnaire. This study was approved and conducted in compliance with the human subject research requirements of Ohio University’s Office of Research Compliance (see Appendix C). No adverse occurrences were observed or reported during data collection.

Instrumentation

*Hoffmann Reflex*

The EMG signals were amplified (500 –1,000x), band-pass filtered (10–500 Hz), and sampled at a high sampling (100 kHz) rate to attain high temporal resolution readings (MP150, Biopac Systems, Goleta, CA). A 1 ms electrical stimulus was used to elicit the H-reflex (Digitimer DS7; Digitimer, Welwyn Garden City, Hertfordshire, UK) (see Figure 1). Recording electrode arrangement is discussed in the procedures. The H-reflex and M-responses were determined by a prescribed increase in the stimulus intensity to identify maximum values. Recruitment curves were recorded to calculate the $H_{\text{max}}$, was normalized to the $M_{\text{max}}$ and reported as a proportion ($H_{\text{max}}/M_{\text{max}} \times 100$).

*Figure 1.* Biopac MP150 and Digitimer DS7 instruments.
Cervicomedullary Magnetic Stimulation

For the CMEP procedure a high-voltage magnetic pulse (50-100 μs duration, up to 750 V) was passed across the cervicomedullary junction using two connected Magstim 200² (The Magstim, Whitland, England) stimulators (see Figure 2) through a 70-mm double-cone coil (see Figure 3). The flexor carpi radialis surface electrodes captured these evoked potentials and were amplified, digitized, and recorded by the Biopac MP 150 (BIOPAC, Systems, Goleta, CA USA).

Figure 2. Magstim 200² stimulators.
Procedures

Individuals interested in participating attended an orientation session with the primary investigator that informed them of study. After the procedures, risks and discomforts, and benefits were fully disclosed to them, participants scheduled two testing sessions on two separate days with the primary investigator. Before the first testing session, participants reread the informed consent document and if they agreed to participate, provided their signature. After the informed consent document, medical history questionnaire, and magnetic stimulation screening were completed, participant information was reviewed for inclusion and exclusion criteria. Once enrolled in the study, testing began.

When participants arrived for testing, their skin was prepared at the site of electrode placement. Participants were shaved over the flexor carpi radialis muscle, cubital fossa, and the medial epicondyle. Isopropyl alcohol swabs and fine sand paper cleaned and lightly abraded the skin surface. For the H-reflex procedure, two surface
EMG adhesive recording electrodes (Ag-AgCl, 35 × 45 mm, No. 2015; Nikomed, Doylestown, Pennsylvania) were placed longitudinally over the flexor carpi radialis muscle of their dominant arm with an interelectrode distance of 2cm (see Figure 4). Two surface EMG adhesive stimulating electrodes were placed over the median nerve, approximately 4cm distal to the medial epicondyle.\textsuperscript{15}

![Flexor carpi radialis EMG electrode placement.](image)

Participants were seated in a semireclined position with their feet flat on the ground. They were instructed to relax their shoulders and place both forearms facing down on a table in front of them while looking straight ahead. Body position was maintained, and the testing environment was maintained at a constant ambient temperature and humidity.

The stimulating electrode placement was identified by recording H-reflex and muscle response amplitudes and normalizing values.\textsuperscript{15} Once the median nerve was found, a series of short-duration (1ms), high intensity (150-200V) stimuli were delivered every
10 seconds in increments of 0.1 to 0.5 mA, starting with 1 mA. To achieve maximal H-reflex and M-response amplitudes, a reflex recruitment curve was conducted by delivering electric stimuli in .2 V increments every 10-20s. After maximum peak-to-peak amplitudes were observed, three pretreatment measurements were recorded for the maximum H-reflex and for the maximum muscle response. When the recruitment curve was completed, the $H_{\text{max}}:M_{\text{max}}$ ratio was used to compare measurements. Figure 5 illustrates sample H-reflex and M-response data collected.

![Figure 5](image)

*Figure 5.* Sample H-reflex and M-response observed on surface EMG.

The CMEP stimulation site was marked by placing three reference stickers to the back of the participant’s head (see Figure 6). The center of the coil was initially placed behind the participant’s head approximately 3.5cm to the right and 2.8cm below the inion. To find the best site of stimulation the coil was moved laterally from the inion. For all participants, stimulus intensity was set at 100% of stimulator output on both
stimulators and was maintained constantly during the stimulation procedure. Figure 7 illustrates sample CMEP data collected.

![Figure 6. CMEP reference markers.](image1)

![Figure 7. Sample CMEP observed on surface EMG.](image2)
Participants had one of two interventions administered following pretreatment baseline measurements. A .75 L bag of crushed ice was applied to the dorsum of the hand and wrist including, proximal to the ulnar and radial styloid processes. The crushed ice bag was replaced with a bag of foam packing pellets to serve as the control sham treatment (see Figure 8). Both interventions were secured comfortably to the dorsum of participant’s hand and wrist distal to the radial and ulnar styloid processes using Flex-I-Wrap (Cramer Products, Gardner KS). Following the 15-minute treatment, the bags were removed and data was recorded posttreatment and 15-minutes posttreatment. Prior to leaving the lab, participants were reminded that if they experienced any discomfort following testing to contact the primary investigator. A chart of the procedures timeline is presented below (see Figure 9).

*Figure 8. Cryotherapy and sham treatments.*
| EMG recruitment Prep curve | H-reflex: M_max | CMEP | 3 Trials | 3 Trials | 3 Trials | 3 Trials | 3 Trials | 3 Trials |
|----------------------------|----------------|------|----------|----------|----------|----------|----------|----------|----------|
| Pre-treatment              |                |      |          |          |          |          |          |          |          |
| Post-treatment             |                |      |          |          |          |          |          |          |          |
| 15 Min Post-treatment      |                |      |          |          |          |          |          |          |          |

*Figure 9. Procedures timeline.*

**Data Analysis**

A 2 x 2 repeated measures ANOVA was used to analyze the data. A probability level of $p < .05$ was established *a priori*. All statistical analyses were computed using Statistical Package for Social Sciences for Windows (version 18.0; SPSS Inc., Chicago, IL) to test the following research questions:

**Q**A$_1$: Does a 15-minute focal wrist cooling intervention facilitate the flexor carpi radialis Hoffmann reflex?

**Q**A$_2$: Does a 15-minute focal wrist cooling intervention facilitate the flexor carpi radialis CMEP?
CHAPTER 4: RESULTS

The results for the research questions presented in Chapter 1 are presented in this section.

Research Question 1

Does a 15-minute focal wrist cooling intervention facilitate the flexor carpi radialis Hoffmann reflex?

A 2 x 2 repeated measures ANOVA revealed no statistical mean differences in the flexor carpi radialis H-reflex ($F_{1.18} = .593, p = .520$, partial $\eta^2 = .062, 1-\beta = .648$). Please refer to Table 1 showing the mean differences. Raw data of $H_{\text{max}}$ and $M_{\text{max}}$ for all participants are presented in Appendix F.

Table 1

$H_{\text{max}}:M_{\text{max}} (V)$ Amplitudes (Mean ± Standard Deviation) for Each Intervention Over Time

<table>
<thead>
<tr>
<th>Time</th>
<th>Cryotherapy (Mean ± SD)</th>
<th>Control (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>49.3 ± 20.3%</td>
<td>43.3 ± 20.2%</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>45.1 ± 16.6%</td>
<td>42.8 ± 19.4%</td>
</tr>
<tr>
<td>15 minutes post</td>
<td>42.1 ± 11.3%</td>
<td>36.2 ± 13.4%</td>
</tr>
</tbody>
</table>
Research Question 2

Does a 15-minute focal wrist cooling intervention facilitate the flexor carpi radialis CMEP?

A 2 x 2 repeated measures ANOVA revealed no statistical mean differences in the flexor carpi radialis CMEP across time and between treatment ($F_{1,18} = 2.807, p = .098$, $\eta^2 = .238$, $1-\beta = .144$; Table 2). Although, no time*treatment interaction was observed, focal wrist joint cooling did appear increase CMEP amplitude measurements immediately post-treatment. Raw data of CMEP for all participants are presented in Appendix G. Though no true differences existed, these data indicate that focal cooling does not decrease upper extremity motoneuron excitability. The CMEP was not affected by the sham treatment ($p > .05$). Figure 10 illustrates an increase in CMEP amplitude.

Table 2

<table>
<thead>
<tr>
<th>Intervention</th>
<th>CMEP Amplitudes (Mean ± Standard Deviation) for Each Intervention Over Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Cryotherapy</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>$42.8 \pm 23.8%$</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>$45.9 \pm 24.5%$</td>
</tr>
<tr>
<td>15 minutes post</td>
<td>$44.8 \pm 22.5%$</td>
</tr>
</tbody>
</table>
Figure 10. Cervicomedullary motor evoked potentials (CMEP) prior to and following the focal wrist joint cooling treatment.
CHAPTER 5: DISCUSSION

The purpose of this thesis was to examine the effects of focal joint cooling on flexor carpi radialis motoneuron excitability. It was hypothesized that focal wrist joint cooling would facilitate both the flexor carpi radialis H-reflex and CMEP. The data indicated that no statistical differences occurred between cryotherapy and control interventions from flexor carpi radialis H-reflex and CMEP over time. However, CMEP amplitudes were facilitated immediately post focal wrist joint cooling ($p = .05$). While the H-reflex literature investigating joint cooling is limited, this thesis is arguably the first to examine the relationship between resting motoneuron excitability and focal joint cooling in the upper extremity.

This thesis provides evidence that motoneuron excitability in the upper extremity, specifically the flexor carpi radialis H-reflex, does not exist during or following focal wrist joint cooling. Although no increases were observed, there was no change in H-reflex or CMEP amplitudes. This suggests that focal joint cooling does not affect these neuromuscular outcome measures. Based on current observations,\textsuperscript{7-10} focal cooling has been reported to increase the neural excitability of dynamic musculature in lower extremity joint segments. Hopkins et al\textsuperscript{7} observed that knee cryotherapy facilitated the vastus medialis motoneuron pool during and following a 30-minute crushed ice bag treatment. Focal joint cooling has been attributed to neuromuscular facilitation in the soleus.\textsuperscript{8,9,10,12} Observations of the soleus motoneuron pool demonstrate facilitation following a 30-minute ankle ice bag application and over 60 minutes during the post-cooling period.\textsuperscript{9} Krause et al\textsuperscript{10} found increases in soleus motoneuron excitability during
and after cooling, suggesting that temperature change affects the state of motoneuron excitability. Pietrosimone et al\textsuperscript{51} discovered that focally cooling the knee joint produces increases in quadriceps central activation ratio immediately after application. These studies provide critical evidence to support the theory that joint cooling increases the resting activation levels of dynamic control musculature. This growing literature over the past decade has provided rehabilitation specialists and clinical researchers with cryotherapy applications beyond decreasing cell metabolism, increasing pain thresholds, and range of motion.

Focal joint cooling is used to minimize pain and cell metabolism following musculoskeletal injury and, in combination with exercise, to restore function.\textsuperscript{1} After sustaining an orthopedic injury, neural mechanisms reduce the amount of motoneurons that can be recruited to elicit a muscle contraction.\textsuperscript{8} The inability to activate a muscle is an outcome of arthrogenic muscle inhibition, which needs to be a focus of rehabilitation. When rehabilitating an inhibited muscle using therapeutic strengthening exercises, new motor patterns from a decreased motoneuron pool are being activated. Not engaging inhibited motor neurons can lead to poor recruitment patterns and increased recovery time. It is imperative to remove arthrogenic muscle inhibition prior to performing therapeutic exercises intended to facilitate musculature.\textsuperscript{8}

There are many factors to be considered in explaining why the present research found no change in the flexor carpi radialis H-reflex. In response to focal joint cooling, four characteristics possibly contributing to this finding include: (1) motor unit recruitment and firing rate properties, (2) location of fast-twitch fibers within a muscle,
(3) noise from signals originating adjacent to the muscle, and (4) agonist-antagonist muscle interaction. Compared to lower extremity muscles, the flexor carpi radialis has a smaller cross sectional area and number of muscle fibers. Smaller muscles tend to rely primarily on firing rate, whereas larger muscles rely primarily on recruitment to modulate force. The relative location of slow-twitch and fast-twitch muscle fibers are an important characteristic because the amplitude of the action potential is proportional to the fiber diameter. The electrical noise from surrounding muscles is more pronounced in smaller muscles because surface electrodes are located near adjacent musculature. The agonist-antagonist relationship is an important factor during isometric contractions. However, the relationship may also be altered by factors such as joint angle and limb position.

In the upper extremity most movement patterns are open-kinetic chain, whereas the lower extremity patterns are primarily closed-kinetic chain. In the open-kinetic chain, distal segments move freely in space and muscle activation occurs in the prime mover and is isolated to muscles of the moving joint. In the closed-kinetic chain, distal segments remain fixed and muscle activation occurs in more than one muscle group, both distal and proximal to the moving joint. Open-kinetic chain exercises rely on external stabilization, whereas closed-kinetic chain exercises rely on internal stabilization by means of muscle action, joint compression, and postural control. Due to the relative position of the body during activity, closed-kinetic chain exercises allow more functional patterns of movement and provide a platform for multi-planar movements. A closed-kinetic chain progression of movements against resistance theoretically results in central nervous
system engram patterning, providing that the emphasis is on precision of movement. In addition, much of the adaptation that occurs during the training will be specific to the type of training that takes place. Closed-kinetic chain rehabilitation also creates an environment for the optimal development of proprioceptors. Developing proprioception is essential for precise and efficient functional performance.\textsuperscript{59}

It has been observed that focal cooling can be used to facilitate therapeutic exercise during rehabilitation without altering functional agility,\textsuperscript{26,31} or proprioception.\textsuperscript{32} During closed-chain activities, which are used during functional rehabilitation such as cryokinetics, many more joints and receptors are involved in proprioception. Clinically, these findings support cryokinetics, because there was no change in neuromuscular outcomes following focal cooling. This supports the idea that feedback and feedforward neuromuscular mechanisms of joint stability remain intact. If this is the case, then cryotherapy and cryokinetics, in particular, may be used safely prior to, during, and after therapeutic exercises.

Both spinal and supraspinal neuromuscular mechanisms appear to be involved in focal joint cooling-induced motoneuron pool facilitation.\textsuperscript{6-10} Increases in motoneuron excitability suggest that focal cooling could possibly have an effect on the afferent signaling of the peripheral nervous system and local effects on the central nervous system. Increased afferent excitatory stimulation from joint mechanoreceptors and thermoreceptors cause increased muscle activation by overriding inhibitory signals transmitted to the central nervous system.\textsuperscript{7,9} It is difficult to define neuromuscular
mechanisms at the spinal level without descending inputs from the central nervous system.

Corticospinal Response to Focal Cooling

New and advanced technology has allowed for noninvasive stimulation of the human corticospinal tract at the level of the cervicomedullary junction. Magnetic stimulation of the corticospinal tract and other motor pathways is thought to evoke a single descending volley in corticospinal axons that can be used to examine corticospinal responsiveness and motoneuron excitability. Muscles of the upper extremity, especially of distal origin like the flexor carpi radialis, have strong monosynaptic components. Since corticospinal inputs to the motoneurons are free from presynaptic inhibition, the degree of response to corticospinal axon stimulation relies on the number of descending axons activated, transmitter release at the synaptic terminals, and the responsiveness of the motoneuron pool.

Evidence for the activation of corticospinal axons by cervicomedullary stimulation comes from investigations showing that ascending antidromic potentials elicited by this stimulus collide with descending orthodromic potentials from cortical stimulation. These experiments cannot rule out the possibility that axons in other pathways were also stimulated. However, corticospinal axons are differentially susceptible to stimulation at this level, because the axons cross at the pyramidal decussation. Activation of corticospinal neurons leads to large monosynaptic compound excitatory postsynaptic potentials in the motoneurons, which innervate smaller muscles, especially intrinsic muscles of the hand and forearm. Monosynaptic input masks later
motoneuron responses to oligosynaptic input, suggesting that the responses in the muscle depend primarily on the monosynaptic pathway. Changes in the size of CMEPs in smaller muscles in the upper extremity are most likely to reproduce changes in excitability of the descending axons to stimulation, changes at the corticomotoneuronal synapse, or changes in excitability of the motoneuron.58

Clinical Relevance

Sports medicine and rehabilitation specialists can gain a valuable edge in therapeutic rehabilitation by increasing motoneuron excitability. If a motoneuron pool is inhibited, smaller amounts of motor units are recruited, resulting in a muscle with decreased functional ability. The proposed benefit of facilitation includes the ability to stimulate larger numbers of motoneurons. Many therapeutic techniques have been developed to safely facilitate neuromuscular control during rehabilitation, but these techniques are of little benefit if arthrogenic muscle inhibition exists.7

Although inconsistent, cryotherapy has been demonstrated to produce strength gains following application.9,10 The result of conflicting findings may lie within application techniques. For instance, ice immersion to the lower extremity demonstrated no differences in strength, compared to other techniques.26 Focal joint cooling using an ice bag, compared to immersion, has been shown to increase an athlete’s performance during therapeutic rehabilitation.9,10 Cold application can potentially increase range-of-motion to a joint, producing an optimal movement and coordination that is more apt to cope with alterations in the environment. At this time, establishing an effective treatment
intervention that decreases the chances for inhibition in both the lower and upper extremities will improve therapeutic rehabilitation.

Future Research

There were several limitations in this thesis. For example, H-reflex and CMEP data were only collected for the flexor carpi radialis muscle. Future research in these areas should use larger sample sizes. Also, during the control intervention, the application of a sham ice bag along with compression to the dorsum of the wrist joint should add a greater level of control. Future research should take these limitations into consideration.

Future research should continue to focus on the effects of focal joint cooling on motoneuron excitability in other muscles of the upper extremity. This investigation chose to focus on the flexor carpi radialis muscle, because limited research has been conducted in the upper extremity. While it is possible that the forearm flexor group would react similarly, it is probable that other muscles surrounding the wrist and forearm or in the upper extremity may not respond to joint cooling like the flexor carpi radialis. Research in these areas will provide further information needed to determine the relationship between focal joint cooling and neuromuscular facilitation.

Additionally, future investigations are warranted to examine means by which induced changes in motoneuron activation impact spinal pathways. Finally, further delineation of the amount of motoneuron facilitation that is beneficial for therapeutic rehabilitation purposes needs to be clarified. More research is needed to support this approach with focal cooling.
REFERENCES


APPENDIX A: MEDICAL HISTORY QUESTIONNAIRE

Name: ___________________________ Age: __________
Sex: _______ Height: _______ Weight: _______ BMI: _______

Medical History Questionnaire

Section 1. Please indicate if anyone in your family has ever had or been diagnosed with the following:
YES NO Epilepsy
YES NO Seizures

Section 2. Please check all that apply to you personally:

☐ Alzheimer’s disease ☐ Fibromyalgia
☐ Amyotrophic lateral sclerosis ☐ Fracture in the upper or lower extremity in the past six months
☐ Amputation of the upper or lower extremity ☐ Frequent faintness/dizziness
☐ Anti-depressants ☐ Frequent pains in heart/chest
☐ Anxiety disorder ☐ Gastrointestinal disorders
☐ Arthritis ☐ Head injury
☐ Asthma ☐ Heart attack
☐ Bleeding disorders ☐ Heart disease
☐ Blindness ☐ Heart murmur
☐ Body mass index ≥ to 32kg/m² ☐ Heart valve
☐ Cancer ☐ Disease/abnormality
☐ Cardiac problems ☐ History of smoking in the past six months
☐ Chronic obstructive pulmonary disease ☐ HIV
☐ Chronic pain syndrome ☐ Hypertension (BP > than 140/90)
☐ Circulation disorders ☐ Kidney disease
☐ Congestive heart failure ☐ Liver disease
☐ Coronary artery disease ☐ Macular degeneration
☐ Dementia ☐ Major psychiatric disease
☐ Depression ☐ Metal plates
☐ Diabetes ☐ Multiple Sclerosis
☐ Drug or alcohol abuse in the past six months ☐ Neurological disorder
☐ Emphysema ☐ Osteoarthritis
☐ Epilepsy ☐ Vascular heart disease

☐ 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?
☐ Do you have any orthopedic limitations such as a recent bone fracture?
☐ Is there a good reason not mentioned here why you should not perform physical activity?
☐ Do you use an assistive device to help you walk (i.e. a walker or cane)?
☐ Have you fallen more than two times in the past year?
☐ Are you currently participating in any other studies where you are receiving any treatment?

Section 3. Please list any medications or supplements that you are currently taking (include over the counter medications and/or supplements).

<table>
<thead>
<tr>
<th>Name</th>
<th>Dose</th>
<th>Reason for taking</th>
</tr>
</thead>
</table>

Section 4. Please list any surgeries or major/persistent medical problems that you have ever had including broken bones.
APPENDIX B: MAGNETIC STIMULATION QUESTIONNAIRE

WARNING: The magnetic stimulation unit may disrupt certain implants, devices, or objects and thus may be hazardous to your health or interfere with the function of the device.

Please indicate if you have any of the following:

☐ Yes  ☐ No  Aneurysm clip(s)
☐ Yes  ☐ No  Cardiac pacemaker
☐ Yes  ☐ No  Implantable cardioverter defibrillator (ICD)
☐ Yes  ☐ No  Electronic implant or device
☐ Yes  ☐ No  Magnetically-activated implant or device
☐ Yes  ☐ No  Neuromodulation implant
☐ Yes  ☐ No  Spinal cord stimulator
☐ Yes  ☐ No  Internal electrodes or wires
☐ Yes  ☐ No  Bone growth/bone fusion stimulator
☐ Yes  ☐ No  Cochlear, otologic or other ear implant
☐ Yes  ☐ No  Insulin or other infusion pump
☐ Yes  ☐ No  Implantable drug infusion device
☐ Yes  ☐ No  Any type of prosthesis (eye, penile, etc.)
☐ Yes  ☐ No  Heart valve prosthesis
☐ Yes  ☐ No  Eyelid spring or wire
☐ Yes  ☐ No  Artificial or prosthetic limb
☐ Yes  ☐ No  Metallic stent, filter, or coil
☐ Yes  ☐ No  Shunt (spinal or intraventricular)
☐ Yes  ☐ No  Vascular access port and/or catheter
☐ Yes  ☐ No  Radiation seeds or implants
☐ Yes  ☐ No  Swan-Ganz or thermodilution catheter
☐ Yes  ☐ No  Medication patch (Nicotine, Nitroglycerine)
☐ Yes  ☐ No  Any metallic fragment or foreign body
☐ Yes  ☐ No  Wire mesh implant
☐ Yes  ☐ No  Tissue expander (e.g., breast)
☐ Yes  ☐ No  Surgical staples, clips, or metallic sutures
☐ Yes  ☐ No  Joint replacement (hip, knee, etc.)
☐ Yes  ☐ No  Bone/joint pin, screw, nail, wire, plate, etc.
☐ Yes  ☐ No  IUD, diaphragm, or pessary
☐ Yes  ☐ No  Dentures or partial plates

☐ Yes  ☐ No  Hearing aid

(Remove before entering MR system room)

☐ Yes  ☐ No  Other implant ________________________

☐ Yes  ☐ No  Breathing problem or motion disorder

NOTE: You may be advised or required to wear earplugs or other hearing protection during the magnetic stimulation procedure to prevent possible problems or hazards related to acoustic noise.
The amendment, detailed below, and submitted for the following research study has been approved by the Institutional Review Board at Ohio University.

Project: Cold Pressor Test Modulates Flexor Carpi Radialis Muscle Activation

Amendment: Modify cold pressor test; Modify total time commitment; add research assistants, ICF revised, Recruitment revised.

Primary Investigator: Shawn Cameron
Co-Investigator(s):

Advisor: Bentley Krause
Department: Recreation & Sport Sciences

Robin Stack, CIP, Human Subjects Research Coordinator
Office of Research Compliance

Date: 02/23/2011

Protocol Expiration Date: 6/2/2011
APPENDIX D: PARTICIPANT DEMOGRAPHICS

Means ± Standard Deviations for Age, Height (cm), and Mass (kg)

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Height</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>23.6 ± .94</td>
<td>182.3 ± 5.1</td>
<td>96.8 ± 15.8</td>
</tr>
</tbody>
</table>

*Note. cm= centimeters; kg= kilograms*
APPENDIX E: INFORMED CONSENT

APPENDIX A
Ohio University Consent Form

Title of Research: Cold pressor test modulates flexor carpi radialis muscle activation.

Researchers: Shawn Cameron & Dr. Andrew Krause Department: School of Applied Health Sciences and Wellness – Athletic Training

You are being asked to participate in research. For you to be able to decide whether you want to participate in this project, you should understand what the project is about, as well as the possible risks and benefits in order to make an informed decision. This process is known as informed consent. This form describes the purpose, procedures, possible benefits, and risks. It also explains how your personal information will be used and protected. Once you have read this form and your questions about the study are answered, you will be asked to sign it. This will allow your participation in this study. You should receive a copy of this document to take with you.

Explanation of Study

This study compares the effects of cortical and corticospinal reorganization of the flexor carpi radialis following the cold pressor test. This experimental design compares this wrist flexor muscle activation, spinal reflexes, and cortical motor evoked potentials before and after a cold pressor test.

Inclusion Criteria:

Healthy, physically active adult males between the ages of 18-27. Physically active will be defined as engaging in physical activity at least 30 minutes a day, 3 days a week.

No history of upper extremity injury or surgery within the past year.

Passes the Magnetic Stimulation Screening (Appendix C).
Exclusion criteria:

- Alzheimer's disease
- Amyotrophic lateral sclerosis
- Amputation of the upper or lower extremity
- Anti-depressants
- Anxiety disorder
- Arthritis
- Asthma
- Bleeding disorders
- Blindness
- Body mass index ≥ to 32 kg/m²
- Cancer
- Cardiovascular problems
- Chronic obstructive pulmonary disease
- Chronic pain syndrome
- Circulation disorders
- Congestive heart failure
- Coronary artery disease
- Deafness
- Depression
- Diabetes
- Drug or alcohol abuse in the past six months
- Emphysema
- Epilepsy
- Failure to give consent
- Fibromyalgia
- Fracture in the upper or lower extremity in the past six months
- Frequent faintness/dizziness
- Frequent pains in heart/chest
- Gastrointestinal disorders
- Head injury
- Heart attack
- Heart disease
- Heart murmur
- Heart valve disease or abnormality
- History of smoking in the past six months
- HIV
- Hypertension (BP > than 140/90)
- Kidney disease
- Liver disease
- Macular degeneration
- Major psychiatric disease
- Metal plates
- Multiple Sclerosis
- Neurological disorder
- Osteoarthritis
- Orthopedic limitation
- Other psychiatric disorder
- Pacemaker
- Parkinson's disease
- Participation in another study that requires treatment or intervention
- Peripheral vascular disease
- Personal or family history of blood clotting
- Reynaud's disease
- Renal disease
- Rheumatoid arthritis
- Seizures
- Severe anemia
- Sickle cell anemia
- Spasticity
- Stroke (< six months)
- Traumatic head injury
- Unable to obtain high quality electrical recordings from their muscle using surface electrodes or unable to observe spinal reflex responses
- Unsafe to enter a strong magnetic field
- Valvular heart disease
Total time commitment for the entire study is approximately 4 hours
(2 hours on 2 separate days)

Testing:
1. You will complete a 10 minute warm up on a recumbent exercise bike at a pace that is comfortable for you.

Muscle Force:
2. You will then sit in a Biodex dynamometer chair in a seated position with the forearm secured with a strap (Figure 1. Data Collection Set up). The Biodex dynamometer measures the amount of force produced by a muscle/muscle group (in this study: the flexor carpi radialis muscle). By flexing your wrist against a fixed attachment, you will then be asked to contract your wrist flexor muscle as hard as you can against an isometric resistance.

3. You will have 3 sEMG adhesive electrodes placed over the belly of your wrist flexor muscles of your dominant arm and complete several trial voluntary isometric grip strength contractions on the Biodex to acclimate to the dynamometer and the amount of force that they will produce.

4. You will have 2 small stimulating electrodes affixed to your wrist flexor muscle belly above and below the sEMG electrodes. Measurement collection will begin, as you will then be asked to contract your wrist flexor muscle as hard as you can against an isometric resistance.

Pre-Testing (Baseline):

Muscle Strength and Central Activation (CAR – 20 minutes): You will be seated in a chair that attaches your forearm securely to a torque motor (BioDex Dynamometer). You will be asked to flex your wrist muscles as hard as possible against a rigid restraint to determine how strong your muscles are. Around 3 totals of each strength test will be performed within 1-2 minutes between trials. We will also determine what percentage of total force generating capacity of the wrist flexors can produce voluntarily, by using a combination of voluntary and electrically stimulated contractions. Here, electrical nerve stimulation to the median nerve will be delivered (two pulses separated by 10-msec) during a maximal contraction as well as 1-2 seconds after the contraction. Around 2 trials of this protocol will be performed. The electrical stimulation will consist of delivering a short duration electrical pulse. All stimuli will be brief (<1sec), and the stimulation intensity will be first increased until a maximal stimulus is obtained (no further increase in the EMG or force response despite an increase in stimulus intensity).

Hoffmann Reflex and Muscle Response (H-reflex – 10 minutes): Hoffmann reflexes (Hmax) and maximum compound action potential (Mmax) recruitment curves will be elicited using 1ms square pulse stimuli to the median nerve. Hmax and Mmax values were recorded and the Hmax:Mmax ratios will be calculated to normalize Hmax values.
5. EMG signals will be collected with both the Bioac system and cervicomедullary motor evoked potentials.

*Cervicomедullary motor evoked potentials (CMEP – 20 minutes):* CMEP induces an electrical current in human tissue by a strong magnetic stimulation performed via a BiStim® stimulator (Magstim Inc., Woburn, MA) using a double-cone coil between electrodes fixed over the mastoid processes can evoke CMEPs in the muscles of the upper limb. A high-voltage magnetic pulse (30-100 μs duration, up to 750 V) will be passed across the spinal cord between electrodes at levels between 2 cm above and 4 cm below the bottom of the mastoids.

**Treatment/Intervention:**

6. You will then have a ~.75 Liter ice pack wrapped comfortably to the back (dorsum) of your wrist for 15 minutes. Measurements will be taken at baseline, immediately post treatment, and 15 minutes post treatment.

**Post – Testing:**

7. After completion of the test, you will once again contract your wrist flexor muscle as hard as you can against isometric resistance and CAR, H-reflex, and CMEP post-treatment measurements will be recorded.

8. When you have completed all of the trials, you will be free to go. Prior to leaving the lab, you will be reminded that if you experience any discomfort following testing to contact the PI directly.

**Contact Information:**

Shawn Cameron  
(617) 763 – 0678 (mobile)  
Sc265809@ohio.edu (email)

**Risks and Discomforts**

Potential risks or discomforts that you may experience range from minimal to moderate.

- You may experience post-exercise muscle soreness due to maximal voluntary contraction; this risk is minimal.

- Should you have discomfort while performing the wrist flexion exercise, you may terminate the session at any time. Should you feel that the protocol is too painful, discomforting or too hard, you may terminate at any time and for any reason without penalty.

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- The sticky electrodes placed on your arm may cause skin irritation. This irritation generally subsides after a few days.

- You may experience some pain/discomfort during the testing. For example, intermittent electrical stimulation to a nerve results in some mild discomforts as the magnetic coil is engaged. This discomfort will be extremely brief (milliseconds), but there are no lasting effects.

- The magnetic coil placed over the base of the skull, posteriorly, will induce an electric current and make the muscles contract is mildly uncomfortable, but it is frequently described as "weird." When applied a sharp 'clicking' sound is heard that could cause a subject's ears to ring. To minimize this effect, ear plugs will be given out to reduce noise. More common complaints during or following this procedure are neck pain or headache. It is unknown if these complaints are due to having to sit still or due to the muscles contracting and creating a tension-type headache. These symptoms may occur in a small percentage of subjects tested and typically dissipate within 24 hours.

- The magnetic coil elicits a magnetic field, so you should not participate in this study if you have any metallic or electrical objects in or on your body (i.e. cardiac pacemakers, metal plates). There is no radiation exposure associated with this.

- There is a theoretical chance that the magnetic stimulation could cause a seizure, although this has never been known to occur using the device in the matter that we will in healthy people. However, if you have a predisposition to seizures or are taking anti-depressant medications you may be susceptible. Therefore, if you have a family history of seizures or epilepsy, or are taking anti-depressant medications, or have experienced a traumatic head injury you should not participate in this study.

- Prior to leaving the lab you will be reminded that if you experience any discomfort following testing to contact the PI directly.

**Benefits**

There are no known benefits to you. For society this study will show how an isometric contraction exercise with and without ice will hopefully increase the knowledge about muscle tissue temperature responses to cooling, the effect of exercise before cooling on muscle tissue temperature.
Confidentiality and Records

All participant information will remain confidential throughout the study and will not be shared with anyone else other than the investigators of this study.

Each participant will have an assigned specific code number for the study. They key codes to connect the participant’s code number to the data files will be destroyed as soon as the connection has been made between data sets by using a micro-shredder. The signed informed consent and data records will be stored for three years after the completion of the study.

All files including confirmed consent, key codes linking participants to the data and the data itself will be stored in a locked cabinet in Dr. Krause’s office. Other investigators will have access to this office if needed Monday through Friday 8:00am to 5:00pm.

Additionally, while every effort will be made to keep your study-related information confidential, there may be circumstances where this information must be shared with:

* Federal agencies, for example the Office of Human Research Protections, whose responsibility is to protect human subjects in research;
* Representatives of Ohio University (OU), including the Institutional Review Board, a committee that oversees the research at OU;

Contact Information

If you have any questions regarding this study, please contact Shawn Cameron (617) 763 - 0678 or email me at sc265809@ohio.edu. You can also contact Dr. Andrew Krause at (740) 593 - 4648 or you may email him at krausea@ohio.edu. 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.

By signing below, you are agreeing that:
* You have read this consent form (or it has been read to you) and have been given the opportunity to ask questions
* Known risks to you have been explained to your satisfaction
* You understand Ohio University has no policy or plan to pay for any injuries you might receive as a result of participating in this research protocol
* You are 18 years of age or older
* Your participation in this research is given voluntarily
* You may change your mind and stop participation at any time without penalty or loss of any benefits to which you may otherwise be entitled.

Signature ________________________________ Date ________________________________

Printed Name ___________________________________________ Version Date: [February/25/2011]

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## APPENDIX F: RAW DATA OF $H_{\text{max}}$ and $M_{\text{max}}$ FOR ALL PARTICIPANTS

**Raw Data for Peak-to-Peak (V) $H_{\text{max}}$ and $M_{\text{max}}$ Sessions**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Intervention</th>
<th>Pretreatment $H_{\text{max}}$</th>
<th>Pretreatment $M_{\text{max}}$</th>
<th>Posttreatment $H_{\text{max}}$</th>
<th>Posttreatment $M_{\text{max}}$</th>
<th>15 Min Post $H_{\text{max}}$</th>
<th>15 Min Post $M_{\text{max}}$</th>
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</thead>
<tbody>
<tr>
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<td>5.16</td>
<td>9.54</td>
<td>5.68</td>
<td>10.11</td>
<td>4.18</td>
<td>9.21</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.54</td>
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<td>3.77</td>
<td>7.37</td>
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APPENDIX G: RAW DATA OF CMEP FOR ALL PARTICIPANTS

*Raw Data for Peak-to-Peak (V) CMEP Sessions*

<table>
<thead>
<tr>
<th>Participant</th>
<th>Intervention</th>
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<th>Posttreatment</th>
<th>15 Min Post</th>
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