### THE PENNSYLVANIA STATE UNIVERSITY SCHREYER HONORS COLLEGE

### DEPARTMENT OF KINESIOLOGY

# COMPARISON OF CONTINUOUS AND INTERMITTENT ICE TREATMENTS AFTER MUSCLE CONTUSION INJURY USING MAGNETIC RESONANCE IMAGING

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A thesis submitted in partial fulfillment of the requirements for a baccalaureate degree in Kinesiology with honors in Kinesiology

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### ABSTRACT

Comparison of Continuous and Intermittent Ice Treatments After Muscle Contusion Injury Using Magnetic Resonance Imaging

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Context: Several recent studies have investigated cryotherapy and its role in the treatment of secondary injury. These studies have used widely varying ice treatment protocols in their study designs. Ice treatment protocols generally fall into two categories: continuous, in which ice is applied for a set length of time and then removed, and intermittent, in which ice is generally applied for shorter amounts of time, but is reapplied several times over the treatment course. To date, continuous and intermittent icing protocols have not been compared. Further research comparing continuous and intermittent icing protocols must be conducted to determine which method provides the greatest therapeutic benefits. **Objective:** To compare two icing protocols (continuous vs. intermittent) in a rat model following contusion of the gastrocnemius by using MRI T2 scans and volumetric calculations. It was hypothesized that the continuous icing protocol would induce less swelling as measured by a lesser change in limb volume and T2 relaxation time, which indicate that edema and inflammation have occurred. Design: T2 MRI scans were taken 0, 2, 4, and 6 hours after injury. The continuous icing protocol involved ice application throughout the entire 6-hour experiment; the intermittent icing protocol involved a pattern of 30 minutes of icing, followed by 60 minutes of no ice, completed 4 times over the 6-hour experiment. T-tests were used to analyze statistical differences between icing protocols and limb status. Setting: Controlled laboratory environment. Subjects: 20 male Wistar rats  $(272g \pm 29 g)$  procured from Harlan-Sprague Dawley (Indianapolis, IN). Interventions: Icing protocol (continuous vs. intermittent), limb status (healthy vs. injured) and time point (0, 2, 4, and 6 hours after injury). Main Outcome Measures: T2 signal was measured throughout the experiment to observe fluid accumulation in the limb due to edema and hemorrhage. Limb volume changes were calculated using AMIRA 3D imaging software (Mercury Computer Systems, Inc., Chelmsford, MA, USA). Data were analyzed using T-Tests to identify within and between group differences. Results: Significant differences existed between the injured and healthy limbs in both protocols except at baseline for both T2 and volume. A significant difference in T2 was found between the two protocols at 2 hours and over the 2-to-4 hour timespan post-injury. While continuous T2 relaxation time significantly increased over all timespans, intermittent T2 relaxation time change was not significant over the 2-to-4 and 4-to-6 hour timespans. Significant changes in volume occurred in the intermittent protocol until the 4-to-6 hour timespan, while significant changes occurred over all timespans in the continuous timespan. A significant difference in limb volume occurred between the two protocols in the injured limb over the 4-to-6

hour timespan. Conclusion: Our findings suggest that no statistically significant difference exists between the two icing protocols, although different swelling patterns may have occurred between the two protocols. This indicates that the intermittent protocol would be a better therapeutic choice, as it can elicit the same swelling responses as continuous icing while placing a patient at a lower risk for developing cold-related injuries. Furthermore, because of its less extreme icing schedule, the intermittent protocol may be superior in maintaining patient compliance.

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#### Chapter 1

## Introduction

Cryotherapy is a technique used commonly by clinicians to lower tissue temperature after acute muscle injury. Cryotherapy involves several methods, the most common being ice, cold packs, and ice massage.<sup>1</sup> Cryotherapy is used frequently by medical professionals, as it is an inexpensive, safe, and well-tolerated treatment for soft tissue injuries, especially skeletal muscle contusions.<sup>2,3</sup> The therapeutic effect of cryotherapy is achieved through tissue cooling. The magnitude of tissue cooling that results from cryotherapy is largely influenced by the cryotherapy technique, the duration of therapy, the muscle temperature prior to therapy, and the thickness of subcutaneous fat.<sup>4</sup> Cryotherapy aims to achieve several treatment goals, which include reduction of inflammation, limitation of edema, reduction of secondary injury within the muscle, and reduction of pain experienced by the patient.<sup>1,4-6</sup> These beneficial effects are achieved by influencing several biological processes that occur within the body after injury.

Cryotherapy addresses primary problems related to muscle damage by decreasing local blood circulation, thereby positively impacting inflammation, edema formation, and hemorrhage effects at the injury site.<sup>1,5,6</sup> In addition, cryotherapy helps reduce pain by depressing peripheral nerve ending sensitivity, resulting in reduced transmission of pain signals from nociceptors and an increased pain threshold.<sup>4,6</sup>

The secondary injury model explains how muscle injury from mechanical trauma can lead to damage in surrounding cells that were otherwise unaffected by the mechanical trauma. The additional damage develops from a series of physiological processes that stem from the negative primary effects of tissue injury.<sup>7</sup> The inflammation responses caused by injury can lead to muscle tissue swelling and increases in intramuscular pressure, which causes further muscle damage. These effects can induce ischemia, leading to hypoxic conditions within the muscle and waste material buildup in otherwise healthy cells.<sup>8</sup> These waste materials include intracellular chemicals and free radicals that, if unchecked, can irritate and damage surrounding healthy cells, thereby spreading the injury beyond the original contusion.<sup>3</sup>

In addition to addressing the effects of the inflammation process associated with the primary muscle injury, cryotherapy helps limit secondary injury by controlling inflammation and reducing the metabolic rate of undamaged cells.<sup>3</sup> Tissue damage often occurs due to the reperfusion of hypoxic tissue, which introduces free radicals to healthy tissue. By reducing the metabolic rate, healthy cells consume less oxygen and produce fewer free radicals, which in turn prevents further damage.<sup>5</sup> The lesser metabolic rate allows the cells to survive hypoxic conditions for longer periods of time.<sup>9</sup> Moreover, cryotherapy induces local vasoconstriction, which limits fluid leaking into interstitial tissues and pressure increases within the muscle.<sup>3</sup>

The efficacy of cryotherapy in the field of physical rehabilitation has recently been scrutinized<sup>8,10</sup>; however, systematic reviews of cryotherapy-based research have demonstrated that, while past experiments may have methodological weaknesses, cryotherapy does seem to have a positive effect, both with regard to pain relief and return to activity outcomes.<sup>2,11,12</sup> It is possible that the discrepancies between studies with regard to effectiveness have resulted from the wide variety of ice treatments and

protocols used, as studies often differ in cryotherapy technique, duration of treatment, area of treatment, and the thickness of subcutaneous fat layers in subjects.<sup>1,4,5,13</sup> Research efforts to study these variables in cryotherapy are warranted to improve the clinical use of cryotherapy. In order to standardize clinical cryotherapy treatment, it is important to identify the most effective ice application protocol while reducing the risk for cold-related injury, such as frostbite.<sup>1</sup>

Schaser et al.<sup>3</sup> conducted a study in rats to investigate cryotherapy effects during long-term ice treatment. After a 6-hour ice treatment, cryotherapy was shown to reduce injury site vascular permeability, limit inflammation caused by leukocyte-perpetuated tissue damage, and improve muscle tissue survival.<sup>3</sup> Although this procedure provided beneficial effects, its treatment protocol differs from conventional protocols, during which cold is applied 20 to 30 minutes at a time.<sup>9</sup> Patient compliance and potential for cold-related injury may limit the use of this protocol in humans.

More traditional intermittent protocols have also been investigated. Bleakley et al.<sup>13</sup> compared the effectiveness of two intermittent protocols (one consisting of 20 minutes of icing every two hours, the other consisting of an intermittent treatment of 10 minutes of ice followed by 10 minutes of rest) in relieving pain related to ankle sprain. Patients who underwent the 10-minute cycle protocol experienced less pain after treatment; however, functional differences between the two groups were insignificant. This protocol may have maintained lower tissue temperatures over a longer time period, which may have preserved the analgesic effects of cold in these participants. To date, continuous and intermittent icing protocols have only been compared by Hochberg.<sup>14</sup>

circumference more than an intermittent ice protocol in carpal tunnel release patients. Differences in injury type and ice treatment protocols prevent Hochberg's work from being directly being compared to this thesis; therefore, the extrapolation of these results to work for muscle contusion injury patients is invalid. Further research comparing continuous and intermittent icing protocols must be conducted to determine which method provides the greatest therapeutic benefits for different types of musculoskeletal injury.

The aim of this study is to utilize magnetic resonance imaging (MRI) technology to compare the effectiveness of two ice treatments (continuous and intermittent) in a rat model. Magnetic resonance imaging scans were taken before and after an acute muscle contusion injury and muscle volume changes were measured to identify which treatment best limits swelling. Magnetic resonance imaging was used because of its ability to create high-resolution T2 maps, which detect the amount of water within an image. This was helpful because the T2 relaxation time for extracellular fluid is different than fluid within healthy tissue; therefore, MRI allows for measurement of increases in extracellular fluid due to trauma. MRI was also used to accurately measure limb volume via segmentation. For more information concerning MRI and its uses, please consult the Literature Review, page 36. The purpose of this study was to identify which icing protocol limits inflammation and resultant swelling, therefore suggesting a greater therapeutic effect. Based on the findings noted by Hochberg<sup>14</sup>, it was hypothesized that the continuous icing protocol would result in less swelling, as measured by a lesser change in limb volume, than the intermittent protocol.

### Chapter 2

# Methods

### Subjects

Twenty male Wistar rats  $(272g \pm 29 \text{ g})$  were studied and all experimental procedures were approved by the University Laboratory Animal Care and Use Committee. The rats were procured from Harlan-Sprague Dawley (Indianapolis, IN), and are descended from animals of the Wistar Institute (Philadelphia, PA). This breed of rat is specially bred to ensure all animals are as identical as possible, including limb symmetry. Animals were housed two per cage fed a commercial rat chow and water ad libitum, and kept on a 12:12-hour light:dark cycle.

## **Injury Model**

An acute injury was induced using a custom-built contusion device (Figure 1); a pilot study verified the reliability and validity of the device.<sup>15</sup> On the day of injury, the hind limb was shaved and the midbelly of the gastrocnemius was marked by identifying the middle of the calf through palpation. The animal was then placed prone into the injury device and secured with Velcro® strap as previously described.<sup>15</sup> A free-falling load (mass: 267 g) was dropped from 60 cm and impacted the leg at the marked region. This weight and drop height were shown to produce the ideal muscle contusion injury in

rat hind limbs in a previous study.<sup>15</sup> Only one of the hind limbs was injured thereby leaving the contralateral limb as an internal control. The side of the injured limb (left and right) was sequentially alternated to allow for an even distribution for each condition.

### **Cryotherapy Protocol**

After injury, each rat randomly received one of two ice treatment protocols: continuous or intermittent. Two rats were injured per day of research; one was assigned the continuous treatment while the other was assigned the intermittent treatment. Crushed ice was placed in small plastic bags and was applied to the injury site and secured with surgical tape. The continuous treatment protocol involved constant icing, with ice bag replacement occurring as needed over 6 hours. In contrast, the intermittent treatment protocol involved a pattern of 30 minutes of icing, followed by 60 minutes of no ice, completed 4 times over the 6-hour experiment.

#### Measures

One baseline T2 MRI scan of each rat was performed the day before injury. Prior to injury and every scan, animals were anesthetized using an inhaled isoflurane/oxygen mix (5% isoflurane and 1000 ml  $O_2$  initially, then reduced to 3% isoflurane and 500 ml  $O_2$  once the animals achieved stable sleep) administered via a nose cone. An acute injury was induced using the contusion device described previously. After injury, each rat received one of two ice treatments. Crushed ice was placed in small plastic bags and was applied to the injury site as described in the treatment protocol. T2 scans of both the injured and healthy hind limb muscles were performed at 2, 4, and 6 hours after injury. Fifty-six slices were collected at the resolution of 215 x 194 x 500 mm<sup>3</sup> over five spin echo sequences. Echo times increased over each sequence (11, 20, 30, 40, and 60 ms). A final spin echo sequence with an echo time of 11 ms was completed to be a control value for volume calculations. Animal temperature was monitored using a rectal temperature probe (SA Instruments Inc., Stony Brook, NY, USA) and respiratory rate was monitored throughout the study. All scans were conducted using a Varian 7 Tesla horizontal MRI unit and a 6 cm diameter birdcage resonator.

Due to financial constraints stemming from the MRI running costs, only one baseline T2 scan was completed. Because T2 relaxation time measurements require data from five T2 scans, T2 data could not be calculated at baseline. To overcome this, it was assumed that the average T2 relaxation time found in the healthy limb at time points 2, 4, and 6 hours would accurately represent baseline T2 data since the healthy limb should not have changed over the experiment. This average T2 data was used as the baseline value for both the healthy and injured limbs, since these limbs were assumed to be similar at this timepoint. This latter assumption is common in animal research as the animals are genetically inbred to ensure similarity and has been used in past research studies.<sup>16,17</sup> In contrast to T2 relaxation time data, limb volume can be determined after only one T2 scan via segmentation. As a result, limb volume data was collected for all timepoints and for all limbs. In order to estimate the extent of injury and treatment influence based on limb volume, the volume of the limb was measured using 3D data visualization and reconstruction software (AMIRA 3D imaging software (Mercury Computer Systems, Inc., Chelmsford, MA, USA)). Segmentation began at the slice below the knee joint and included the next thirty slices to generate volume information for healthy and injured hind limbs.

### **Statistical Analyses**

Normal probability plots were computed to determine if the data met the assumptions for t-tests. The plots demonstrated that the data points were normally distributed. Thus, the use of t-tests was appropriate for statistical analyses. Separate one-tailed, paired t-tests were used to identify within group (healthy versus injured) differences per timepoint for each treatment protocol. Analyses of T2 relaxation time at baseline in the continuous and intermittent groups were not completed due to the assumptions in limb T2 mentioned previously. We used paired t-tests to assess bilateral differences because all bilateral measures were conducted on the same subject and because clinical convention is to compare the injured to the uninjured side assuming dependence. All group comparisons only consisted of two levels of the independent variable. Separate one-tailed, two-sample t-tests were used to identify between group (continuous versus intermittent) differences per timepoint for both the healthy and injured limbs. The a priori significance level was defined as  $p \leq 0.05$ .



Figure 1: Custom-made Muscle Contusion Device.

The weight is raised to a specific height (A) and is released upon the push of a button. The weight falls onto a steel plunger, which induces the muscle contusion injury (B).



Figure 2: Volumetric Segmentation of an Injured Limb Using AMIRA

MRI of injured limb volume with continuous treatment at 2 hours post-injury. The purple area indicates the area used to calculate limb volume from 30 adjacent slices.

### Chapter 3

### Results

### **T2 Relaxation Time**

T2 relaxation time can be utilized as a measure of tissue damage with MRI because it quantifies increases in extracellular water due to edema. T2 is able to measure this change, because the relaxation time of extracellular water is longer than the water within tissues.<sup>16,18</sup> T2 relaxation time increases in areas of high swelling as a result of this effect (Figure 3). Bilateral differences of T2 relaxation time existed between the healthy and injured limbs at 2, 4, and 6 hours post-injury for both the continuous and intermittent icing protocol groups (Table 1, Figure 4). Baseline measures were assumed to be identical for the healthy and injured limbs of each rat. Between protocol differences existed only in the injured limb at the 2 hour timepoint. No other significant differences in T2 relaxation time were found at any timepoint between the intermittent and continuous icing protocols for the healthy and injured limbs (Table 2).

Between group t-tests of T2 relaxation time change scores were calculated by sequentially subtracting the measure of the prior timepoint from the measure of the subsequent timepoint for each time interval. Bilateral change scores were significantly different for both treatment protocols except for the 2-to-4 and 4-to-6 hour timespans in the intermittent group (Table 3, Figure 5). Furthermore, the intermittent icing protocol showed a lower T2 relaxation time from 2-to-4 hours post-injury compared to continuous

icing (Table 4, Figure 5). Only one significant relationship was found using this test; the timespan from 2 to 4 hours showed a significant difference in T2 relaxation time change between the two injured populations. All other change score between group analyses were not significant.



Figure 3: Representative T2 Maps for Each Timepoint Visually Indicating Extracellular Fluid Accumulation in Both Icing Protocols.

The left leg was injured in the intermittent images, while the right leg was injured in the continuous images. The red area superior to the injured leg in the continuous sample is an ice bag.

60 50 40 Mean T2 (ms) 30 т 20 10 0 0 2 4 6 Timepoint (hours) Healthy Continuous Injured Continuous Healthy Intermittent Injured Intermittent

# Figure 4: Mean Limb T2 Relaxation Time at Each Timepoint.



Figure 5: Mean T2 Relaxation Time Change Over Experimental Timespans.



Mean T2 Relaxation Time Change Over Experimental Timespans

■ Healthy Continuous
■ Injured Continuous
■ Healthy Intermittent
■ Injured Intermittent
\* Denote significant differences (p ≤ 0.05).

### **Limb Volume**

Limb volumes were calculated based on 3D images processed from the MRI. Limb volumes were converted from pixels into mm<sup>3</sup> by using the formula:

Volume 
$$(mm^3) = 0.0176 * pixels$$

Limb volume increased in the injured leg due to the swelling effects of muscle injury (Figure 6). Bilateral differences of limb volume existed between the healthy and injured limbs at 2, 4, and 6 hours post-injury for both the continuous and intermittent icing protocol groups (Table 1, Figure 7). No significant differences in baseline limb volume were found. No significant differences in limb volume were found at any timepoint between the intermittent and continuous icing protocols for the healthy and injured limbs (Table 2).

Limb volume change scores were calculated by sequentially subtracting the measure of the prior timepoint from the measure of the subsequent timepoint for each time interval. Bilateral change scores were significantly different for both treatment protocols except for the 4-to-6 hour timespan in the intermittent group (Table 3, Figure 8). One significant between protocol group change score was found in the injured limb at the 4-to-6 hour timespan (Table 4, Figure 8). All other change score comparisons were not significant.



Figure 6: MR Images from Each Time Point Visually Indicating Tissue Swelling in Both Icing Protocols.

The left leg was injured in the intermittent images, while the right leg was injured in the continuous images. The large white area superior to the injured leg in the continuous sample is an ice bag.



Figure 7: Mean Limb Volume at Each Time Point for Both Icing Protocols.

Mean Limb Volume At Each Timepoint

Figure 8: Mean Limb Volume Change Over Experimental Timespans.



Mean Limb Volume Change Over Experimental Timespans

		- ``	•	
Protocol	Timepoint	Healthy (Mean±SD)	Injured (Mean±SD)	P-Value
T2 (ms)				
Continuous	Baseline	27.0 ± 0.7	27.0 ± 0.7	
	2	26.7 ± 0.7	40.7 ± 1.4	<0.001*
	4	27.2 ± 0.9	43.4 ± 2.1	<0.001*
	6	27.2 ± 0.5	44.4 ± 2.0	<0.001*
Intermittent	Baseline	27.6 ± 0.8	27.6 ± 0.8	
	2	27.4 ± 1.0	43.0 ± 3.6	<0.001*
	4	27.9 ± 1.0	44.0 ± 3.2	<0.001*
	6	27.6 ± 0.9	45.4 ± 4.3	<0.001*
Volume (mm <sup>3</sup> )				
Continuous	Baseline	3595.7 ± 382.3	3602.8 ± 337.3	0.467
	2	3536.4 ± 433.5	5098.0 ± 518.9	<0.001*
	4	3474.6 ± 384.0	5229.4 ± 492.9	<0.001*
	6	3479.5 ± 345.9	5355.1 ± 500.3	<0.001*
Intermittent	Baseline	3524.8 ± 523.8	3391.9 ± 513.7	0.089
	2	3518.6 ± 529.6	4991.7 ± 816.3	<0.001*
	4	3552.8 ± 536.5	5221.6 ± 799.3	<0.001*
	6	3477.1 ± 482.5	5215.8 ± 855.9	<0.001*

Table 1: Limb Status T2 and Volume Comparisons (n=18)

Note: P-values were omitted for T2 baseline measurements due to the assumptions outlined in the Methods. \* Denote significant differences ( $p \le 0.05$ ).

		Continuous	Intermittent	
Limb	Timepoint	(Mean±SD)	(Mean±SD)	P-Value
T2 (ms)				
Healthy	Baseline	27.0 ± 0.7	27.6 ± 0.8	0.059
	2	26.7 ± 0.7	27.4 ± 1.0	0.059
	4	27.2 ± 0.9	27.9 ± 1.0	0.082
	6	27.2 ± 0.5	$27.6 \pm 0.9$	0.132
Injured	Baseline	27.0 ± 0.7	27.6 ± 0.8	0.059
	2	40.7 ± 1.4	$43.0 \pm 3.6$	0.046*
	4	43.4 ± 2.1	44.0 ± 3.2	0.319
	6	44.4 ± 2.0	45.4 ± 4.3	0.262
Volume (mm <sup>3</sup> )				
Healthy	Baseline	3595.7 ± 382.3	3524.8 ± 523.8	0.374
	2	3536.4 ± 433.5	3518.6 ± 529.6	0.469
	4	$3474.6 \pm 384.0$	3552.8 ± 536.5	0.363
	6	3479.5 ± 345.9	3477.1 ± 482.5	0.495
Injured	Baseline	3602.8 ± 337.3	3391.9 ± 513.7	0.159
	2	5098.0 ± 518.9	4991.7 ± 816.3	0.373
	4	5229.4 ± 492.9	5221.6 ± 799.3	0.490
	6	5355.1 ± 500.3	5215.8 ± 855.9	0.340

Table 2: Icing Protocol T2 and Volume Comparisons (n=18)

Treatment Protocol	Timespan (T <sub>Late</sub> -T <sub>Early</sub> )	Healthy Change (mean±SD)	Injured Change (mean±SD)	P-Value
T2 (ms)				
Continuous	Baseline to 2	$-0.4 \pm 0.2$	13.7 ± 1.3	<0.001*
	Baseline to 4	$0.2 \pm 0.3$	16.4 ± 2.0	<0.001*
	Baseline to 6	$0.2 \pm 0.2$	17.3 ± 2.2	<0.001*
	2 to 4	$0.6 \pm 0.5$	2.7 ± 1.6	<0.001*
	2 to 6	$0.6 \pm 0.4$	3.7 ± 1.5	<0.001*
	4 to 6	0.0 ± 0.5	0.9 ± 1.2	0.009*
Intermittent	Baseline to 2	-0.3 ± 0.6	15.4 ± 3.7	<0.001*
	Baseline to 4	$0.3 \pm 0.7$	16.4 ± 3.4	<0.001*
	Baseline to 6	$0.0 \pm 0.6$	17.8 ± 4.5	<0.001*
	2 to 4	0.5 ± 1.2	$1.0 \pm 0.9$	0.238
	2 to 6	$0.3 \pm 0.8$	2.4 ± 2.9	0.033*
	4 to 6	-0.3 ± 1.2	1.4 ± 3.0	0.079
Volume (mm <sup>3</sup> )				
Continuous	Baseline to 2	-59.3 ± 201.4	1495.2 ± 203.8	<0.001*
	Baseline to 4	-121.1 ± 216.2	1626.6 ± 195.8	<0.001*
	Baseline to 6	-116.2 ± 201.5	1752.2 ± 199.5	<0.001*
	2 to 4	-61.8 ± 147.9	131.4 ± 188.4	<0.001*
	2 to 6	-56.9 ± 210.7	257.1 ± 175.7	<0.001*
	4 to 6	4.9 ± 168.2	125.7 ± 164.0	0.012*
Intermittent	Baseline to 2	-6.1 ± 199.6	1599.7 ± 445.3	<0.001*
	Baseline to 4	28.1 ± 164.2	1829.7 ± 439.6	<0.001*
	Baseline to 6	-47.7 ± 256.4	1823.8 ± 485.2	<0.001*
	2 to 4	34.2 ± 81.8	230.0 ± 138.6	<0.001*
	2 to 6	-41.5 ± 167.4	224.1 ± 187.1	0.002*
	4 to 6	-75.8 ± 137.9	-5.9 ± 104.3	0.118

Table 3: Limb Status T2 and Volume Change Per Timespan Comparisons (n=18)

Limb	Timespan (T <sub>Late</sub> -T <sub>Early</sub> )	Continuous Change (mean±SD)	Intermittent Change (mean±SD)	P-Value
T2 (ms)				
Healthy	Baseline to 2	-0.4 ± 0.2	$-0.3 \pm 0.6$	0.267
	Baseline to 4	$0.2 \pm 0.3$	$0.3 \pm 0.7$	0.387
	Baseline to 6	$0.2 \pm 0.2$	$0.0 \pm 0.6$	0.226
	2 to 4	$0.6 \pm 0.5$	0.5 ± 1.2	0.454
	2 to 6	$0.6 \pm 0.4$	$0.3 \pm 0.8$	0.184
	4 to 6	$0.0 \pm 0.5$	-0.3 ± 1.2	0.293
Injured	Baseline to 2	13.7 ± 1.3	15.4 ± 3.7	0.101
	Baseline to 4	16.4 ± 2.0	$16.4 \pm 3.4$	0.489
	Baseline to 6	17.3 ± 2.2	17.8 ± 4.5	0.394
	2 to 4	2.7 ± 1.6	$1.0 \pm 0.9$	0.006*
	2 to 6	3.7 ± 1.5	2.4 ± 2.9	0.124
	4 to 6	0.9 ± 1.2	1.4 ± 3.0	0.350
Volume (mm <sup>3</sup> )				
Healthy	Baseline to 2	-59.3 ± 201.4	-6.1 ± 199.6	0.291
	Baseline to 4	-121.1 ± 216.2	28.1 ± 164.2	0.059
	Baseline to 6	-116.2 ± 201.5	-47.7 ± 256.4	0.269
	2 to 4	-61.8 ± 147.9	34.2 ± 81.8	0.054
	2 to 6	-56.9 ± 210.7	-41.5 ± 167.4	0.433
	4 to 6	4.9 ± 168.2	-75.8 ± 137.9	0.141
Injured	Baseline to 2	1495.2 ± 203.8	1599.7 ± 445.3	0.265
	Baseline to 4	1626.6 ± 195.8	1829.7 ± 439.6	0.112
	Baseline to 6	1752.2 ± 199.5	1823.8 ± 485.2	0.344
	2 to 4	131.4 ± 188.4	230.0 ± 138.6	0.112
	2 to 6	257.1 ± 175.7	224.1 ± 187.1	0.352
	4 to 6	125.7 ± 164.0	-5.9 ± 104.3	0.030*

Table 4: Icing Protocol T2 and	Volume Change Per	Timespan Comparisons (n=18	;)

#### Chapter 4

### Discussion

T2 relaxation time and limb volume can both be utilized as measures of tissue damage. The vasodilation and increased vascular permeability that occur with the inflammatory process after an injury result in increased fluid in the limb and the interstitial tissues. The increased water content in the limb as a result of hemorrhage and edema formation produces increased limb volume. T2 relaxation times lengthen as a result of increased extracellular water within tissues.<sup>16,18</sup> Since edema is the result of increase as a result. T2 relaxation time and volume have both been successfully used in past studies to evaluate muscle swelling due to a variety of injuries.<sup>15,16,19-23</sup>

No significant differences were found in the healthy limb T2 and volume measurements at any timepoint or over any timespan. Furthermore, no significant differences in baseline T2 relaxation time and volume were found between healthy and injured limbs. These results demonstrated no cross-over effect of post-injury swelling and justified our use of average T2 relaxation time measures from the healthy limb as the baseline measure for the injured limb.

As expected, increased water content was detected in the injured limb of all rats but not in the healthy limb. This increase in water content, indicating swelling, significantly increased both the T2 relaxation time and the limb volume in the injured limb while the healthy limb revealed no significant changes in either measure. When all injured limbs were combined, significant increases in T2 relaxation time and limb volume were measured at hours 2, 4, and 6. This data supports the conclusion that the injury device successfully induced a muscle contusion injury and the associated physiological effects within the subject population for both treatment protocols and that the limbs responded as expected to muscle injury. This effect is consistent with the outcome created during the injury device validation study.<sup>15</sup> The results also agree with other studies found in the literature, which report injury after exercise induced muscle swelling<sup>24</sup> and that muscle injury leads to a longer T2 relaxation time<sup>22</sup>. Further, this analysis indicates that ice treatments are unable to completely prevent swelling and fluid accumulation at the injury site.

Although overall swelling was not significantly different between the two icing protocols, different patterns of fluid accumulation for each protocol were noted over the 6 hour experiment. T2 data for the continuous protocol indicated significant increases over each timespan. In contrast, T2 data for the intermittent protocol showed no significant change over the 2-to-4 hour and 4-to-6 hour timespans, although change over the 2-to-6 hour timespan did show significant change. This effect was likely due to the significantly greater T2 relaxation time at the 2 hour timepoint in the intermittent protocol compared to the continuous protocol. These findings indicated the continuous protocol continually underwent significant increases in T2 relaxation time, while T2 relaxation time from the intermittent protocol increased more quickly until 2 hours, after which it slowed to a rate similar to the healthy limb. Continued change in the intermittent protocol was only significant over longer spans of time. This difference may involve the shunting reflex or

other mechanisms and may be a fertile area for future research. There were significant changes in volume across all timespans.

Generally, the accepted time course for swelling after injury is a large acute increase in swelling during the first hour after injury and then gradual continued swelling during the next 1 to 5 days, with some MRI studies finding peak T2 relaxation times up to 7 days after injury.<sup>19,25,26</sup> More specifically, a study by Takahashi et al. measured the time course of swelling over the first hour and then over the next 7 days.<sup>22</sup> This study notes that eccentric exercise induced injury caused two peaks in T2 and muscle swelling (as measured by cross sectional area) during this timespan, one within the first hour after injury, the other 12 hours after injury, in most muscles examined. Our data indicate that some slowing in T2 may have occurred after the initial peak; indicating a non-linear increase in swelling under intermittent icing protocol as found by Takahashi et al. The researchers note that these two peaks are likely caused by two different factors; the first by an increase in extracellular water at the injury site, while the second may be caused by additional injury to the muscle tendon itself, resulting in further edema.<sup>22</sup> Since we didn't have a second injurious event, the different pattern of increase swelling may be due to different vascular responses under the different icing protocols.

In contrast to these T2 findings, the limb volume changes over all timespans reacted more similarly for both icing protocols. Significant differences between injured and healthy limbs were found across all timespans in the continuous protocol. Significant differences were also found in the intermittent protocol across all timespans except the 4to-6 hour timespan, which indicates that swelling had significantly slowed after hour 4 for this protocol. Interestingly, the intermittent protocol showed a small mean loss of volume in the injured limb over this timespan, which may indicate that the intermittent protocol is able to limit swelling faster than the continuous one. This result may fit the muscle swelling time course shown by Howell et al., which found that muscle swelling elevated soon after injury, subsided after 6 hours, and then increased over the next three days.<sup>24</sup> These results are somewhat reinforced by several other studies, which found that muscle swelling peaks between 3 and 5 days.<sup>27,28</sup> In contrast to the findings of Howell et al., Nosaka and Clarkson found that muscle swelling as measured by muscle circumference increased continually after injury, finally reaching a plateau around 4 to 5 days after injury.<sup>26</sup> It is important to note that the muscle injuries examined in much of the literature are eccentric exercise-induced injury which may differ from contusion injuries with regard to swelling time course.

There was no significant difference in the amount of swelling accumulated between the treatment protocols at any time point as indicated by T2 relaxation time and volume data. Although there was a similar pattern of change in swelling across most timespans, a significant difference in T2 relaxation time change was found between the two protocols from 2-to-4 hours, with the intermittent protocol resulting in a smaller increase in swelling than the continuous protocol. Mean changes in limb T2 seem to indicate that the continuous group experienced significantly more fluid accumulation during this time than the intermittent group. However, given that the total T2 relaxation time and limb volume changes (baseline to 6 hours) measured in both protocols were not significantly different, this finding indicates a different pattern of swelling under the two treatment protocols but no superiority between the protocols in terms of overall amount of swelling.

Our findings suggest that both icing protocols acted similarly in influencing extracellular fluid accumulation and swelling within the injured limb. This is a beneficial finding, given the safety risks and inconvenience associated with continuous ice treatment protocols. Long-term exposure to ice treatments, such as that experienced during the continuous treatment, increase the risk of experiencing cold-related injury.<sup>6</sup> Some studies indicate cold-induced injuries may develop after as little as 20 to 30 minutes of icing.<sup>5</sup> Intermittent ice treatments of shorter duration may limit the possibility of sustaining a cold-related injury. Furthermore, since ice application time would be shorter, patient compliance with treatment application is more likely. Akgun et al. note that shorter icing times improve patient compliance and cite a 15 minute long treatment as inducing minimal patient discomfort and maximizing compliance.<sup>1</sup> While this recommendation is half the time used in the intermittent protocol in this study, it still lends practical support to the clinical use of intermittent icing protocols over continuous protocols. Belitsky et al. note that intermittent ice treatments of shorter length (between 10 and 45 minutes) are typically used by health professionals to treat muscle contusion injuries; our study's data supports the continued use of intermittent protocols by clinicians.29

Only two studies have been identified that compare cryotherapy treatments. One study conducted by Hochberg compared the continuous application of a temperature controlled cooling blanket for 12 hours a day to an intermittent cyclical application of ice for 20 minute and rest for 20 minutes for 12 hours.<sup>14</sup> The cooling blankets were found to reduce pain sensation and wrist circumference 3 days post-operation more than the intermittent ice treatment.<sup>14</sup> Hochberg's study differs considerably from this study with

regard to injury, as all subjects were carpal tunnel release surgery patients. Furthermore, the cooling blankets were set to maintain temperature at 45°F; considerably higher than the temperature of ice. Measurements were also made at longer timepoints than in our study. Bleakley et al. compared two intermittent icing protocols, one consisting of icing 20 minutes every 2 hours, the other consisting of a cycle of 10 minutes of ice and 10 minutes of rest.<sup>13</sup> The 10-minute protocol was found to reduce patient pain the most, especially during the first week of treatment. No significant differences between groups with regard to swelling were found; however, the authors note that these measurements were obtained at least 4 days after the initial application of ice, so immediate treatment effects of the two protocols could not be evaluated.<sup>13</sup> Unlike these previous studies, our data suggests no difference between icing protocols in the early stages of swelling after a muscle contusion injury thereby favoring the intermittent icing protocol based upon safety issues and patient compliance.

There were several limitations to the current study. First, the study design did not include an injured control group. While the healthy limb of each animal was used as a baseline, no control group measuring T2 relaxation time and limb volume change after injury without an ice intervention was designed. The inclusion of an injury control group would have allowed for measurement of the effectiveness of each icing protocol to limit swelling and the magnitude of any swelling reduction, which are important aspects of cryotherapy. No judgments regarding the absolute effectiveness of the icing protocols in limiting swelling can be made from this study.

Another weakness of the study is the assumptions that were made regarding baseline T2 relaxation time measurements for the healthy and injured limbs. These
measurements were not made due to financial constraints associated with the additional time needed to complete additional T2 scans. Baseline T2 data was approximated by averaging the T2 data from the 2, 4, and 6 hour timepoint scans of the healthy limb. This baseline data was used for both the healthy and injured limbs under the assumption that no significant difference in limb T2 would be found between the two limbs at baseline. An ideal study design would have allowed for T2 relaxation time measurement at baseline for both limbs, thereby strengthening the statistical analysis of T2 change for both limbs. We believe that the breeding of the rats for uniformity, lack of T2 or volume increases in the healthy leg during the experiment, lack of differences between the baselines measures of volume between the injured and healthy limbs and previous studies justify the use of the averaged T2 data from the healthy limb as our T2 baseline measure.

A third weakness of the study is its small sample size. Of the 20 rats purchased for use in the study, 2 died before completion of the entire experiment; consequently, the data from these two rats were not used in the study analyses. Because of this, only 9 animals were in each treatment protocol group. This small sample size may have introduced hidden potential treatment response differences due to the large standard deviations calculated from the data. Larger sample sizes may reduce the variability of the measurements and clarify trends seen in the data. In spite of the sample size, the data did meet assumptions for normality.

Future studies should implement an injury control into the study to investigate the absolute physiological changes that are elicited by icing protocols compared to no intervention. Larger sample sizes could reduce variability of the measurement and clarify treatment effects. Additionally, given that fluid accumulation and swelling are

physiological processes largely based on chemical effects within the injury site, studies investigating chemical biomarkers of injury should also be performed to compare these two treatment protocols. It is possible that while the T2 and limb volume changes measured in this study were similar between the treatment protocols, chemical effects could have been significantly different and translated to differing long-term treatment outcomes. Finally, this study only investigated crushed ice pack cryotherapy treatments. Given the wide variety of treatments available (i.e. ice massage, gel packs, etc.), future studies could compare the effectiveness of different cryotherapy treatments across a wide range of injury sites.

### Chapter 5

## Conclusion

Little is known about the relative effectiveness of the various cryotherapy treatment protocols utilized in rehabilitation of musculoskeletal injuries. Information about the relative effectiveness of treatment protocols with regard to patient risk and the likelihood of patient compliance can help clinician make better treatment decisions with their patients. The findings of this study indicate that little difference exists between the continuous and intermittent icing protocols in terms of overall changes in limb volume and T2 relaxation time. Given that the risk for developing cold-related injury increases dramatically when ice is applied for long periods of time, this study supports the use of an intermittent icing protocol, as it showed similar changes in T2 relaxation time and muscle volume as the continuous protocol without the increased risk for cold-related injury. Furthermore, given the extreme length of icing time needed to complete the continuous protocol compared to the shorter icing times of the intermittent protocol, the intermittent protocol would likely show greater patient compliance. Further research is needed in determining the ideal intermittent icing protocol design to optimize the limitation of swelling while reducing the risk of cold-related injury. Additionally, investigations into the biological effects and biomarkers of injury development and recovery should be undertaken for both continuous and intermittent icing protocols in order to evaluate the ability to influence these factors during patient healing.

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## **Literature Review**

### Overview

Magnetic Resonance Imaging (MRI) is an imaging technique currently used in medical and research facilities worldwide to diagnose and learn more about a wide variety of soft tissue injuries and conditions. The fundamental mechanics of MRI are based on atomic nuclear spin and the magnetic field that may develop from the spin. Certain specific atomic nuclei, which include <sup>23</sup>Na, <sup>19</sup>F, and, most importantly, <sup>1</sup>H (hydrogen), have magnetic moments that react and align to magnetic fields.<sup>30,31</sup> These fundamentals were discovered in 1946 by Felix Bloch and Edward Purcell, who later shared the 1952 Nobel Prize in Physics for this breakthrough.<sup>32</sup> Medical MRI research became mainstream in the 1970s and 1980s as MRI scanners were developed and refined and the human body was imaged; making the once novel discovery more useful in the laboratory.<sup>32</sup> MRI technology eventually spread to commercial hospitals as imaging techniques and technology improved.<sup>32</sup>

A typical MRI scanner is comprised of three components: a high strength magnet, a radiofrequency coil, and a computer that interprets the signals collected by the coil and creates an image. The magnet strength of MRI scanners found in most hospitals usually ranges from 1.5 to 3 Teslas [T], although machines that reach up to 9.4 T can be found in some research facilities.<sup>33</sup> Even stronger magnets are being used currently in animal research.<sup>33</sup> While the magnets used in developing the first MRI scanners in the 1970s

were large permanent magnets, modern MRI scanners almost always use electromagnets because of their higher strength. These magnets are cooled to 4 Kelvin (-269.15° Celsius) with liquid helium in order to take advantage of the superconducting properties of the magnet material.<sup>34</sup> This allows for a significant reduction in electrical resistance and thereby produces a stronger magnet.<sup>34</sup>

As a subject is placed within the magnet bore of the MRI scanner, specific atomic nuclei such as <sup>1</sup>H align with the magnetic field due to their magnetic moment.<sup>30,31,34</sup> Within the magnetic field, these nuclei position themselves in either a parallel or antiparallel configuration.<sup>34</sup> While the ratio of aligned nuclei to reversed nuclei is close to 1:1, the number of nuclei aligned with the magnetic field will be slightly greater since this configuration requires less energy to maintain.<sup>34</sup> Since the amounts of parallel and anti-parallel nuclei are almost equal, they effectively cancel each other out *except* for the few extra parallel nuclei. These uncancelled nuclei are what the MRI eventually detects.<sup>34</sup> Ultimately, the abundance of <sup>1</sup>H in the body, primarily contained in water, allows MRI to be possible.

To create an image, the radiofrequency coil emits radio waves at the Larmor frequency, which is the frequency at which <sup>1</sup>H nuclei resonate.<sup>35</sup> At this point, the uncancelled, parallel nuclei absorb these waves, gain energy, and move to the higher energy, anti-parallel position.<sup>34</sup> When the radio waves cease, these nuclei release the stored energy and revert to their original alignment. The energy is released back toward the coil, which detects the energy and sends this data to a computer where the image is constructed.<sup>34</sup>

One problem that develops from this technique stems from the fact that both lean and adipose tissue contain <sup>1</sup>H atoms. If an image needs to show both of these tissue varieties as separate and distinguishable entities, some adjustments are necessary. This is accomplished through T1 and/or T2 weighting.<sup>30</sup> T1 and T2 weighting are related to the rate at which the excited nuclei change to their original configurations after tissues are subjected to the radio waves from the coil. In T1 weighting, the rate at which the uncancelled nuclei revert from the high energy level (the –z direction) to the low energy level (the +z direction) is measured.<sup>36</sup> Different tissues have different T1 values based on their chemical compositions. When a second radio wave pulse is emitted from the coil after a set length of time, the amount of low energy nuclei in each type of tissue varies due to the inherent differences in T1 values.<sup>36</sup> By detecting these variations, the computer can create an image that differentiates tissue types; short T1 tissues are bright, while long T1 tissues are dark.<sup>36</sup> T1 is also known as the proton spin lattice relaxation time.<sup>34</sup>

In contrast to T1 weighting, T2 weighting operates by applying radio wave pulses at a 90° angle to the magnetic field.<sup>36</sup> These pulses cause changes in nuclei orientation in the x and y planes.<sup>36</sup> Similar to T1, T2 is the rate at which these newly oriented nuclei revert to the +z direction.<sup>36</sup> It is important to note, however, that while T1 is a positive rate (the amount of nuclei going into the +z direction is increasing over time), T2 is a negative rate (the amount of nuclei oriented in the x and/or y planes is decreasing as they align with the +z direction).<sup>36</sup> This effect results in an opposite shading trend for T2 weighted images; short T2 tissues are dark, whereas long T2 tissues are bright.<sup>36</sup> Because of their abilities to emphasize different aspects of a patient's anatomy, both T1 and T2 weighting techniques are useful in producing informative MR images.<sup>30,31,36</sup> For example, T1 generally shows a high quality image of a patient's anatomy, which is useful in identifying problems like intervertebral disc degeneration, while T2 is more adept at displaying irregularities within tissues, such as problems associated with the cerebrospinal fluid in the spinal cord and intervertebral disc herniation.<sup>35</sup> Ultimately, T1 and T2 techniques allow medical professionals to comprehensively examine their patients for abnormalities, thereby further educating their decisions regarding diagnosis and treatment.

To investigate certain physiological structures, MRI contrast agents have been developed and are used both in diagnostic and research settings. Contrast agents are molecules whose structures interact with specific tissues and change the magnetic properties of the tissue.<sup>37,38</sup> As a result of this effect, different frequencies are used to react with the contrast agents in the tissue, thereby distinguishing the tissue region from the rest of the image. Contrast agents have been used in cancer diagnosis, as well as in joint injury assessment, to name a few applications.<sup>38,39</sup> Most contrast agents currently in use are metal complexes and the most commonly used agent in research is gadolinium-DTPA (diaminotetra-ethyl penta-acetic acid).<sup>37,38</sup>

Compared to other types of imaging technologies, MRI has several advantages toward its use. MRI's biggest advantage is its lack of radiation. While X-ray and CT scans apply ionizing radiation at the body, MRI is radiation-free and currently shows no harmful side effects due to its use.<sup>40</sup> Because of this, certain populations, such as infants and pregnant women, are often examined using MRI rather than other imaging techniques.<sup>41</sup> No known physiological side effects stem from exposure to MRI treatments; therefore, the technique is convenient for patients and MRI specialists, since these individuals may withstand many procedures without needing to be concerned about developing cancer or other disorders. MRI is a painless, non-invasive imaging technique that accurately provides a three-dimensional image of soft tissues in the body, which makes it a popular technology for injury diagnosis.<sup>42</sup> Finally, MRI is an effective tool for both researchers and medical professionals alike, as it allows for a relatively fast accumulation of high resolution data.<sup>40</sup>

While MRI has many advantages, it also has some limitations. MRI may be an uncomfortable experience for certain groups of people; specifically obese individuals, whose large size may prevent them from safely fitting within the magnet bore, claustrophobics, who may experience fear from being inside the scanner, and individuals that have pacemakers or other metallic implants, which could be attracted by the magnetic field and cause safety hazards.<sup>40,41</sup> Additionally, the subject in the scanner must be very still and may need to hold his/her breath during portions of the scanning procedure in order to produce good quality images. Given that a scan may take around 30 minutes to complete, maintaining stillness may prove difficult or impossible for some individuals (i.e. infants).<sup>40,41</sup>

## **Diagnostic MRI**

Many studies have investigated the use of MRI in diagnosing injury. Combined with a clinical exam, MRI is a powerful tool in patient evaluation and has been found to be helpful in diagnosing muscle strain injuries to the quadriceps and hamstrings and injuries to the ulnar collateral ligament (UCL).<sup>39,43-45</sup> MRI has also been shown to be helpful in diagnosing some acute knee injuries.<sup>46</sup>

MRI can supplement conventional clinical examinations of muscle strain injuries (i.e. range of motion and strength measurements) by showing the size of the muscle strain injury. MRI can accurately identify the location and degree of muscle injury because it can provide a detailed picture of muscle anatomy and edema.<sup>44</sup> Injuries are identified by the presence of high signal intensity during a T2 weighted scan with fat suppression.<sup>43</sup> Injury size is found by measuring the injury length and cross-sectional area.<sup>43</sup> The "site of injury" can be identified using MRI by finding the region within the injured area where there is the greatest measured cross-sectional area.<sup>43</sup> While injury size and site both impact medical prognosis for recovery length, the exact anatomical position of a muscle strain injury most significantly impacts the rehabilitation time.<sup>43</sup> Ultimately, MRI is helpful with making precise muscle strain injury diagnoses and prognoses for rehabilitation in professional athletes; however, due to high procedural costs, MRI is not recommended for recreational athletes.<sup>43</sup>

During hamstring examinations of Australian Rules Football players reporting symptoms such as pain, muscle tenderness and pain during resisted contraction, Verrall et al. found that MRI was able to detect hamstring injuries in most of the patients.<sup>45</sup> The injuries not detected by MRI were generally mild in severity and were associated with a shorter amount of time lost from competition due to recovery. Furthermore, in the hamstring injuries that were detected by MRI, the mechanisms of injury were reasonably clear to the investigators. The underlying causes of hamstring injuries that went undetected were more difficult to identify.<sup>45</sup> Hamstring pain does not always indicate

muscle injury; often pain is referred from the lumbar spine. In this case, MRI would be unable to detect the cause of hamstring pain, as the real injury is elsewhere, leading to a false negative diagnosis. Because MRI data and clinical symptoms did not correlate in some cases, Verrall et al. note that MRI is useful in evaluating many hamstring injuries; however, a clinical exam must rule out more subtle causes of hamstring pain that would be undetectable by MRI.<sup>45</sup>

While the correlation of clinical exam and MRI diagnosis of posterior thigh injury is not perfect, diagnosis of other injuries has been widely successful with MRI. For example, MRI has been used in UCL injury diagnosis since the early 1990s and can accurately identify both partial and complete UCL tears.<sup>39</sup> To make the diagnosis even clearer, a contrast material is sometimes administered within the joint; if an incomplete tear exists, the contrast agent will pass outside the normal joint capsule limits, but not beyond the UCL itself; if a complete tear exists, the contrast agent will pass outside the normal agent will pass beyond both the joint capsule and the UCL.<sup>39</sup> The contrast material can be visualized in MRI, thereby enhancing the diagnostic accuracy for this condition.

MRI evaluation of knee problems is sometime inconclusive. This can be problematic due to inaccurate image interpretation by radiologists; errors may be due to the radiologist's inexperience or lack of context from clinical evaluations.<sup>42</sup> Image clarity as well as human error create the difficulty associated with interpreting MR images. Luhmann et al. argue that orthopedic surgeons should be shown MRI images for their patients, because research shows that surgeons who are provided with MRI images, radiographs, and physical examination results are able to make the most accurate diagnoses.<sup>42</sup>

Conversely, Frobell et al. observed that MRI combined with a clinical exam were considerably more effective at diagnosing acute knee injuries than a clinical examination by an experienced orthopedic surgeon alone, noting that half of acute ACL injury patients are at risk for an incorrect diagnosis unless MRI tests are prescribed in the sub-acute phase, which allows swelling to subside.<sup>46</sup> This assessment conflicted with previous studies that had shown that MRI was no more effective than clinical assessment; however, the previous studies were conducted five weeks after injury. Since the pain and swelling in the acute phase of knee injury makes these cases more difficult for surgeons to examine, MRI is a more accurate diagnostic method than clinical examination alone in these cases.<sup>46</sup> Furthermore, when there is an uncertain clinical diagnosis of a knee injury, reports have shown that patient satisfaction and management are improved by using MRI.<sup>46</sup> Ultimately, Frobell et al. recommend that MRI evaluations are made with patients with severe knee injuries in order to ensure that correct diagnoses are made and the according treatments are prescribed.<sup>46</sup> This recommendation is important since early and correct diagnosis of knee injuries can improve treatment and subsequently reduce the risk of the further complications.

While MRI may be helpful in diagnosing acute knee injuries, evidence has shown that diagnoses based on knee images from children and adolescents may actually provide incorrect conclusions. Imaging of the child or adolescent knee is difficult because growth changes are particularly evident in the knee, which may lead to false positive or negative diagnosis.<sup>42</sup> These growth related changes are especially evident in the meniscus. As a result, a normal, healthy meniscus in a child may appear to be injured, even though it is

not.<sup>42</sup> In this case, MRI diagnoses may be detrimental as they could lead to unnecessary surgical procedures.

Sensitivity of MRI for knee injuries ranged from 66.6% for lateral meniscal (LM) injuries to 81.8% for posterior cruciate ligament (PCL) injuries while specificity varied from 86.2% for LM injuries and 100% for PCL injuries.<sup>47</sup> These values were lower than sensitivity and specificity ranges found using arthroscopy, although the authors maintain that MRI still has diagnostic value in investigating knee injuries. In contrast, the sensitivity and specificity of MRI have been found to be considerably lower when investigating the cartilage injuries at the wrist joint.<sup>48</sup> Sensitivity ranged from 18% to 41% and specificity ranged from 75% to 93% based on injury location when compared to arthroscopy.

MRI has been found to be highly sensitive to detecting breast cancer. Sardanelli et al. found that the overall sensitivity of MRI to detecting multiple breast tumors was 81% compared to 66% for mammography.<sup>49</sup> Sensitivity of MRI towards residual breast cancer after surgery is also high (96%).<sup>50</sup> Ultimately, MRI has been found to have excellent diagnostic abilities for many types of musculoskeletal injury and soft tissue conditions; however, its accuracy depends somewhat on the regions and tissues being examined, as well as error caused by examiner inexperience.<sup>42</sup> While MRI has become a gold standard for evaluating soft tissue injuries, especially of muscle, arthroscopy remains the gold standard of knee and wrist examination for conclusive diagnoses in both children and adults, even though it is an invasive procedure and MRI is not.<sup>42,46,48</sup> Nonetheless, the role of diagnostic MRI has been established as a useful tool within the medical community.

## **Clinical Applications**

## Cryotherapy

Cryotherapy is a technique used commonly by clinicians to lower tissue temperature. Cryotherapy involves several methods, the most common being ice, cold packs, and ice massage.<sup>1</sup> Cryotherapy is used frequently by medical professionals, as it is an inexpensive, safe, and well-tolerated treatment for soft tissue injuries.<sup>2</sup> These injuries include sprains, strains, lacerations, skeletal muscle contusions, and fractures.<sup>3</sup> Cryotherapy has several effects; it can reduce inflammation and limit edema formation and hemorrhage effects caused by injury because cold decreases local blood circulation.<sup>1,5,6</sup> Cryotherapy also depresses the sensitivity of peripheral nerve endings, which can lead to a reduction in pain sensation and an increase in the pain threshold.<sup>4,6</sup> When treating an acute injury, cryotherapy is most successful if initiated as quickly as possible after injury.<sup>2</sup> Overall, the tissue cooling that results from cryotherapy is largely influenced by the cryotherapy technique, the duration of therapy, the muscle temperature previous to therapy, and the thickness of subcutaneous fat.<sup>4</sup>

Cryotherapy aims to reduce subsequent damage to healthy tissues as a result of injury. This effect is known as the secondary injury model, which is the currently accepted explanation concerning why cryogenic treatments work.<sup>7</sup> This model states that structural damage to a muscle results in inflammation responses within the muscle, thereby causing secondary damage. Muscle tissue swelling, intercapillary distance, and intramuscular pressure all increase due to injury and lead to a slower recovery. These factors may lead to ischemia in cells surrounding the injury site. Ischemia damages cells

by creating hypoxic conditions, leading to insufficient fuel sources within the cell and waste build up.<sup>8</sup> The intracellular chemicals and free radicals released by neutrophils during reperfusion irritate healthy cells and lead to further damage.<sup>3</sup>

Cryotherapy helps address these problems by controlling inflammation and lowering oxygen consumption by injured tissues, thereby lowering the amount of harmful free radicals.<sup>3</sup> By lowering tissue temperature with cryotherapy, the metabolic rate of the cells decreases, which allows them to persist through the damaging hypoxic period without dying.<sup>9</sup> Additionally, because cold leads to vasoconstriction, cryotherapy may reduce intramuscular pressure and the risk of compartment syndrome by limiting internal bleeding and thus prevent further damage of healthy tissues.<sup>3</sup>

Reviews of cryotherapy studies have shown that, although many studies have methodological problems, cryotherapy appears to be helpful in patient recovery.<sup>2,11,12</sup> Further, a recent study investigating a 6 hour ice treatment after contusion of the extensor digitorum longus in rats showed that cryotherapy was able to reduce vasodilation and permeability at the injury site, limit inflammation, and improve muscle tissue survival.<sup>3</sup> However, it should be noted that the experimental procedure for this study differs from conventional treatments, during which cold is applied 20 to 30 minutes at a time.<sup>9</sup> Hochberg compared continuous and intermittent cryotherapy protocols in carpal tunnel release surgery patients and was the only study found in the literature comparing these two types of treatments.<sup>14</sup> This study compared the continuous application of a temperature controlled cooling blanket for 12 hours a day to an intermittent cyclical application of ice for 20 minute and rest for 20 minutes for 12 hours. Interestingly, the cooling blankets were set to maintain temperature at 45°F; considerably more than the temperature of ice, which is the most commonly used cryotherapy. The cooling blankets were found to reduce pain sensation and wrist circumference 3 days post-operation more than the intermittent ice treatment.<sup>14</sup>

While cryotherapy has been shown to be beneficial, there are some drawbacks regarding its use. For example, excessive ice application times and temperatures can lead to frostbite, nerve damage, and burns.<sup>1,2</sup> Additionally, cryotherapy sessions that last too long may impede healing.<sup>1</sup> Recently, the effectiveness of cryotherapy has come into question as few studies have investigated this issue and much evidence seems to be anecdotally based.<sup>8,10</sup>

Part of the reason why the effectiveness of cryotherapy is in question is due to the fact that icing protocols vary widely within the literature; studies may differ in cryotherapy technique (ice vs. cold pack, crushed ice vs. partially melted ice, etc.), duration of treatment, area of treatment, and the thickness of subcutaneous fat layers in subjects.<sup>1,4,5,13</sup> As a result, finding the most effective amount of icing time that does not cause cold-related injuries is important in order to improve cryotherapy treatments.<sup>1</sup> Bleakley et al. compared the effectiveness of two common protocols on human ankle injuries; one consisting of 20 minutes of continuous icing every two hours (the commonly recommended protocol used in most literature), the other consisting of an intermittent treatment of ten minutes of ice followed by ten minutes of rest.<sup>13</sup> In this study, subjects using the intermittent protocol experienced less pain than subjects in the continuous group during the first week of treatment. It was suggested that the ability of the intermittent protocol to maintain lower tissue temperatures for longer periods of time than the continuous method led to diminished swelling, inflammation, and secondary cell

injury.<sup>13</sup> It is thought that since further secondary cell injury is prevented with cryotherapy, less overall damage occurs, which could lead to a faster recovery.<sup>11</sup> In spite of the decrease in pain, both groups exhibited similar ankle mobility and measures of function throughout the study.<sup>13</sup> As a result, the functional effectiveness of the intermittent protocol was not demonstrated. Further research is needed to define the parameters of successful cryotherapy application in terms of desired outcomes to demonstrate that lowering tissue temperature positively affects functional measurement outcomes.

## Volumetrics

Volumetrics refers to techniques that are used to determine volume. There are several volumetric techniques, including MRI, bioelectrical impedance, optoelectronics, tape measure, and water volumetry, which is the current gold standard for measuring volume.<sup>51</sup> The popularity of water volumetry stems from its low cost and the three-dimensional nature of the measurements acquired using this technique (volume itself is measured, rather than calculated).<sup>51</sup> While MRI shows higher measurement precision, shortcomings such as higher operating costs and the technology's reliance on constructing volume from a series of two dimensional images with computer aid decrease its popularity.<sup>51</sup> Volumetrics is important because research performed using volumetric measurements can evaluate the effectiveness of treatments on volume reduction.<sup>51</sup>

The accuracy of MRI as a volumetric technique has been verified. By comparing MRI measurements with cadaver measurements, it was found that error was low (1%),

even when spaces from 10 to 40 mm existed between the images.<sup>52</sup> A contiguous image (one with no spaces between the images) is estimated to be even more accurate, as the computer calculating the volume does not have to approximate as many values; however, the error found in contiguous scanning was not investigated during the study.<sup>52</sup> Since MRI carries no known side effects, prolonging an MRI procedure to get more images is typically a safe process.<sup>52</sup> In addition to low overall error, MRI was found to be effective in determining interstitial adipose tissue, adipose tissue-free skeletal muscle, and subcutaneous adipose tissue volumes when compared to a cadaver measurements. Error in these measurements was found to be about 2%.<sup>52</sup> Ultimately, MRI was found to be effective in measuring small muscle tissue volume changes in vivo.<sup>52</sup>

Three studies were found that investigated the volumetrics of acute muscle injury with MRI. McBrier et al. focused on the effect that buprenorphine, an analgesic, had on healthy and injured Wistar rat muscle volume.<sup>17</sup> This study found that buprenorphine increased the volume of both injured and healthy limbs, which could potentially lead to difficulties in detecting injury.<sup>16</sup> However, MRI scans using T2 maps were found to be accurate, even when using the drug, which supports the use of MRI in injury diagnosis.

Silder et al. used MRI to investigate volumetric changes in hamstring muscle and tendon volume associated with scar formation and muscle atrophy after injury.<sup>53</sup> This study compared hamstring MRI scans of previously injured athletes to similar scans in healthy control subjects, allowing the researchers to notice changes in tendon volume and scar presence. Finally, Slavotinek et al. investigated hamstring muscle volume change in athletes shortly after injury and examined the relationship between abnormalities in

muscle cross-section, volume, and return to competition.<sup>54</sup> These studies indicate that MRI can be successfully used for volumetric analyses of muscle tissue.

Outside of skeletal muscle injury, MRI volumetrics has been used to study a variety of topics. Topics of interest include the measurement of muscle atrophy in heart failure patients and its effect on exercise intolerance<sup>55</sup> and measurement of brain atrophy following traumatic brain injury<sup>56</sup>.

## **Fluid Accumulation**

MRI has been used in the past to measure fluid accumulation in muscle tissue after injury. Much of the past research has focused on injuries caused by eccentric exercise. T2 relaxation time is typically used to measure fluid accumulation after injury because T2 changes depending on the amount of water that is present within or outside of tissues. T2 relaxation times are longer for extracellular fluid than for water within tissues.<sup>16,18,21</sup> After injury, water accumulates in the limb due to edema. This increases the extracellular water in the injured limb, as a result, the T2 relaxation time increases in the regions experiencing edema. T2 relaxation time and volume have both been successfully used in past studies to evaluate muscle swelling in a variety of injury sites.<sup>15,16,19-22</sup>

A study conducted by Takahashi et al. measured the time course of swelling over the first hour after injury and then over the next 7 days by using MRI.<sup>22</sup> The study found two peaks of T2 relaxation time and swelling over the experiment in most muscles examined; one within the first hour post-injury, and the other 12 hours after injury. Interestingly, the measurements also indicate that some decrease in T2 relaxation time may have occurred between the two peaks. The researchers note that the two peaks are likely caused by two different factors; the first by an increase in extracellular water at the injury site and the second by additional injury to the muscle tendon itself, resulting in further edema.<sup>22</sup> Other MRI studies investigating peak T2 relaxation times after injury have shown that these peaks can occur up to 7 days after injury.<sup>19,25,26</sup>

T2 MRI scans have also been used to identify muscle injury location. Lovering et al. utilized MRI to identify the location of muscle injury within a rat model.<sup>20</sup> McCully et al. used MRI to identify muscle injury location and follow changes in muscle metabolism after injury in humans.<sup>21</sup> Nosaka and Clarkson investigated muscle swelling with T2 relaxation time and with biological inflammatory markers.<sup>26</sup> This study showed considerable fluid accumulation at the injury site, although no significant changes in inflammatory markers were measured. The authors indicate that this finding may differ from other analyses of inflammatory markers after injury due to procedural differences; past studies had not differentiated biologically active markers from inactive ones.<sup>26</sup> Like volumetric applications, MRI T2 measurement has proven to be a valuable tool in evaluating and researching muscle injury.

## **Future Applications**

While MRI and cryotherapy have established applications in the medical field, current research is leading to new possibilities of novel applications. MRI contrast agents have been improved upon in recent years and may lead to unique future applications. Gadolinium-DTPA is currently the most commonly used contrast agent in research; unfortunately, gadolinium is toxic within the human body.<sup>38</sup> Pharmaceutical companies are currently developing new markers that may offer a lower toxicity to humans and better imaging quality.<sup>38</sup> Additionally, more advanced contrast agents in the form of micro-fabricated, geometric particles are currently being developed and explored.<sup>37</sup> These molecules are being designed with the hope that they will enable full color MRI images in the future.<sup>37</sup> While specific future applications for color MRI are still unknown (researchers are still unsure of how these agents will react in physiological systems), it is possible that this advancement could lead to many new discoveries in the medical and rehabilitation fields.<sup>37</sup> Finally, as MRI technology continues to advance, future MRI units will likely provide greater image contrast and clarity at a quicker rate, making MRI a quicker, more sensitive, and more effective method of imaging.

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## Appendix 1

# IACUC Research Application

PENNSTATE			Office f	or Research Protections	
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PLEASE NOTE: Incorporat	te ALL Protocol related in	formation into this :	application – NC	ATTACHMENTS	
Project Title: Evalua	ation of cryotherapy on the	e extent of injury d	uring the first 6 h	nours	
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Principal Investigator: N	icole McBrier				
Email: Nmm13@psu.edu	Penn St	ate Access UserID	(e.g., abc123): r	nmm13	
Mailing Address: 146 Recre	ation Building				
University Status (For exan	nple Faculty, Staff, Post-	-doc, Grad. Studer	nt): Faculty	<b>Telephone:</b> 863-9732	
Dept: Kinesiology	: Kinesiology College: HHD Campus: UP				
Co-Investigator: Andrew W	'ebb		-		
Email: agw@engr.psu.edu	Penn State Acc	ess UserID (e.g., a	bc123): agw3		
Mailing Address: 205 Hallo	well Build				
University Status (For exan	nple Faculty, Staff, Post-	-doc, Grad. Studer	nt): Faculty	<b>Telephone:</b> 865-0459	
Dept: Bioengineering	College: ENG	,	Campus: UP	•	
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Is there anyone you wish to in	clude on correspondence r	elated to this proto	col (e.g., a study c	oordinator, etc.)?	
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Dept:	College:		Campus		

1. Is this application for the renewal of a 3-Year Closeout (Is this protocol replacing a study that will expire this year)? If NO, go to 2.

□ YES ⊠NO

1a. What is the IACUC number of the protocol that you are replacing?

1b. If all of your animals were purchased through ARP then go to 1c. Please complete the following table for the protocol that is expiring:

Species (Common Name)	Total used during the LAST research year		

1c. Are animals still on hand for the expiring protocol?  $\Box$  YES  $\Box$  NO, go to 2.

1d. Please indicate how many animals of each species you currently have on hand.

Species (Common Name)	Total on hand

1e. How will these animals be handled?

□ According to the methods described in the approved protocol **Remaining animals will continue to be used under this research protocol** □ Animals will be transferred back to a PSU herd/flock (i.e. dairy herd) □ Other, please explain

Is this project funded through external sources? 2.

□ YES (provide a copy of the grant and cover page with this submission)

### **I** NO, this project is funded internally (i.e. departmental or start-up funds)

2a. Provide the name and mailing address of each funding source:

PI will fund project through department funds and salary release money

2b. Regarding your external funding, please complete the following table for each funding source:

Grant or Fund Number:	
Period of support (start to end):	
Grant Manager (if known):	

2c. Do you have funding for the procurement and care of the animals to be used under this study?

### $\square$ YES $\square$ NO

 Please provide the following information in lay terminology: 3a. A general description of the experiment or activity. Explanation and examples.

The purpose of the proposed experiment is to compare two forms of ice treatments (continuous and intermittent) on the extent of injury that occurs within the first 6 hours following trauma. Cryotherapy (ice treatments) is a common practice and recommended by health profession in order to minimize the amount of inflammation and secondary injury that may occur following trauma. Our goal is to determine what treatment parameters would best benefit in reducing the amount of inflammation that occurs.

3b. The reason for the experiment or activity and a description of the benefits to be gained. Examples.

Currently medical professionals recommend intermittent ice treatments following injury but the timing ratio of when to put ice on and when to remove it are varied. Current literature supports the use of ice in reducing secondary injury however these studies used ice continuously.(Merrick 1999, Schaser 2007) The goal of this project is to determine if intermittent icing will be just as effective as continuous icing as well as the appropriate time needed to ice following injury.

### 4. Animal Procedures

4a. Will animals be euthanized (See the ARP website for <u>AVMA euthanasia methods</u>)?

### ☑ YES □ NO, go to c

4b. Provide the method of euthanasia (if the method is conditionally acceptable scientific justification must be provided).

Animals will be euthanized following tissue extraction via  $CO_2$  inhalation. To insure that unintended recovery does not occur after  $CO_2$  euthanasia, cervical dislocation may be performed

4c. Will wildlife be trapped or captured in any way?

**ZNO** YES, please describe how injured animals will be handled.

4d. Will samples be collected from the animals following euthanasia?  $\Box$  YES  $\blacksquare$  NO

4e. How will the animals be disposed of once the research is complete?

DEuthanized and placed in a freezer/cooler until they are picked up for disposal

# □ Animals will be transferred back to a PSU herd/flock (i.e. dairy herd) □ Animals will remain with their owners

□ Other, please explain

4f. Will any procedures or tests be performed on live animals? What is considered a procedure or test?
☑ YES □ NO, go to Question12– Animal Husbandry

4g. Will animals be anesthetized or sedated for any of the procedures?

☑YES, See the ARP website for common anesthetic agents and dosages.

 $\Box$  NO, go to Question # 5.

4h. Please describe the anesthetic procedures **and** complete the table below.

Anesthetic Agent	Name of Procedure Requiring Anesthesia	Dose (mg/kg)	Route of Administration
isoflurane	Injury, MRI	5%/1000ml O <sub>2</sub> for initial; 3%/500ml O <sub>2</sub> prn	Nose cone, inhaled
Pentobarbital	surgery	40-50mg.kg	IP

 Will surgery be performed on live animals? (See IACUC Guideline VIII, <u>Guidelines on Survival Surgery of</u> <u>Rodents</u> or consult with an Animal Resource Program veterinarian at 865-1495).

☑YES, if yes, you <u>must</u> discuss your surgical plan and post operative care with an attending ARP veterinarian (865-1495) prior to submitting this proposal.

☑ I discussed my surgical plan and post operative care with the veterinarian

 $\Box$  NO, go to question #6.

5a. Will animals recover following the surgical procedure?  $\Box$  YES  $\blacksquare$  NO

 5b.
 Will an animal be subjected to more than 1 major surgical procedure?

 □ YES, explain below
 ☑ NO

5c. Please describe each surgical procedure to be performed.

Following the MR scanning animals will receive an IP injection of pentobarital. Once anesthetized, the hair will be clipped from the hindlimb. Plane of anesthesia will be periodically monitored through checking reflexes (eye blink & leg withdrawl). Presence of reflexes will indicate inadequate anesthesia and therefore distress. Additional anesthetic will be administered as needed to maintain place of anesthesia. The Achille's tendon of the animal will be severed with scissors. The blunt edge of the scissors will be used to separate the skin of the posterior calf from the underlying deep fascia and tendon, then the skin will be incised, distal to proximal, over the posterior calf to a point just distal to the tibiofemoral joint. After reflecting the skin medially and laterally, the proximal tendinous attatchments of the gastrocnemius and soleus muscles will be severed with scissors and/or a scapel and the muscle tissue removed. This procedure will be repeated on the contralateral extremity. Due to the nature of the proteins being assessed especially those associated with the inflammatory stages of healing; it is necessary to have the animals alive and under anesthetic for surgeries. Euthanizing the animals would largely influence the time availabe to harvest tissue appropriately. The lack of blood flow to the injured area would influence the amount of necrotic tissue and therefore if the muscle is not sampled quick enough our results would be inaccurate and potentially contamenated. The surgery procedure using anesthesia has been used previously in our work and in others in the field. After the samples have been removed, the animals will be euthanized via overdose of CO2 and in cases of unintended recovery cervical dislocation may be performed

5d. Will analgesics be provided to control post-operative pain?

**YES, provide the name of the analgesic medication & list the details in Q7** 

### **☑** NO, please explain.

This is a non-survival surgery; animals will be euthanized prior to regaining consciousness.

5e. Please describe the care provided during the post-operative period (i.e. animals observed until recovered from the procedure, daily observation until sutures are removed at 7-10 days post surgery).

6. `	Will	blood	be	collected	from	live	animals?

N/A

🗆 YES

Frequency:

☑ NO, go to #7

- 6a. Please describe the method of blood collection (i.e. tail vein, jugular vein, retro-orbital sinus, etc.) See the ARP website for common methods of blood collection.
- 6b. Provide the volume and frequency of blood collection. (**NOTE:** Guidelines call for a maximum of 1% body weight collected no more frequently than every two weeks.)

6c.	Will the animals be anesthetized for blood collection?	

Volume:

□ YES, please make sure the anesthesia has been described in # 4h. □ NO

7. Will substances, other than the anesthetics described in section 4g, be administered to animals?

$\Box$ YES (complete the table below)			☑NO, go to # 8		
Substance	Dose (mg/kg)	Route of Administration	Volume to be Administered	Frequency of Administration	

8. Will any other samples be collected from live animals?

□ YES, please describe the sample collection □ INO

9. Will animals be prevented by physical restraint from making normal postural adjustments for a period of greater than 15 minutes? (See IACUC Guideline XVIII, <u>The Use of Physical Restraint of Research Animals</u>)

### ØNO, go to # 10.

**YES,** please describe the method of prolonged restraint and explain why prolonged restraint is required (prolonged physical restraint is considered to be USDA pain category E).

10. Other than the anesthesia, surgery, and blood collection procedures described above, please list and provide a brief description for each of the procedures or tests that will be performed on **live animals**. Provide enough information to allow the reader to understand what the procedure(s) or test(s) involve(s).

A previously IACUC approved (#22126) closed skin crush injury will be made to their hind limbs. The crush injury will be produced using a drop-weight method in which a weight is dropped from a deteremined height onto the gastrocnemius muscle. Two 1/2in steel drill rods will track a free fall weighted device as it hits an impactor that will compress the posterior side of the muscle. Animals will be anesthetized throughout the entire procedure. Plane of anesthesia will be periodically monitored through checking reflexes (eye blink & leg withdrawl). Presence of reflexes will indicate inadequate anesthesia and therefore distress. Additional anesthetic will be administered as needed to maintain place of anesthesia. We will examine the animals and any animals that do receive a fractured bone will be euthanized immediately. Immediately following injury animals will be undergo MRI imaging. MRI standard operative procedures will be followed. Animals will be anesthetized via inhaled anesthetic througout this procedure and plane of anesthesia will be monitored as described previously. MRI imaging will commence immediately post-injury and will be performed for 6 hours to accurately track the injury response and the treatment effect. The first 6 hours seems to be the critical time to apply some form of cryotherapy previous work in this area in animal model show that ice treatments are effective in decreasing secondary injury and other biological markers. (Merrick 1999, Schaser 2007) In order to best visualize the changes associated with icing 6 hours of imaging will be needed. The animal will be in the magnet for the entire time and anesthetized for the duration. There is one imaging session (6 hours) and then the animal's hind limb tissue will be harvested.



11. Are any of the procedure(s) listed above or the substances administered to the animals likely to cause anything more than momentary pain or distress (See Appendix C for examples of procedures from each pain category.)

 $\square$  YES  $\square$  NO, go to Question 12.

11a. Please describe how the animals may be affected.

In this particular study the time frame following injury is much shorter and animals will remain anesthetized for the duration of the procedures. Tissue will be immediately harvested and the animals will be euthanized. From our previous research we have found following the crush injury that the animals will remain sedentary (guarding their hind limb) for approximately  $\pm 20$ min in the cage immediately following the injury. Then they will begin to move around the cage and return to a normal cage activity level. The repeated visits following the injury, the animals showed no outward signs of injury (no limp, they interacted with the other animal in the cage) and had normal behavior similar to the animals that were uninjured. We also know from previous research that diet is not affected by the injury after that initial bought animals maintain weight and many will gain weight over a week period.

11b. Please provide the specific criteria (endpoints) that will be used to identify animals that will be removed from the study prematurely. See the ARP website for examples of humane endpoints.

Since the animals will be anesthetized for the procedures removal will include respiratory distress, significant core body temperature decreases and heart rate decreases.

11c. Please describe the procedures and frequency for monitoring these animals.

Animals will be constantly monitored with a heart rate and breathing monitor while in the **magnet.** Core body temperature will be monitored throughout the entire MRI process. An anal probe thermometer will be inserted and used to monitor throughout the duration. Warm air will be blown on they to help them maintain normal body temperature while MR scanning

#### 12. Animal Husbandry

12a. Will deviation from standard housing and care be required (i.e. special caging such as hanging wire cages or metabolism cages, working with biohazards, radioisotopes, altered schedule for care, etc.)?

□ YES, please describe any special housing or care requirements. ☑ NO

12b. Will a deviation from the standard diet be required?

□ YES, please explain □ INO, go to question #13

12c. How will the diet differ from the standard diet?

- 12d. Who will be responsible for the formulation of the special diet?
- 12e. For concentrates, what will be the physical form of the diet (i.e., powder, mash, crumbles, pellet)?
- 12f. Who will prepare the diet? (commercial or university personnel) If commercial, provide the name and city of the feed company.
- 12g. Where will the special diet be stored?

13. Will the availability of food or water require a deviation from normal husbandry procedures?

```
□ YES   ☑ NO, go to Question 14.
```

- 13a. Provide the details of food and/or fluid restriction.
- 13b. Will food or fluid be withheld for greater than 18 hours?
  - □ YES If YES, then this is considered a Pain Category E procedure. (See IACUC Guideline XIX, Food or Fluid Restriction for Traditional Laboratory Animal Species)

### □ NO, go to question # 14.

14. List the proposed TOTAL number of vertebrate animals anticipated to be used for the **<u>full duration of the</u> <u>project (3 vear period)</u>**.

Common Name	No.	Strain (if applicable)
Male Wistar Rats	20	

14a. Why are you using this particular species?

This species is appropriate because the animals are relatively small, easily cared for, relatively inexpensive, frequently used in similar lines of research, and provide adequate amounts of tissue for analysis. The PI has previous experience with this species and the biochemistry assays to be used in this study has been previously used with this species.

14b. What will be the source of these animals?

Harlan (all ordering through ARP)

**NOTE:** It is assumed that the data from your experiments will be subjected to an appropriate statistical test. If relevant, you may discuss the relationship between your statistical analysis and the design of your experiments. You may wish to address this item by discussing previous pilot studies, related experimental designs, or reports published by yourself or your collaborators, which preclude a marked deviation from an established design. Conversely, unsuccessful approaches to specific questions may necessitate the adoption of alternative methods. The following is NOT sufficient: "The number of animals selected is necessary to ensure a valid statistical result." For each species, please be sure that the total in this table matches the number provided in the table above and in the budget portion of the research proposal or course outline.

14c. Construct a table or outline that details the use of animals in your experimental design. Your design should be described so that both scientific and non-scientific members of the Committee will understand it. See an explanation of experimental design.

The table should include:

- 1) The number of different experiments proposed;
- 2) The number of experimental groups;
- 3) The number of animals per group; and
- 4) The comparison to be made.

Experiment	# of experiment groups	# of animals	Comparisons
Effect of icing on acute	2x6 matrix design to		MRI images; clinical
injury	compare 2 different ice		signs of injury
	treatments on injury over 6		
	time points. Injury will be	20	
	performed on one limb		
	other limb control		

14d. If not done so in the table or outline above, provide a rational for the number of animals per group and the number of groups.

This study will examine the influence of ice treatments on inflammatory and healing response over the initial 6 hour period following injury. Twenty (20) animals will be used in a 2x6 repeated measures design (treatment x time for MRI). The injury and MRI will take place in Chandlee. Animals will receive a unilateral contusion injury under inhaled anesthetic to their left hind limb and their right hind limb will serve as a control. Previous work in our lab has shown that the use of analgesics, to decrease pain influences our dependent variables. (McBrier, 2008) In addition we want to evaluate the effect of treatment alone so no pain medication will be administered but every animal will receive a treatment on the injured limb. Cryotherapy has analgesic effects and will be administered to each animal (Knight 1995). One group (n= 10) will receive ice for the entire duration 6 hours. This ice will be at 32°F and will be replaced as needed. Previous work in this area (Merrick 1999) so no sign of damage to the skin or muscle for icing for long periods of time. This type of treatment has also been used readily in human studies and applied directly to the skin. The other group (n=10) will receive ice intermittently 30min application time and 1 hour of no ice for the 6 hour duration. Ice has been demonstrated to be an analgesic (Knight 1995) so every animal will receive one form of pain control during this experiment. MR imaging will commence immediately post-injury up to 6 hours. Animals will maintain anesthetic via inhaled anesthetic and be monitored. Both hind limbs will be scanned for location of injury and severity of injury. This portion of the study will rely on the use of clinical markers (hematoma; water diffusion) to determine the level of injury.(McBrier 2008b) Animals will then receive an IP injection of sodium pentobarbital 40-50mg/kg) and be transported to Noll Laboratory for tissue extraction (gastrocnemius muscle). Only one animal will be transported at a time and will be directly monitored during transportation. Tissue will be trimmed of excess fat and connective tissue and weighed to determine mass. Animals will be euthanized via CO<sub>2</sub> inhalation and if needed cervical dislocation may be utilized.

The first 6 hours seems to be the critical time to apply some form of cryotherapy previous work in this area in animal model show that ice treatments are effective in decreasing secondary injury and other biological markers. (Merrick 1999, Schaser 2007) In order to best visualize the changes associated with icing 6 hours of imaging will be needed. The animal will be in the magnet for the entire time and anesthetized for the duration. There is one imaging session (6 hours) and then the animal's hind limb tissue will be harvested. The data collected from the MRI studies will provide us with better insight about the timing associated with inflammatory and muscle regenerative markers and how current acute care influences injury and inflammation.

14e. Are any of the animals known to express a phenotype associated with signs of disease or a shortened lifespan?

☑ NO □ YES, describe the phenotype & how the animals will be handled.

15. Will the animals be used to duplicate research?

ØNO

□ YES, Please explain why it is necessary

16. List the animals according to their Level of Pain or Distress and Consideration of Alternatives to Painful or Distressful Procedures. <u>See Appendix C for examples of procedures from each pain category.</u> **PLEASE NOTE:** Animals should be categorized based on the most severe procedure to which they will be subjected. **DO NOT list or count animals twice**.
| Category  | Number of Animals |
|---|-------------------|
| <u>USDA Category C</u> : Slight or momentary pain or distress or no pain or distress. This category includes breeding colonies and non-invasive teaching protocols. |                   |
| ** <u>USDA Category D</u> : Pain or distress appropriately relieved by analgesia, tranquilization or anesthesia.  | 20                |
| ** <u>USDA Category E</u> : Unrelieved pain or distress.  |                   |

Identify each of the procedures that you plan to use by Category.

Category C:

Category D:

**Contusion injury** under anesthesia with post procedural treatments; non-survival surgery to remove muscle tissue; ice treatments will be provided to every animal.

Category E:

- 17. Literature Search for Alternatives to Painful or Distressful Procedures. For any procedure that is likely to cause more than slight or momentary pain or distress, a **literature search** is required to determine if other methods are available that could reduce or eliminate pain or distress experienced by the animal. A literature search should consider the following questions:
  - Could procedures be refined to reduce the level of pain or distress (such as the use of a less invasive technique or establishing humane endpoints)?
  - Are non-animal models available (such as computer simulation or in vitro testing)?
  - Could the study be conducted on a less sentient species such as invertebrates?

# Complete the following as your written summary of your literature search for alternatives to painful or distressful procedures.

See examples of literature searches for alternatives to painful procedures. Follow this link to read IACUC Guideline I: Documentation of Consideration of Alternatives.

17a. Sources used: [NOTE: At least two sources should be listed!]

PubMed, AWIC (http://www.nal.usda.gov/awic/index.html)

17b. The date the search was completed:  $\lfloor$ 

**5/5/2008** 

17c. The years covered by the search:

17d. Keywords and/or the search strategy used:

animal use alternatives, laboratory animal replacement, laboratory animal reduction, refinement of animal research, refinement of techniques, animal model alternatives, muscle injury, contusion injury, cryotherapy

1990-present

17e. Brief statement summarizing the outcome of your research stating that no acceptable alternatives were found or why potential alternatives cannot be used.

Alternative methods were explored through database searching and reference books. This study examines a phenomenon that involves interactions of multiple physiological systems, and as such, no adequate alternative models were located.

18. Identify the following locations. Include all locations where housing will be for >12 hours or overnight. Also, provide the name of the University facility coordinator or farm facility manager (It is expected that these individuals have been informed of this application prior to submission).

Activity	Building	<b>Room Number</b>	<b>Facility Manager</b>
Housing	Noll Physiological Research Center	14	Doug Johnson
Survival Surgery			
Non-Survival Surgery	Noll Physiological Research Center	6	Doug Johnson
Non-Surgical Procedures	Chandlee	Basement, MRI	Andrew Webb

19. If using non-University facilities for housing, provide the name, address and telephone number of the facilities owner who approved the use of these facilities.

N/A

20. Will this research involve any privately owned animals?

#### 

20a. Name and address of the owner who approved the use of the facilities and animal(s).

20b. Name and phone number of the veterinary clinic or hospital.

20c. Name(s) and experience of the person(s) who will provide daily care and feeding.

21. Including <u>yourself</u>, complete the information in the following table for each individual involved with this protocol:

Name: Nicole McBrier	PSU e-mail ID: nmm13	Hours of animal per week: >25hr/wk			
Type of animal contact with each species used (for example: mouse tail snips): <b>Majority of animal interaction</b> relative to this protocol, contusion injury, non-survival surgeries, tissue removal					
Experience working with each species: I have been working with this type specie of animal for over 5 years prior					
Experience with procedures and/or training the individual will receive: <b>During doctoral studies I was trained to</b> perform the procedures associated with this protocol. I have performing these protocols for 5years prior					
Name: Andrew Webb PhD	PSU e-mail ID: agw3	Hours of animal per week: ~20			
Type of animal contact with each species used (for example: mouse tail snips): <b>Performing the MRI imaging of the animals</b>					
Experience working with each species:	Experience working with each species: He has previous experience with this species				
Experience with procedures and/or training the individual will receive: He is Director of Huck Imaging Magnetic Resonance Center and therefore very experienced with this protocol and able to train others to acquire images.					
Name: Thomas Neuberger	PSU e-mail ID: tun3	Hours of animal per week: ~20			
Type of animal contact with each species used (for example: mouse tail snips): <b>Performing the MRI imaging of the animals</b>					
Experience working with each species:	He has previous experience	e with this species			
Experience with procedures and/or training the individual will receive: He performs imaging for Huck Imaging MR Center and therefore is very experienced with this protocol and able to train others to acquire images.					
Name: Eric Fontaine	PSU Access ID: Etf5009	Hours of animal per week: <b>20</b>			
Type of animal contact with each species used (for example: mouse tail snips): Handling of animals and assisting with contusion injury, MRI images and harvesting of tissue					
Experience working with each species: limited					
Experience with procedures and/or training the individual will receive: He will be trained by the PI and Co-PI regarding the proper way to handle animals and how to assist in the protocols outlined in the IACUC protocol.					

22. Has each person listed in Question #21 completed the Occupational Health Training?

## ØYES

 $\square$  NO, all personnel listed in #21 must complete this training before the IACUC can approve the protocol

23. Will Animal Resource Program (ARP) personnel, the University Veterinarian or others be expected to provide technical services beyond routine care?

ØNO

 $\Box$  YES, list these services and provide the name of the ARP representative who agreed to provide these services. Injections, sampling, euthanasia and technical services are beyond routine care.

24. Is another institution (or Hershey Medical Center) involved in this project?

⊠NO

**YES**, provide a copy of the IACUC approval from that institution's committee.

25. Is there potential for University animals to be exposed to any of the following materials derived from off campus sources? This information is used to help prevent unplanned introductions of infectious agents to University animals. (If yes, please provide the details)

Animal tissue, fluids or cells	□ YES	ØNO
Human tissue, fluids or cells	□ YES	⊠NO
Custom antibodies	□ YES	⊠NO

26. Will this project involve animal contact with any of the following Hazardous Agents?

Hazardous Agent	Check all that apply	Name of Agent
Carcinogens		
Infectious Agents		
Toxic Chemicals		
Isotopes		
Recombinant Organisms		
Mutagens		
Other Hazardous Agents		

**NOTE:** If you checked any of the items listed in Questions #26, you MUST submit an application to either the University Isotopes Committee (UIC)

http://www.ehs.psu.edu/radprot/rad\_authorization.rtf or the Institutional Biosafety Committee (IBC) http://www.research.psu.edu/orp/areas/biohazardous/applications/index.asp

If applicable, IACUC approval will not be granted without prior approval from the UIC and/or IBC Committees. Contact the Office For Research Protections for Guidance.

#### 27. Will the animals be infected with any viable organism?

#### ☑NO, go to # 28

□ YES – Please discuss your project with Dr. Jeffery Dodds, D.V.M. (865-1495 or jwd12@psu.edu), and develop a written description of safety procedures by completing an Animal Biohazard Safety Protocol, <u>http://www.research.psu.edu/orp/areas/biohazardous/applications/animal\_bio.rtf</u>. (PLEASE NOTE: This is a different form than the Application for the Use of Biohazardous Materials.)

Provide the Animal Biohazard Safety Protocol with your submission.

28. Will the animals, caging, or bedding material be contaminated with hazardous materials that will pose a risk to personnel caring for or working with the animals?

### ⊠NO

#### UNKNOWN, please consult with the Attending Veterinarian for Laboratory Animals

□ YES – Please discuss your project with Dr. Jeffery Dodds, D.V.M. (865-1495 or jwd12@psu.edu), and develop a written description of safety procedures by completing an Animal Biohazard Safety Protocol, <u>http://www.research.psu.edu/orp/areas/biohazardous/applications/animal\_bio.rtf</u>. (PLEASE NOTE: This is a different form than the Application for the Use of Biohazardous Materials.)

#### Provide the Animal Biohazard Safety Protocol with your submission.

AFTER COMPLETION, SEND THIS FORM TO THE OFFICE FOR RESEARCH PROTECTIONS, 201 KERN GRADUATE BUILDING.

Project Title:

Evaluation of cryotherapy on the extent of injury during the first 6 hours

#### ASSURANCES FOR THE HUMANE CARE AND USE OF ANIMALS

As the principal investigator on this project, I assure...

- 1. I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
- 2. all individuals named in this application who are at risk will be registered in the Occupational Health and Safety Program.
- 3. the information provided in this application reasonably summarizes the nature and extent of the proposed use of animals. If funded by an extramural source, I assure that this application accurately reflects all procedures involving animal subjects described in the proposal to the noted funding agency.
- 4. all individuals will have successfully completed the IACUC Basic Training. I assure that all individuals performing animal procedures described in this application are technically competent and have been (or will be) properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures.
- 5. I will obtain review and approval from the IACUC before initiating any changes to the approved protocol.
- 6. I will notify the IACUC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and the IACUC.
- For all procedures that fall under USDA Category D or E, I have reviewed the pertinent scientific literature and the sources and/or databases and have found no valid alternative to any procedures described herein, which may cause more than momentary pain or distress, whether it is relieved or not.
- 8. I am familiar with and will comply with Penn State's Policy RA15, "Care and Use of Vertebrate Animals."
- 9. I will maintain appropriate animal records (e.g., census, health, veterinary care, euthanasia, surgery, diagnostic, anesthesia, etc.)
- 10. all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc. have been addressed in the preparation of this application and the appropriate reviews have been initiated.
- 11. I will do everything within my power to safeguard the health and well-being of each animal under this protocol.

Signature of Principal Investigator/Instructor, REQUIRED	Date
Signature of Faculty Advisor/Unit Leader, IF APPLICABLE	Date

I hereby confirm that I have read this protocol and my signature denotes departmental approval of this project.

Signature of Department Head, REQUIRED

Date

THIS APPLICATION <u>WILL NOT</u> BE PROCESSED WITHOUT THE PROPER SIGNATURES! SEND TO 201 KERN UPON COMPLETION!

## VITA

## **Eric Thomas Fontaine**

558 Brittany Drive, State College, PA 16803 etf5009@psu.edu

## **Education:**

Bachelor of Science Degree in Kinesiology, Penn State University, Spring 2011

Honors in Kinesiology Thesis Title: Comparison of Continuous and Intermittent Ice Treatments After Muscle Contusion Injury Using Magnetic Resonance Imaging Thesis Supervisor: Dr. Nicole M. McBrier

## **Related Experience:**

Certified in American Red Cross CPR/AED Adult

Research Assistant in Penn State Athletic Training Laboratories Supervisor: Dr. Nicole M. McBrier Summer 2008 – Spring 2011

Mentor for The Second Mile Friend Fitness Program, State College Branch

Physical Therapy Observational Internship at Fit For Play, State College, PA Supervisor: Dr. Traci Richardson October – November 2008; August 2010

Physical Therapy Observational Internship at Lemont Physical Therapy, State College, PA Supervisor: Amy Flick October – November 2009

## Work Experience:

Commons Desk Student Employee Penn State Housing and Food Services May 2010 – Current

Dining Commons Student Employee Penn State Housing and Food Services Summer 2008

## Awards:

Evan Pugh Senior Scholar Award Deans List (Fall 2007, 2008, 2009, 2010; Spring 2008, 2009, 2010) Schreyer Honors College Endowment for Academic Excellence Scholarship College of Health and Human Development Academic Achievement Scholarship Jean Phillips Shibley Memorial Health Education Scholarship Second Mile Scholarship The Golden Key International Honour Society The Phi Kappa Phi Honor Society

## **Presentations:**

Neuberger, T.; Webb, A.; Fontaine, E.; McBrier, N. "Quantitative MRI analysis of two icing techniques in skeletal muscle injury in a rat limb model." Presented at the 17th Scientific Meeting of the International Society for Magnetic Resonance in Medicine. Honolulu, HI, 2009.