8-2010

Systemic and strength effects : experimental concentrations of topical menthol.

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SYSTEMIC AND STRENGTH EFFECTS: EXPERIMENTAL CONCENTRATIONS OF TOPICAL MENTHOL

By

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B.A., Purdue University, 2008

A Thesis
Submitted to the Faculty of the
College of Education and Human Development of the University of Louisville
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Department of Exercise Physiology
University of Louisville
Louisville, Kentucky

August 2010

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A Thesis Approved on

April 12, 2010

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ACKNOWLEDGMENTS

I would like to thank the principal investigator, Dr. Dean Jacks for his assistance and guidance over the course of this thesis project. Thank you to Dr. Charles Lambert and Dr. Robert Topp for being part of my thesis committee and providing excellent assistance with the research. I would also like to extend thanks to the rest of the department faculty and my colleagues for their support in completing this project. Also, many thanks to my wife, Diana, for her encouraging attitude, even during this busy time in our life.

ABSTRACT

SYSTEMIC AND STRENGTH EFFECTS: EXPERIMENTAL CONCENTRATIONS OF TOPICAL MENTHOL

Lee J. Winchester

August 9, 2010

Menthol has been regularly utilized as a treatment in sports related injuries for many years, yet little is known about its physiological interactions or its effect on performance. Our previous study indicates that topically applied menthol may cause an acute decrease in blood flow and may improve strength capabilities. The purpose of this study is to examine the effects of two different concentrations of menthol (3.5% and 10%) on systemic blood flow and strength. 16 subjects participated in this 4 week study examining blood flow and strength effects after menthol or control treatment. Results indicate that the 3.5% menthol causes a significant (<.05) decrease in blood flow, and arterial diameter in the treated leg, and decreased blood flow in the untreated leg. 10% menthol treatment attenuates the statistically significant increase in blood flow observed with Control treatment, and significantly decreases arterial diameter in the treated leg. Results indicate that menthol is capable of suppressing arterial blood flow locally and systemically.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES AND GRAPHS

CHAPTER 1

INTRODUCTION

Menthol is a heavily researched agent that is found in a variety of different products. Menthol is typically found as the active ingredient in many pain/burn relief gels such as Biofreeze, IcyHot, etc. It is a terpene compound that is well known for its cold or heat sensations as well as its anti-nociceptive and counterirritant properties [1-3]. As an additive in topical ointments, it is utilized to soothe the effects of burns, muscle soreness, and even joint pain [1, 3]. This could play a crucial role in sideline treatment of soft tissue injuries during an athletic event [4, 5]. If an athlete becomes injured, immediate and effective treatment must be applied to help sustain their performance capabilities and prevent further injuries. Although these effects of mentholated ointments are of common knowledge, detailed mechanisms behind these effects, as well as other potential effects, are not well established.

With mentholated products being so widely utilized by consumers, one has to contemplate what impact menthol application has on the individual's normal physiological functions and physical performance. It has been demonstrated that menthol may affect the functional ability of certain cellular ion channels, leading to variations in nerve action potential formation [2, 6-12]. More

recent research has demonstrated that menthol may even impact blood flow in the arterial and cutaneous circulation [13-15]. These effects have led our lab to investigate the impact that menthol may have on performance capabilities.

CHAPTER 2

LITERATURE REVIEW

Menthol effects regular physiological function in numerous ways. It has been shown to alter the nervous responses of somatosensory receptors [1-3, 6, 7, 9, 11, 12, 16-18], elicit changes in local and cellular vasoconstrictor responses [8, 10, 13, 15, 19-22], and consequently attenuates the natural physiological inflammatory cascade [23-25]. Complete mechanisms for each of these responses are not clearly understood. Nevertheless, recent research has started to explore menthol's physiological interactions that display these functional characteristics.

It is now understood that pain is sensed when an effector stimulates somatosensory receptors. Nociceptors, or pain receptors, trigger an action potential that stimulates the body to make adjustments either via the spinal cord, or the upper CNS. This action potential is caused by depolarization of the nerve cell body through an influx of sodium ions via voltage-operated sodium channels [2]. Research has shown that the possible antinociceptive effects of menthol could be due to the blocking of these sodium channels [2]. Human skeletal muscle tissue was sampled, and electric stimulus was applied after tissue was exposed to menthol. At various application strengths, inactivated sodium channels were measured to determine the effect on depolarization. It was

demonstrated that the menthol acted as a block of the alpha subunit of voltage gated sodium channels, therefore causing hyperpolarization of the nervous membrane and a block in the signal of pain transduction [2].

Another study demonstrates the antinociceptive effects of menthol by placing mice on a hotplate and determining pain threshold via observation of painfully stimulated behavior. This study suggests that the temporary pain relief from menthol was as effective as morphine for the hot plate test [1]. It is suggested that menthol selectively activates central κ-opioid receptors, which led to this increase in pain threshold [1].

Menthol has many other noted effects as well, such as temperature sensations, which are a display of thermoreceptor excitation [6, 7, 11, 12, 17, 26]. Menthol generates feelings of cold via the transient receptor potential family of ion channels or (TRP's). TRP's are found throughout the body, but TRPM8, found mainly within thermosensitive neurons, is known to be commonly effected by menthol [9, 16, 18, 27-29]. Utilization of calcium imaging techniques has demonstrated that upon the application of menthol to cloned TRPM8 cells, a heavy intracellular influx of calcium ions caused depolarization due to the opening of non-selective calcium permeable cation channels [9, 16, 18, 27-29]. This nervous transduction leads to the thermo-sensation that is recognized from menthol application. However, menthol is not a direct effector of this calcium channel stimulation. Phosphatidylinositol 4,5 bisphosphate or $PI(4,5)P_2$ is a compound that is thought to activate TRPM8 channels, as well as other calcium cation channels throughout the body . Rohacs et al has demonstrated that

application of menthol causes a direct sensitization of TRPM8 to the effects of $PI(4,5)P_2$ [28]. This increase in sensitization is what leads to the excitation properties of thermosensation via menthol application.

Under certain circumstances, menthol exacerbates cold response to the point of innocuous cold nociception, typically referred to as cold allodynia [6, 7, 11, 12, 17, 26]. It has been demonstrated that menthol propagates painful cold stimuli by attenuating the actions of A-delta sensory fibers [12]. A-delta fibers are responsible for painless cold transmission and their inhibitory effects on C nociceptor fibers. With menthol present, A-delta fibers become inactive, and C nociceptors transmit cold pain.

Group III and IV muscle afferents physically correspond to A-delta and C nociceptor fibers respectively [10]. The vascular pressor response is controlled in part by the group III and IV afferent fibers. Several studies demonstrate that menthol's indirect inhibition of the group III afferents attenuate the pressor response elicited by muscle contraction and cold stimuli [8, 10]. Significant decreases in mean arterial pressure were observed at 20 minutes post application [8, 10], with one study showing significant effects even after 40 minutes post treatment [8].

These findings correspond with menthol's ability to cause arterial vasoconstriction [19-22]. Cold stimulation causes an increased production of mitochondrial reactive oxygen species (ROS) [20]. ROS have been shown to activate a Rho A, an inactive protein that binds with GTP and forms Rho kinase [19]. Rho kinase then translocates α_{2c} adrenergic receptors from the golgi to the

cell plasma membrane. Norepinephrine activates the α_{2c} adrenergic receptors via sympathetic reflex of the muscle afferents, creating arterial vasoconstriction [21]. Rho kinase elicits further vasoconstriction by inhibiting actions of myosin light chain phosphatase in vascular smooth muscle (VSM) cells [19, 30]. This allows myosin light chain kinase to phosphorylate regulatory myosin light chain proteins, allowing for sensitization of calcium and contraction of the VSM cells.

Adrenergic stimulation is an established generator of arterial vasoconstriction [21, 22]. However, data suggests that vasoconstriction can still occur in the absence of adrenergic neurotransmission [22]. Nitric oxide (NO) is a potent vasodilator of vascular smooth muscle. It is created by nitric oxide synthase (NOS) and is released from the endothelial cells that line the VSM, causing vasodilation. Hodges et al. have demonstrated that the cold reflex inhibits NOS enzyme and other unclear mechanisms downstream in the NO system; thus, disrupting the normal vascular dilation [22]. Decreased arterial blood flow has been observed due to reflex vasoconstriction [8, 10, 13, 15, 22, 31], most likely as a result of increased total peripheral resistance [8, 10].

Decreased blood flow to a localized area proves to be beneficial in the event of an injury [25, 31]. Studies suggest that decreased blood flow from topical cryotherapies, such as menthol, consequently blunt local inflammatory response [23, 25]. Cryotherapy elicits a hepatic overexpression of interleukin-10 (IL-10), an anti-inflammatory cytokine [23]. Hypothermic tissue response increases activation of the Janus kinase signaling cascade, resulting in overproduction of IL-10 via the STAT-3 cytokine transcription pathway [23].

Normal response to soft tissue injury results in the hepatic transcription of inflammatory cytokines, such as tumor necrosis factor-α, via nuclear factor kappa B (NF-κB) separation from its inhibitory protein IκB [24]. However, the resulting hypothermic stimulation of IL-10 overexpression allows for the inhibition of NF-κB translocation by preventing the phosphorylation of IκB [23]. This mechanism provides the tissue protective properties of cold induced blood flow reduction.

A study by Hollis et al. directly demonstrates the effects of menthol and ice on arterial blood flow reduction [13]. Subjects attended two morning trials approximately one week apart, during which forearm blood flow from the brachial artery was collected. Blood flow was measured for 15 minutes to serve as a baseline measure. Blood flow was measured continuously for 5 minutes before and for 10 minutes after the topical application of either ice or menthol ointment [13]. The remaining treatment was applied on the second visit. Mean arterial pressure was measured at baseline and throughout the entire treatment session to prevent distortions in blood flow due to blood pressure fluctuation.

Hollis et al reported a significant time effect for ice and menthol treatments, but did not display any significant treatment interactions [13]. It was found that blood flow had been significantly ($p<.05$) reduced in both groups from baseline at 60, 120, 180, 240, and 600 seconds post treatment. At 60 seconds the blood flow was reduced by approximately 43% compared to baseline, and by 24% for the rest of the testing period [13]. These findings suggest that menthol and ice cause similar significant reductions in blood flow, indicating that menthol is as effective as ice for arterial vasoconstriction.

Despite evidence supporting local arterial vasoconstriction after topical cryotherapy, some studies demonstrate increases in cutaneous blood flow after menthol application [12, 14]. Hong and Shellock found that with Eucalyptamint, a mentholated topical product, individuals displayed a 4 fold increase in cutaneous blood flow 5 minutes post application, with a slight gradual decrease until 45 minutes post application when compared to baseline [14]. Skin and muscle temperature were measured via infrared thermometer and 25g thermistor needle, respectively. Skin temperature was measured every 5 minutes for 45 minutes, and had an average, significant change of 0.8 degrees C higher than the base temperature. Muscle temperature was significantly higher at 30 minutes post application as well [14]. Differences in methodology, such as treatment and location of blood flow measurement could be accountable for the discrepancies between data. It is possible that the increase in cutaneous cellular stimulation could elicit this increased flow, despite decreased flow to the local muscle tissue. Besides obvious differences in cutaneous and arterial blood flow, Eucalyptamint has several active ingredients that are not found in the other menthol ointments.

During a previous study, our research team discovered that mentholated ointment elicits a statistically significant decrease in arterial blood flow, as well as an practically improved strength measurements [15]. During this study, subjects attended 3 experimental sessions approximately 1 week apart. Subjects received at random, either a topical ice pack, Biofreeze, or Control (no application) conditions applied to the underside portion of the forearm. During each of the three sessions, blood flow was measured at 5 minute intervals for 20

minutes via Phillips HDI 5000 Ultrasound Doppler (Seattle, WA). This was followed by 4 sets of 30 maximal concentric wrist extension/flexions on a Biodex System 3 dynamometer [15].

Statistical analysis demonstrated that radial arterial blood flow was markedly decreased after the first 5 minutes of the Biofreeze treatment, followed by a statistically significant increase in blood flow at 20 minutes, when compared to control condition [15]. However, treatment with a standard ice pack demonstrated a gradual decrease in blood flow, with significance occurring at 15 minutes post treatment. These results suggest that a topical menthol product would be better suited for a situation that required short-term, immediate effects, such as a slight muscle injury or chronic joint pain, allowing an individual to experience a rapid recovery [15]. Nevertheless, these results further support the belief that topical cryotherapy is an effective arterial vasoconstrictor, regardless of its effects on cutaneous blood flow.

The most interesting findings are the effects that the Biofreeze treatment had on strength performance. When compared to the ice and control conditions, Biofreeze elicited a statistically insignificant (p= .83; p=.79; for flexion and extension respectively) increase in average peak strength across all 4 sets of extensions and flexions [15]. During the first set of each Biofreeze trial, flexion and extension strength were increased by approximately 18% and 14%, respectively, when compared to Control conditions. Even during the 4^{th} and final set, the strength remained elevated by 8.5% and 13% respectively for flexion/extension [15].

Although these findings are not statistically significant, increases in performance upwards of 8.5% may be practically significant in an athletic event. Due to the rapid increase in force production, these effects would most likely be due to increased neurological activity, and recent discoveries on the mechanistic actions of menthol could help support this concept.

A topical neurological strength enhancement product would be a valuable asset to any individual looking to improve their athletic ability. Due to its rapid effects, it could be utilized shortly before sprinting, weight lifting, or any other power related events. Although overall strength enhancement could potentially be an effect, its already recognized pain relief properties could play a role in functional capacity as well. Kraemer et al. demonstrates that with the usage of a cetylated fatty acid – mentholated topical cream, 10 subjects suffering from arthritic pain in the knee, were able to improve the times of their stair climbing tests (14.85$\pm$6.3s pre; 13.05$\pm$5.3s post), as well as their up-and-go tests (9.37$\pm$2.9s pre; 8.22$\pm$2.4s post) to statistically significant levels after topical application 2 times daily for 1 week [3]. Although this is more likely due to pain alleviation rather than strength enhancement, this study suggests that menthol may enhance performance by preventing the physiological transduction of the nociceptive pain sensory pathway.

However, many studies report that many terpene compounds, such as menthol, are extremely efficient at penetrating human skin, including the dermal and epidermal layers [32-36]. Terpenes are compounds that are typically derived from essential plan oils, making them rather lipophilic, which allows for easy

penetration of cellular membranes [36]. A range of 10 to 100 fold increases in skin permeation have been observed with the use of topical terpene products [36]. Menthol, in specific, has been show to be effective at skin penetration [34, 35]. Martin et al. demonstrate that menthol can actually be measured in the bloodstream (19.0 $\pm$ 5.4 ng/mL) after topical patch application, and retains a half life of approximately 4.7 hours [34]. The penetration effects of topical menthol suggest that it may even alter intracellular mechanisms of muscle cells.

As the skeletal muscle sarcolemma depolarizes, it stimulates the dihydropyridine – ryanodine receptor complex of the terminal cisternae, leading to a release of calcium ions from the sarcoplasmic reticulum into the sarcoplasm [37-39]. This intracellular influx is what eventually causes the excitation-contraction coupling of muscle tissues. Phosphoinositides are phospholipids that are involved in this intracellular calcium release and regulation [37-42]. As stated earlier, menthol has been shown to sensitize TRPM8 calcium channels to $PI(4,5)P_2$, causing channel activation and calcium influx [28]. $PI(4,5)P_2$ and other similar phosphoinositides are found in muscle tissues throughout the body, including skeletal muscle [37-40, 43, 44]. Phosphoinositides are already known to activate TRP cation channels in pulmonary artery and aortic smooth muscle cells [40-42]. Menthol application to this muscle tissue evoked significant increases in intracellular calcium concentration [42]. Sensitization of calcium channels to these compounds may promote intracellular calcium influx in skeletal muscle tissue as well, provided menthol is present. This increase in calcium

could elicit an even greater force of muscle contraction, due to an increase in excitation-contraction coupling.

An investigation examining the effects of topical menthol ointments, such as Biofreeze, on maximal muscle contraction could help clarify menthol's impact on performance. It is common knowledge that some of the strength gains due to neurological adaptations from training one limb are displayed in the contralateral limb during times of injury or other disuse. It is possible that the effects of menthol could display a systemic effect through the same or similar neuromuscular mechanism. Topical menthol's ability to enter the bloodstream [34] suggests even greater promise that a systemic effect could occur.

CHAPTER 3

PURPOSE

The purpose of this study is to determine the systemic and non-systemic effects of topical Biofreeze application on peak torque production and arterial blood flow. Heart rate and blood pressure will be measured to help determine mechanisms for these effects. Since no specific concentration of menthol has been deemed as the most effective, an experimental Biofreeze compound of 10% menthol and the standard 3.5% menthol Biofreeze will be utilized to determine dosing effects. It is hypothesized that application of Biofreeze will cause an increase in peak torque production on the treated limb, and will cause lesser, yet still significant increases in the peak torque production of the non-treated limb. It is also hypothesized that the increased concentration will lead to an even greater increase in peak power output.

CHAPTER 4

METHODS

This study follows a randomized, repeated measures design. 8 male and 8 female subjects, (mean age 24.2 $\pm$ 3.0 years), completed this study. Subjects were apparently healthy individuals, with no pre-existing cardiovascular disorders or limb injuries that could impact the results of the study, as assessed by a Medical History Questionnaire. Subjects were asked to participate in four sessions, approximately 1 week apart. Session 1 lasted approximately 30 minutes, while sessions 2 through 4 lasted approximately 1 hour.

During the first session, all subjects were consented for participation in the study using an informed consent document approved by the University of Louisville Institutional Review Board. Once consent was obtained, each subject was measured for demographic information by completing a demographic data collection sheet. This demographic sheet was administered at the beginning of the study and included age, gender, type and duration of exercise engaged in, and basic physical measurements. Basic physical measurements include the 3 site Jackson-Pollock skin fold measurements and waist to hip ratio.

A familiarization trial was also administered during the first session. Sample blood flow and strength measurements were demonstrated to the subjects in order to clarify the procedure. A single blood flow sample was taken

on each leg using the Philips HDI 5000 Ultrasound Doppler (Seattle, WA). Blood Pressure and Heart Rate measures were taken prior to the blood flow measure with an Omron automatic blood pressure cuff (Omron Healthcare Inc., Illinois). Subjects received a test run of one set per leg on the Biodex System 3 (Shirley, NY) dynamometer. No data was collected during the familiarization trial.

A Philips HDI 5000 Ultra Sound Doppler (Seattle, WA) was used to quantify blood flow measures of the popliteal artery. Subjects were instructed to lie down in prone position and remain as still as possible on a padded table during blood flow collection. Using the automatic blood pressure cuff (Omron Healthcare Inc., Illinois), heart rate and blood pressure measures were taken and recorded prior to the first blood flow measurement, and immediately after the second blood flow measurement. Once identified manually, the Doppler sensor head was positioned over the artery until the vessel could be accurately visualized on the device's video screen. The blood flow through the vessel was averaged over a full plot of arterial pulsations in order to determine the volume flow (ml/min) and arterial diameter (cm). Recorded arterial pulsations varied due to variations in subject heart rates. Pain measurements, based on a 0 to 10 scale (0 = no pain; 10 = worst pain imaginable), were taken after blood flow measurements to determine pain sensations elicited by menthol treatment. This same protocol was repeated on the contralateral leg immediately after successful completion of the ipsilateral side.

During experimental sessions two through four, one of three randomly selected experimental applications was applied directly to the Ipsilateral (right)

thigh, with no treatment being administered to the contralateral (left) leg. Biofreeze (3.5% menthol), 10% Biofreeze (10% menthol), or control (no treatment) were the three treatment possibilities selected at random. The treatment was introduced immediately following baseline data collection. Due to the nature of the individual products, subjects were unable to be blinded to the administered treatments. However, subjects were blinded expected outcomes of the study. A pre-calculated amount of Biofreeze topical ointment was applied to the entire surface area of the subject's right thigh. A pilot subject was utilized to determine the quantity of Biofreeze needed to fully saturate their right thigh. Biofreeze ointment was applied topically 1 mL at a time until the leg was saturated with the ointment (subject mean = 7.0 $\pm$ 1.1 mL). Saturation was determined by the skins inability to quickly absorb the ointment. The length and circumference of the thigh were measured and applied to the cylinder surface area formula. Thigh measurements were taken from each consented subject, and the quantity of Biofreeze was extrapolated from the known pilot subject data.

The concentrated Biofreeze was administered in a pre-packaged wipe form. Subjects were instructed to apply the menthol wipes in a clockwise fashion using even strokes. Wipes were continuously applied until the leg was saturated, which typically did not require more than 2 wipes total. Subjects were required to wear gloves during menthol application to avoid absorption into the hands. During the control condition no treatment was applied to the leg.

Blood flow data was collected on the ipsilateral then contralateral leg before treatment and 5 minutes after the experimental application. Baseline

strength data was collected immediately following the baseline blood flow data collection using a Biodex System 3 dynamometer. The same protocol was repeated for trials two through four at the 15, 25 and 35 minute data collection points. A data collection timeline for this protocol can be seen in figure 1.

Each subject was seated and appropriately restrained on the Biodex System 3 dynamometer. The subjects were asked to complete 1 set of 3 maximal voluntary knee extensions and flexions at a rate of 90 degrees per second. The Biodex System 3 manual recommends speeds of 60 degrees per second in a clinical setting, and 120 degrees per second for athletes. Since this study involved the general population, the median speed, 90 degrees per second, was the speed chosen for the protocol. In a study by Rothstein et al., four isokinetic speeds (30, 60, 90, and 120 degrees per second) were tested to determine applied subject effort and force based on integrated electromyography (EMG) using an isokinetic dynamometer [45]. Their data demonstrated minimal differences in EMG activity based on rate isokinetic speed. This demonstrates that the selected isokinetic speed of 90 degrees per second will have minimal impact on the results of our peak torque readings. Range of motion was analyzed for each subject to account for individual variations in flexibility. This assessment was performed on the right and then the left leg. This same protocol was repeated for all 4 time trials. The 3 highest peak torque curves for flexion and extension were recorded to obtain the subject's maximum leg extension and flexion strength for both the right and left leg. The highest value recorded for each extension and flexion was then used for analysis of peak torque (N*m).

Collected data for blood flow and peak power was then analyzed for statistical significance using SPSS statistical software. Repeated measures analysis of variance (ANOVA) were calculated to determine the effect of the various treatments on blood flow and peak torque production over time. Significant effects were further addressed through LSD post hoc comparisons.

CHAPTER 5

RESULTS

HEART RATE AND BLOOD PRESSURE

Repeated measures analysis of variance (ANOVA) indicates a significant ($F=15.432$, $p=.000$) linear time effect on mean subject heart rate, with no significant time and treatment interaction present (see table 2). Post Hoc analysis indicates that the drop in Biofreeze (69.938 ± 3.204, 65.313 ± 2.648) and control (70.875 ± 3.204, 65.687 ± 2.648) trial heart rates are statistically significant between the pre and post treatment blood flow measurements. The 10% Biofreeze treatment demonstrates a decline in mean heart rate, but is not statistically significant ($p>0.05$) (see graph 1). No significant time or time and treatment interactions were observed with systolic blood pressure ($F=0.078$, $p=0.781$; $F= 1.189$, $p=0.314$) or diastolic blood pressure ($F=0.425$, $p=0.518$; $F=1.198$, $p=0.311$) for any trial.

BLOOD FLOW AND ARTERIAL DIAMETER

A significant time and treatment interaction ($F=11.489$, $p=.000$) is observed with blood flow in the treated popliteal artery (table 3). Least Significant Differences ($LSD=16.39$) reveals a decrease in blood flow during the 3.5% Biofreeze trial (128.950 ± 10.234; 103.669 ± 10.940), while blood flow during

the control trial (114.625±10.234; 141.025±10.940) significantly increases (table 4). No statistically significant change in blood flow was observed from 10% Biofreeze trial data when compared to Control or 3.5% Biofreeze treatments. However, the ability of the 10% Biofreeze treatment to demonstrate a slight, insignificant decrease in blood flow could be perceived as practically beneficial since it attenuates the typical blood flow response as displayed by the statistically significant increase in blood flow observed with the control treatment (graph 2). Blood flow data in the untreated popliteal artery demonstrates a significant time*treatment interaction (F=4.755; p=0.013). LSD analysis (LSD=16.8) indicates that 3.5% Biofreeze treatment results in a significant decline in left popliteal blood flow, with control trial almost breaching a significant increase (MD=16.369) (tables 5 and 6). The untreated popliteal artery demonstrates a similar pattern to the treated limb, with the 10% Biofreeze trial eliciting an insignificant decline in blood flow, as observed in the treated popliteal artery (see graph 3).

Statistical analysis suggests that treated popliteal arterial diameter displayed a statistically significant time and treatment interaction (F=8.062, p=0.001) (table5). Both 3.5% Biofreeze (.751±.029; .708±.027) and 10% Biofreeze (.683±.029; .637±.027) treatment groups demonstrate significant declines in arterial diameter, or vasoconstriction (LSD=0.04). Control trial data elicits an increase in arterial diameter (.688±.029; .734±.027) which demonstrates vasodilation (tables 7 and 8, graph 4). No significant changes were observed in the arterial diameter of the untreated popliteal artery.

Pain measures taken during the two blood flow trials showed significance for time (F=15.666, p=0.000) and time treatment interactions (F=3.937, p=0.027). The pairwise comparisons analysis displays a significant mean difference (MD=.500) in pain, indicating an increase in pain for both 3.5% Biofreeze and 10% Biofreeze trials over time. For the time treatment interaction, Least Significant Differences (LSD=0.46) suggests that pain sensation increases in 3.5% Biofreeze and 10% Biofreeze groups when compared to control. (Data displayed in tables 9-11)

Maximal Torque Production and Perceived Pain

Repeated measures ANOVA reveals no significant time or time treatement interactions for any part of the Biodex trial. All treatments resulted in similar trends in peak torque production for Maximal Voluntary Extension and Flexion of the treated limb (graphs 5 and 6), and for the untreated limb (graphs 7 and 8), producing insignificant differences.

Perceived pain data was collected for each leg after each completed set during the four trials to determine how menthol effects pain response to exercise. No significant changes were observed for perceived pain over time or between treatments.

CHAPTER 6

DISCUSSION

The purpose of this study was to investigate the effects of two different concentrations of menthol (3.5% and 10%) on: a) arterial blood flow in the treated leg. b) peak torque production in the treated leg. c) arterial blood flow and peak torque production in the untreated leg to determine if menthol is capable of producing systemic effects. d) changes in heart rate, blood pressure, and pain sensation.

The results have produced several major findings. 1) Heart rate was significantly reduced during Control and 3.5% Biofreeze trials, but not in the 10% Biofreeze trial. 2) Blood flow to the treated limb was significantly reduced during 3.5% Biofreeze trial and significantly elevated during Control. 3) Blood flow to the contralateral limb was significantly reduced by 3.5% Biofreeze treatment. 4) All 3 trials demonstrate significant changes in arterial diameter of the treated limb. 5) Peak torque production was not influenced by treatment for either leg.

HEART RATE

Data collected during the blood flow portion of the protocol demonstrates mean heart rate reductions for all three trials, with a significant ($p<0.05$), 10% decrease in mean heart rate for Control and 6.6 % decrease for 3.5% Biofreeze

trials. 10% Biofreeze heart rate reduction was not significant, demonstrating a 2.4% decrease. These results are expected given the vast quantity of research available on heart rate rebound effect after acute bouts of exercise. Some studies illustrate significant decreases in heart rate and blood pressure after acute bouts of low oxygen consumption (~30% VO_2 max) exercise [46, 47]. This supports our findings due to the 3 repetition maximal voluntary resistance bout that occurred between blood flow data collection points. However, this does not explicate the greater significant decrease observed in Control trial heart rate. One explanatory mechanism for this result is the increased adrenergic stimulation due to the sympathetic reflex associated with menthol application [19, 21, 22]. Increased sympathetic stimulation, therefore increased heart, would explain why a lesser rebound effect was observed in the two trials involving menthol treatment. With this mechanism originating in the skin, it makes sense that the more 10% Biofreeze displayed a non-significant decrease, unlike the standard 3.5% Biofreeze product.

BLOOD FLOW AND ARTERIAL DIAMETER

Blood flow and arterial diameter were both significantly ($p<0.05$) decreased, 19.6% and 5.7% respectively, in the treated popliteal artery due to 3.5% Biofreeze treatment. This agrees with the findings of other studies, which also expressed menthol induced decreases in arterial blood flow [13, 15, 22] and increased arterial vasoconstriction [19-22]. Previous work demonstrates that menthol is as effective as ice for decreasing blood flow [13]. Topical menthol and

ice treatments are known to excite α_{2C} adrenergic receptors via sympathetic stimulation [19, 21] and inhibit the effects of the NOS enzyme [22], leading to arterial vasoconstriction. Some studies have shown that arterial vasoconstriction elicits decreases in mean arterial pressure for 20 minutes after application due to increases in total peripheral resistance (TPR) [8, 10]. This is not consistent with our findings, which indicate no significant changes in blood pressure for any trial during blood flow analysis.

Control treatment resulted in statistically significant increases in blood flow and arterial diameter or the treated popliteal artery (23.0% and 6.6% respectively). This increase in blood flow and arterial diameter can be attributed to the maximal resistance exercise that occurred between blood flow trials. Arterial vasodilation normally occurs in response to exercise training, allowing for increased blood flow. Exercise induced vasodilation occurs due to nitric oxide release with increasing vascular shear stress [48]. This explains why lack of treatment resulted in increased arterial diameter and blood flow. Increased arterial diameter would indicate an overall decrease in TPR, which provides a larger stroke volume due to increased ventricular filling. Such an increase in stroke volume further supports why increased flow was observed, despite the significant decrease in Control heart rate.

No significant effects were observed with blood flow of the ipsilateral limb due to 10% Biofreeze treatment. Nevertheless, the 10% Biofreeze results did follow the general trend of the 3.5% Biofreeze trial, with a 8.3% reduction in blood flow and a statistically significant decrease in arterial diameter of 6.7%. It is

difficult to explain why a more concentrated menthol product would display lesser results. Closer examination of the two Biofreeze products could explain this outcome. The Biofreeze gel (3.5% menthol) and Biofreeze wipes (10% menthol), contain many of the same compounds. However, the Biofreeze gel contains two ingredients, propylene glycol and glycerol, that are not found in the wipes. Studies have shown that propylene glycol and glycerol are very effective at increasing the absorption rate and permeability of topical products [49-51]. One study in particular demonstrates that propylene glycol increases the dermal translocation of hydrogel drugs [49]. This clearly supports why the product lacking these ingredients is less effective.

In the contralateral artery, 3.5% Biofreeze treatment resulted in a statistically significant, 14.7% decrease in arterial blood flow. However, no significant changes in blood flow were found in the contralateral limb for Control or 10% Biofreeze treatments. Despite the lack of significance, Control and 10% Biofreeze treatments still displayed similar effects as observed in the treated leg, with Control group demonstrating a 15.2% increase in blood flow, which almost breaches statistical significance. 10% Biofreeze treatment elicits a 5.4% decrease in blood flow, which is similar to the 8.3% observed in the treated limb. These alterations in arterial blood flow support the concept that the sympathetic reflex from menthol causes an increase in TPR [8, 10], indicating a systemic effect of topical menthol application. Although the results are insignificant, 10% Biofreeze and 3.5% Biofreeze treatments decreased arterial diameter by 3.6% and 2.1% respectively, while Control treatment increased arterial diameter by

2.5%. These results follow the same pattern as the treated limb, but to a lesser extent, indicating that the systemic effect is relatively mild.

RESTING PAIN RESPONSE

A significant increase in pain rating was observed during blood flow analysis for the 3.5% Biofreeze and 10% Biofreeze trials over time and when compared to Control. Menthol is known to elicit sensations of acute cold allodynia [11] by stimulation of thermal TRP channels [9, 16, 18, 28, 29]. This TRP channel stimulation creates an inhibitory effect on the A-delta nerve fibers, allowing for thermal transmission via C nociceptors [6-8, 10-12]. This demonstrates an increase in innocuous cold pain due to menthol application.

MAXIMAL TORQUE PRODUCTION

Results from the maximal voluntary extension and flexion trials did not agree with the hypothesized results. There were no significant differences between any of Biodex trials for any treatment, indicating that topical menthol products have no effect on peak torque production. These findings agree with our previous findings that display no stastically significant changes in peak torque production due to topical Biofreeze applications [15]. However, the treatment location and strength protocols are very dissimilar, which may be the cause of the insignificant increases observed previously. The other study applied Biofreeze treatment directly to the forearm instead of the thigh, and utilized sets of 30 repetitions with the forearm [15]. The much smaller muscle mass volume,

or differences in fiber type composition could have provided the observed results when testing the forearm. Whether menthol could reveal performance enhancement through its analgesic properties [1-3], allowing a person to feel less pain during exercise; or through its ability to cause calcium influx by activating skeletal muscle calcium channels [18, 28, 42], further investigation is necessary to truly determine its effects on muscular performance.

CHAPTER 7

CONCLUSION

In conclusion, it is further supported that topical menthol causes a decrease in arterial blood flow by means of arterial vasoconstriction and increased total peripheral resistance. Although the results are less profound, it appears that menthol may actually cause a systemic reduction on blood flow. This study further supports menthol's actions on increased thermosensation, with significant increases in observed cold pain. It was also demonstrated that menthol concentration was not as important as the added ingredients to enhance its absorption. 3.5% Biofreeze displayed greater significance than 10% Biofreeze wipes in almost every category. This was likely attributed to the 3.5% Biofreeze's increased penetration due to its added ingredients, glycerol and propylene glycol.

Menthol application displayed no apparent changes in performance. The known physiological effects make performance enhancements from topical menthol seem promising. Due to the nature of our measurements, our study was subject to human error and variation in subject effort. Therefore, further study is needed to confirm whether or not menthol impacts muscular performance.

Figure 1. Time Line of a Single Testing Session Which Includes Three Data Collection Points

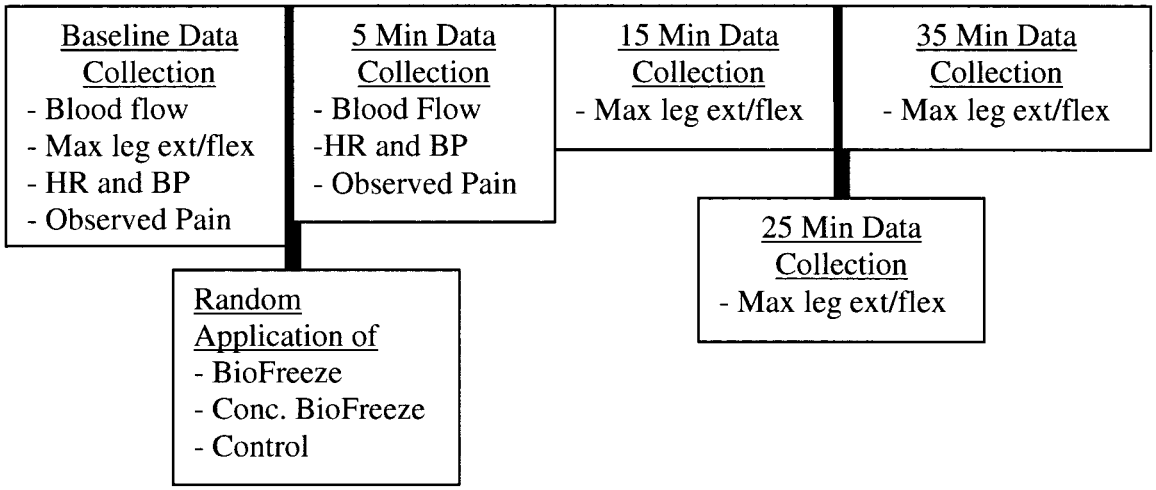


Table 1. Descriptive Statistics

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age	16	22.00	35.00	24.1875	2.97139
Height.IN	16	61.25	73.00	66.6094	3.43265
Weight.LBS	16	113.00	221.00	157.5000	34.36083
RestingHR	16	47.00	92.00	66.5000	13.20101
RestingBP.SYS	16	105.00	143.00	121.7500	12.57776
RestingBP.DIA	16	54.00	73.00	66.6250	5.48787
BodyFatPercentage	16	5.90	28.00	17.9437	6.28267
Waist.cm	16	60.00	85.00	72.8125	8.47914
Hips.cm	16	91.00	110.00	98.0625	6.18028
AerobicExercise.min	16	.00	410.00	200.7375	122.22126
ResistanceExercise.min	16	.00	410.00	150.5125	125.37704
Valid N (listwise)	16				

Table 2. Significant time effect observed during Biofreeze and Control trials from baseline measure.

Tests of Within-Subjects Contrasts

Heart Rate: Initial Compared to 5 Minutes Post Application

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	341.260	1	341.260	15.432	.000
Time * Treatment	Linear	63.146	2	31.573	1.428	.250
Error(Time)	Linear	995.094	45	22.113		

Graph 1. Heart rate response by treatment. Significant time effect observed during Control and Biofreeze trials from baseline measure.
* Indicates Significance at .05 alpha level

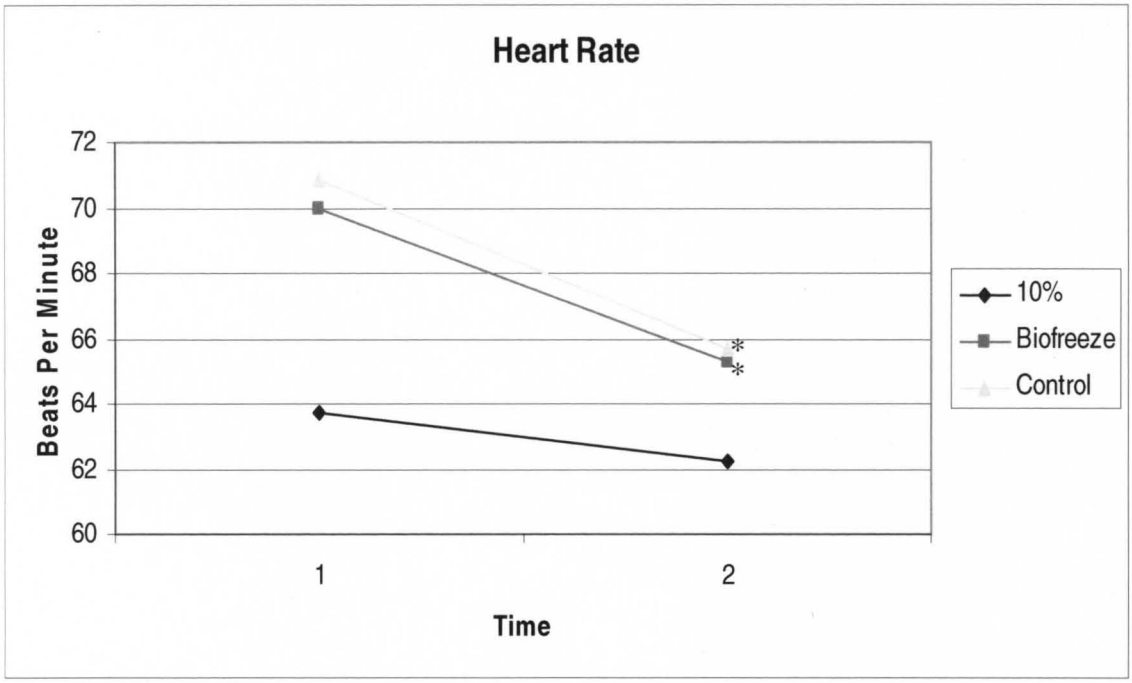


Table 3. Right popliteal blood flow response to treatment. Significant time*treatment effect observed.

Tests of Within-Subjects Contrasts

Blood Flow Right Popliteal Artery

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	140.893	1	140.893	.291	.592
Time * Treatment	Linear	11110.721	2	5555.361	11.489	.000
Error(Time)	Linear	21758.311	45	483.518		

Table 4. Right popliteal blood flow response to treatment. Significant time*treatment effect observed during Biofreeze and Control Trials. LSD=16.39

4. Treatment * Time

Blood Flow Right Popliteal Artery

Treatment	Time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
B	1	128.950	10.234	108.338	149.562
	2	103.669	10.940	81.634	125.703
10%	1	100.913	10.234	80.301	121.524
	2	92.525	10.940	70.491	114.559
C	1	114.625	10.234	94.013	135.237
	2	141.025	10.940	118.991	163.059

Graph 2. Right popliteal arterial blood flow response to treatment. Significant time*treatment effect observed during Biofreeze and Control trials.
* Indicates Significance at .05 alpha level

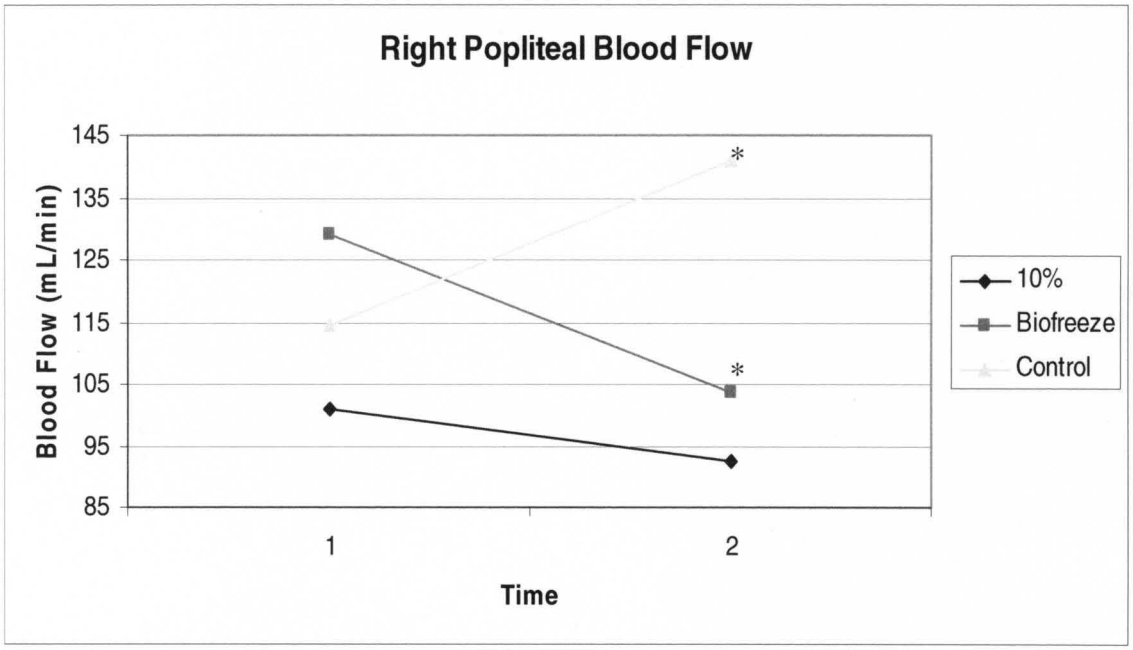


Table 5. Left popliteal blood flow response to treatment. Significant time*treatment effect observed.

Tests of Within-Subjects Contrasts

Blood Flow Left Popliteal Artery

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	138.000	1	138.000	.272	.604
Time * Treatment	Linear	4822.834	2	2411.417	4.755	.013
Error(Time)	Linear	22822.441	45	507.165		

Table 6. Left popliteal blood flow response to treatment. Significant time*treatment effect observed during Biofreeze trial.
LSD=16.8

4. Treatment * Time

Blood Flow Left Popliteal Artery

Treatment	Time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
B	1	121.556	10.523	100.362	142.750
	2	103.669	11.096	81.320	126.018
10%	1	104.962	10.523	83.768	126.157
	2	99.288	11.096	76.938	121.637
C	1	107.700	10.523	86.506	128.894
	2	124.069	11.096	101.720	146.418

*Control approaching significance with Control Difference of 16.3

Graph 3. Left popliteal arterial blood flow response to treatment. Significant time*treatment effect observed during Biofreeze trial.
* Indicates significance at .05 alpha level.

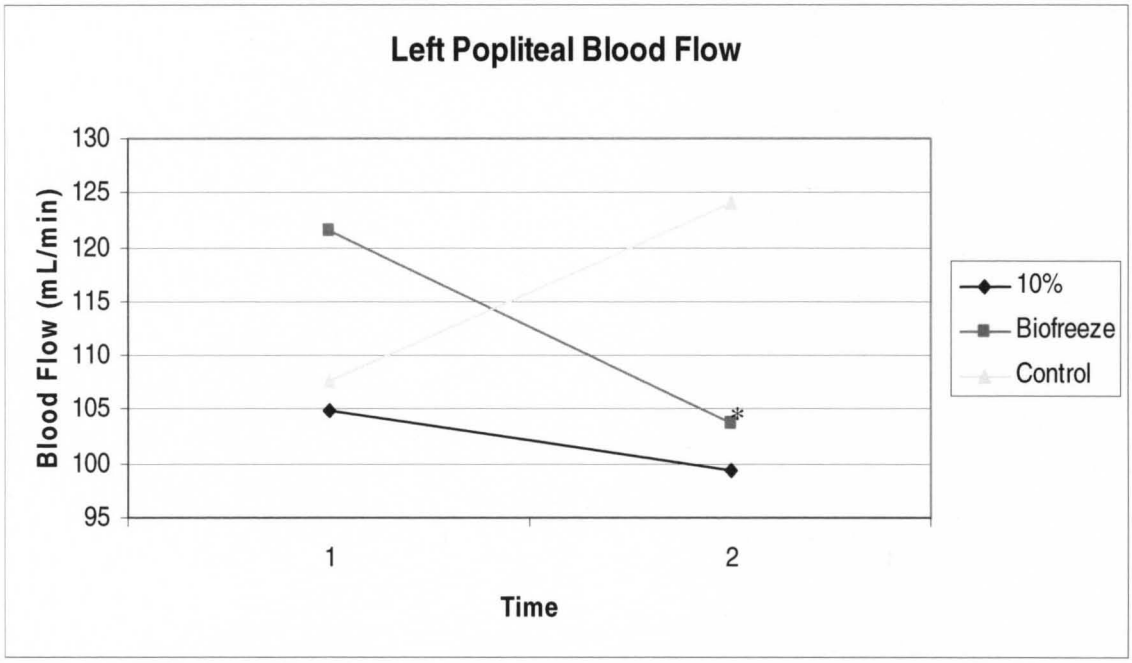


Table 7. Right Popliteal Arterial Diameter response to treatment. Significant time*treatment effect observed.

Tests of Within-Subjects Contrasts

Right Popliteal Arterial Diameter

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	.005	1	.005	1.809	.185
Time * Treatment	Linear	.043	2	.021	8.062	.001
Error(Time)	Linear	.120	45	.003		

Table 8. Right Popliteal Arterial Diameter response to treatment. Significant time*treatment effect observed in all treatments. ($p<0.05$)
LSD=.04

4. Treatment * Time

Right Popliteal Arterial Diameter

Treatment	Time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
B	1	.751	.029	.693	.808
	2	.708	.027	.655	.762
10%	1	.683	.029	.625	.740
	2	.637	.027	.583	.690
C	1	.688	.029	.630	.746
	2	.734	.027	.680	.787

Graph 4. Right popliteal arterial diameter response to treatment. Significant time*treatment effect observed during all trials.
* Indicates Significance at .05 alpha level

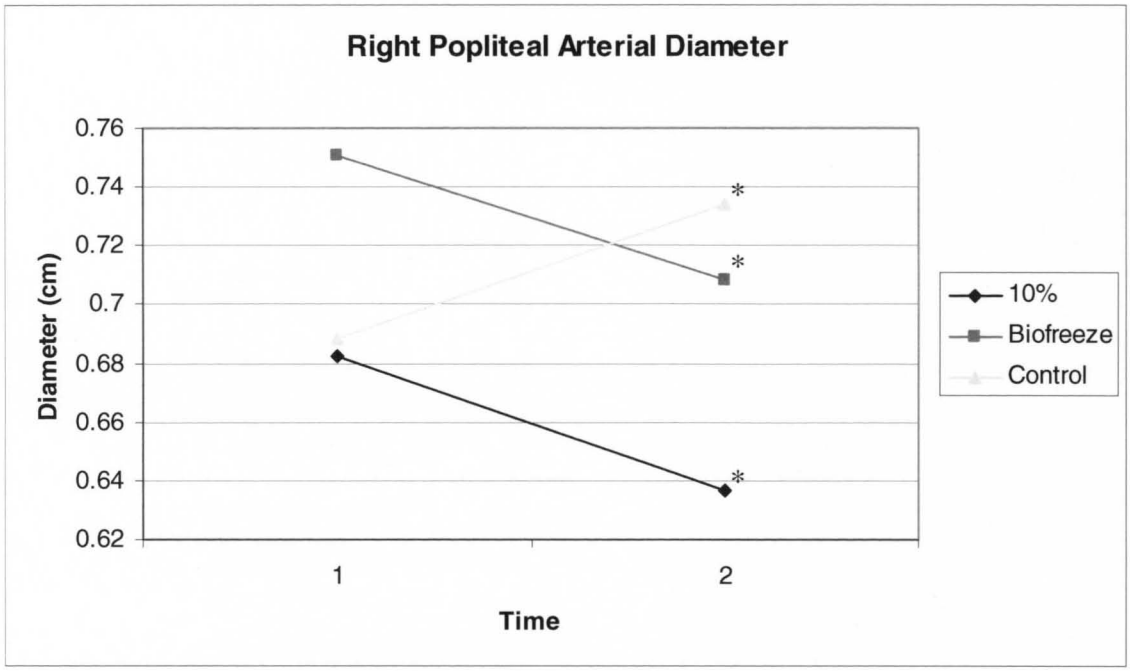


Table 9. Pain response to treatment during blood flow data collection. Significant time and time*treatment effect observed.

Tests of Within-Subjects Contrasts

Perceived Pain From Treatment During Blood Flow Data Collection

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	6.000	1	6.000	15.666	.000
Time * Treatment	Linear	3.016	2	1.508	3.937	.027
Error(Time)	Linear	17.234	45	.383		

Table 10. Pain response to treatment during blood flow data collection. Significant time and time*treatment effect observed.

Pairwise Comparisons

Perceived Pain During Blood Flow Data Collection

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-.500*	.126	.000	-.754	-.246
2	1	.500*	.126	.000	.246	.754

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

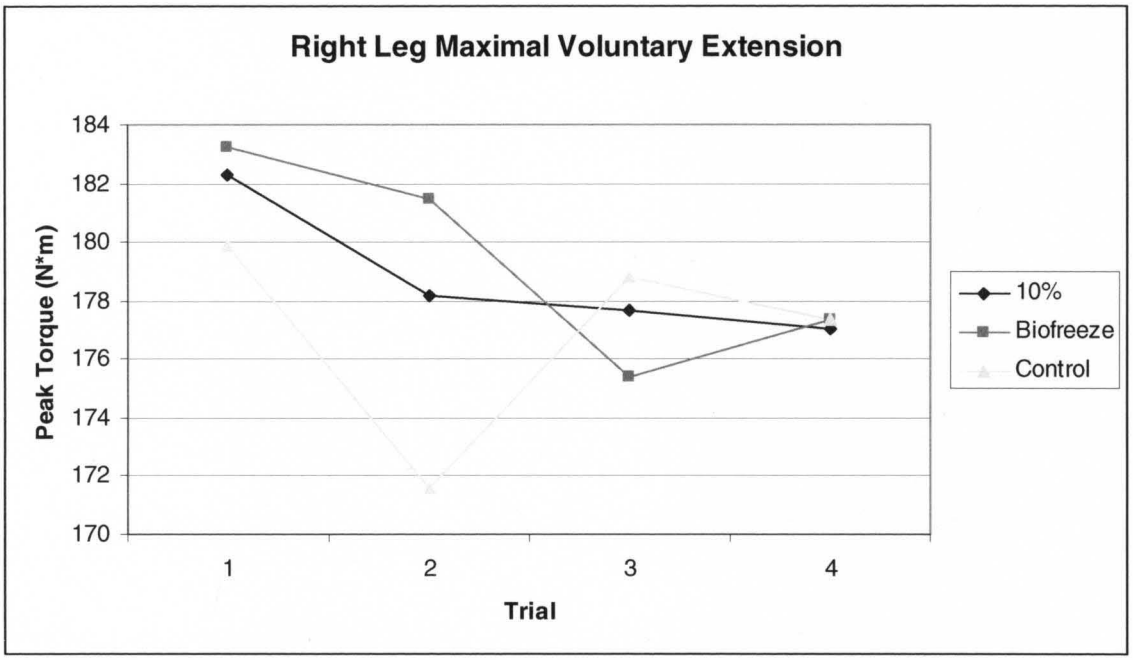
Table 11. Pain response to treatment during blood flow data collection. Significant time and time*treatment effect observed during Biofreeze and 10% Biofreeze trials. ($p \leq 0.05$)
LSD=.46

4. Treatment * Time

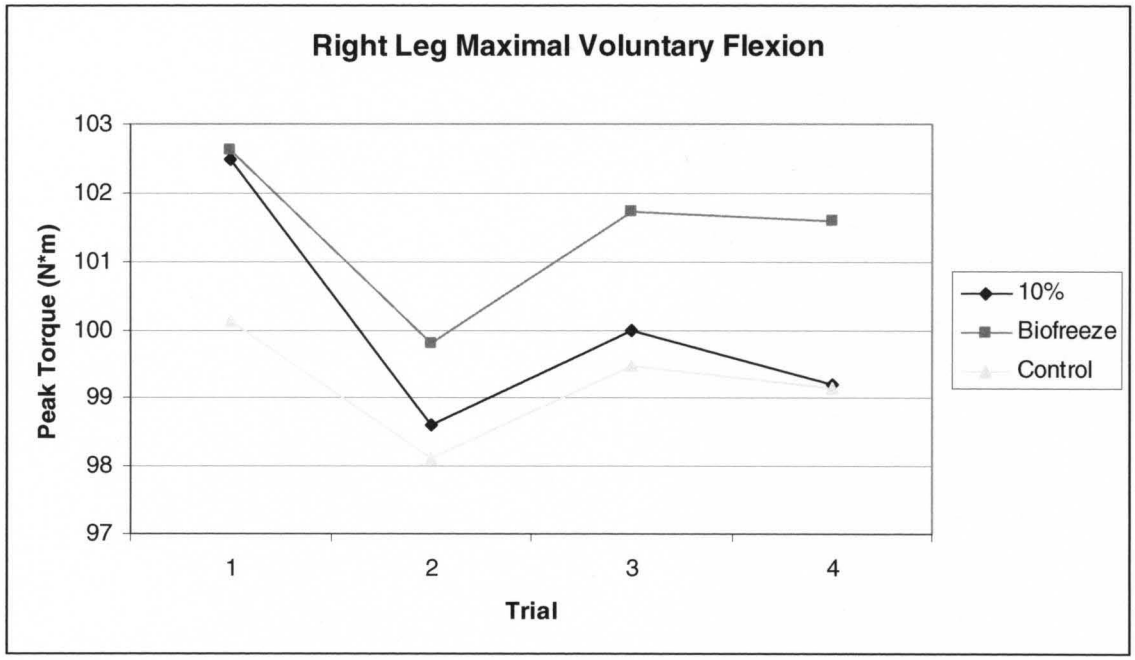
Perceived Pain During Blood Flow Data Collection

Treatment	Time	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
B	1	.000	.000	.000	.000
	2	.781	.219	.341	1.222
10%	1	.000	.000	.000	.000
	2	.719	.219	.278	1.159
C	1	.000	.000	.000	.000
	2	1.110E-16	.219	-.441	.441

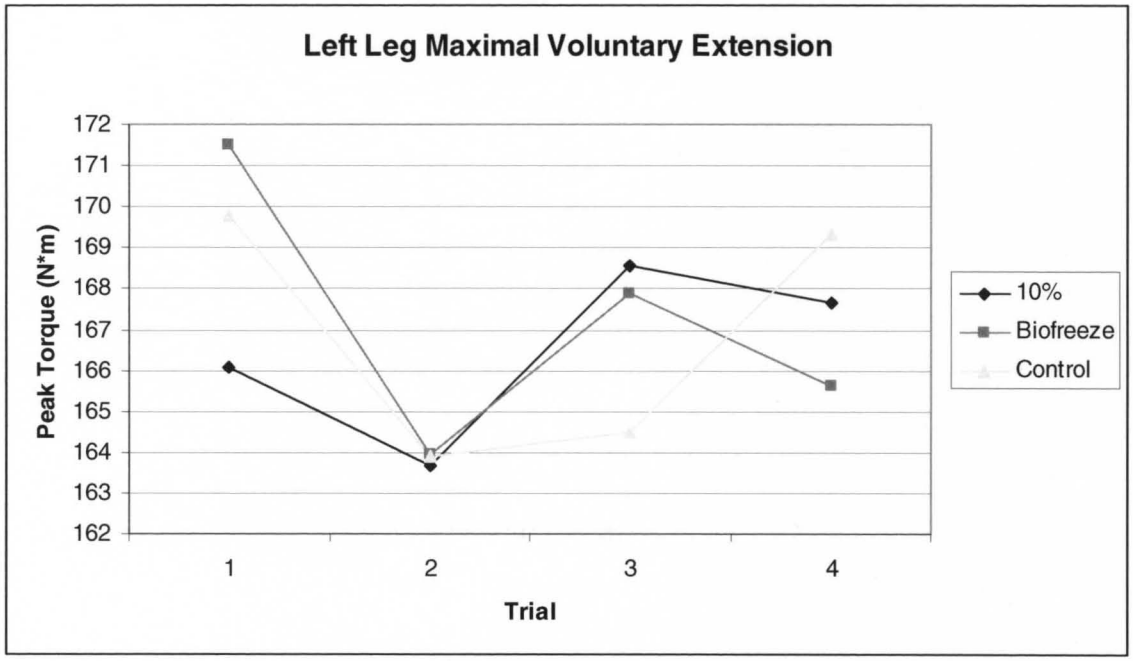
Graph 5. Right leg maximal voluntary extension response to treatment. No Significance observed.



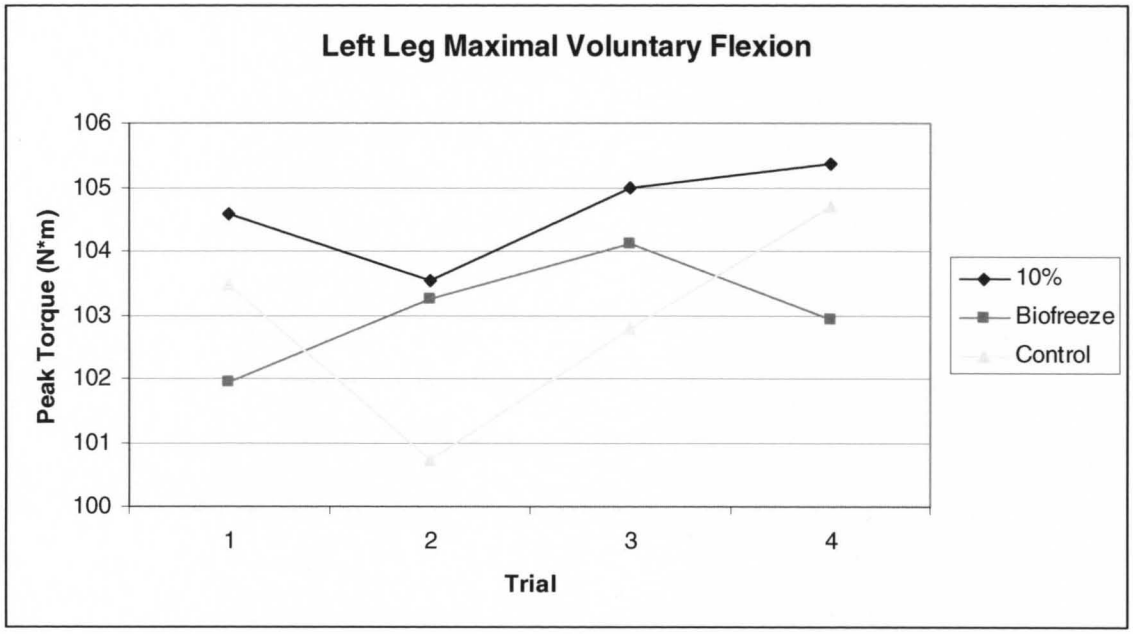
Graph 6. Right leg maximal voluntary flexion. No significant effects observed.



Graph 7. Left leg maximal voluntary extension. No significant effects observed.



Graph 8. Left leg maximal voluntary flexion. No significant effects observed.



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CURRICULUM VITAE

NAME: Lee Winchester

ADDRESS: 56 Ashby Lane
Sellersburg, IN 47172

DOB: Cincinnati, OH – July 30, 1984

OBJECTIVE:

My objective as an exercise physiologist is to improve my capability as a researcher and to further my education within this field to help me accomplish my long-term goal of becoming a Professor and researcher of Exercise Physiology.

EDUCATION:

University of Louisville - Louisville, KY
Masters of Science May 2010
Exercise Physiology
3.837/4.0 Current Graduate GPA

Purdue University- West Lafayette, IN
Bachelors of Liberal Arts May 2008
Health and Fitness Major/ Health Promotion Major
3.2/4.0 Major
2.81/4.0 cumulative

Significant Coursework: Advanced Endocrinology, Systemic Physiology, Human Physiology, Lab Methods in Exercise Physiology, Advanced Exercise Physiology, Exercise Physiology/Human Bioenergetics, Graduate Statistics, Anatomy and Physiology, Exercise Physiology, Exercise Physiology 2, Human Diseases and Disorders, Motor Development, Biomechanics, Worksite Health Promotion, Health and Fitness Program Planning, Health and Fitness Program Management, Health and Fitness Research Methods, Substance abuse and Human Health,

Epidemiology, Health and Fitness Assessments and Exercise Prescription, Health Behavior Change, Health Psychology, Stress and Human Health, Sexuality and Human Health, Women's Health, Essentials of Nutrition, Communications, Spanish to level 4

Computer Skills: Microsoft Office, SPSS

PROFESSIONAL EXPERIENCE

Graduate Research/Teaching Assistant, University of Louisville
Louisville, KY 08/08 – Present
Data collection and analysis
Protocol development and submission
Subject recruiting and scheduling
Anatomy and Physiology course assistance and instruction
Graduate Lab Methods course assistant

Fitness Consultant/Personal Trainer, Riverside Health Care
Bourbonnais, IL 05/08 – 08/08
Perform Fitness Assessments
Give equipment orientations
Develop various fitness programs
Assist with membership and sales
Personal training

Assistant Manager and Personal Trainer, Levee Plaza Health and Fitness
West Lafayette, IN 05/06 – 05/08
Help patrons with membership needs and questions.
Maintained a clean environment for patrons to workout in.
Supervise employees during the evening hours.
Educate patrons about proper equipment usage

Personal Trainer/Fitness Advisor, Purdue University Colby Fitness Center
West Lafayette, IN 01/06-08/07
Interacted with clients by providing a solid workout schedule
Instructed the patrons with proper exercise form and technique
Educated clients about the importance of physical activity.
Perform standard fitness tests on clients to determine health status.

Student Assistant, Purdue University WorkLife Programs
West Lafayette, IN 02/07 – 07/07
Assisted with overall organization of a program titled Targeted Communication.
Helped organize mass mailings for the Targeted Communication program.
Helped to lead patrons through a walking program.
Assisted in numerous other office related tasks

Head Fitness Center Supervisor, Purdue University Colby Fitness Center
West Lafayette, IN 01/05 – 11/05
Insured the safety of patrons.
Organized the fitness center staff duties and schedules
Performed duties such as Payroll and kept track of the number of patrons.

Personal Trainer and Supervisor, Harrison Barbell Fitness Center
Harrison, OH 05/05 – 08/05
Interacted with clients by providing a solid workout schedule and diet.
Instructed the patrons with proper form and technique of exercises.
Assisted in the organization of the gym as well as the patrons memberships.

ACTIVITIES AND HONORS:

Graduate Student Council Representative
I am currently the student representative for the Health and Sports Sciences Department on the Graduate Student Council at the University of Louisville. Meetings discuss issues related to graduate student activities, primarily travel funds. In February 2009, I organized the graduate student funding procedures for the exercise physiology departments trip to the Southeast ACSM conference.

Hallowellness Health Fair Committee Board Member
I assisted in the organization of the entire health fair from the beginning to the end of October 2005 at the Purdue University Division of Recreational Sports. During this Health Fair I organized displays and recruited volunteers in order to allow the fair to run smoothly.

Fitness Center Supervisor Employee of the Year
This is an honorary reward that I received from the Purdue University Division of Recreational Sports in May of 2005, which recognized my dedication to the job I held as a Fitness Center Supervisor in the Colby Fitness Center.

Infant/Child/Adult First Aid and CPR/AED Certified
I am certified by the American Red Cross

Student Lab Assitant, Purdue University Wastl Exercise Physiology Lab
I am currently voluntarily assisting with research in the Wastl Exercise Physiology lab in the basement of Lambert Hall, primarily dealing with carbohydrate studies. I assist in preparation procedures as well as various techniques during the VO2 max tests, including blood collection and analysis.

ACSM Member
I am currently a student member of the National and Southeast American College of Sports Medicine