

CHANGE IN MUSCLE STIFFNESS USING SHEAR WAVE ELASTOGRAPHY,
RANGE OF MOTION, AND PERCEIVED PAIN FOLLOWING A CUPPING
THERAPY TREATMENT IN PHYSICALLY ACTIVE ADULTS

by

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ABSTRACT

Introduction- Cupping therapy originated in Eastern medicine practices, became renowned in international sports, and is now utilized as a therapeutic device for treating musculoskeletal issues in a wide variety of physically active individuals. As with any modality, there is a question of efficacy. This study focused on the effect of cupping therapy on muscle stiffness (MS), active dorsiflexion (DF), and perceived pain. **Purpose-** The purpose of this study was to examine changes in MS, active DF, and perceived pain on the medial gastrocnemius following a cupping therapy treatment. **Methods-** Twenty physically active, healthy participants completed an exercise protocol to induce delayed onset muscle soreness (DOMS) on both lower legs. Participants received a 5-minute cupping treatment on the dominant leg and 5-minutes of rest on the non-dominant leg. DF, MS, and perceived pain were measured at baseline, pre-treatment, post treatment, and 5-minutes post treatment on the medial gastrocnemius muscle. **Statistical Analysis-** A repeated measures ANOVA was used to analyze the main effect and interaction of the conditions and time. **Results-** Active DF was significantly different from baseline to pre-treatment, post treatment, and 5-minute post treatment ($p < 0.001$; $p < 0.001$, $p = 0.01$). Pre-treatment and 5-minute post treatment DF were also significantly different ($p = 0.05$). Active DF was significantly higher at the post treatment and 5-minute post treatment measurements. MS was not significant at any of the time points ($p = 0.398$) nor between conditions ($p = 0.140$). Baseline pain was significantly different than pre-treatment, post treatment, and 5-minute post treatment measurements ($p < 0.001$, $p < 0.001$, $p < 0.001$). Pre-

treatment pain was significantly different than post treatment and 5-minute post treatment ($p=0.09$, $p<0.001$). Post treatment pain was also significantly different than 5-minute post treatment ($p=0.07$). **Conclusion-** After a cupping treatment, active DF was improved in the experimental leg. Participants also reported pain improved following the cupping treatment. No significant difference in MS was observed following the treatment. Thus, a single cupping therapy treatment is a useful modality for individuals experiencing pain and restrictions in ROM.

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LIST OF ABBREVIATIONS

ROM	Range of Motion
MS	Muscle Stiffness
SWE	Shear Wave Ultrasound
DOMS	Delayed Onset Muscle Soreness
VAS	Visual Analog Scale
DF	Dorsiflexion

CHAPTER ONE: INTRODUCTION

The evolution of cupping therapy has led to alternative treatments for pain relief and muscle stiffness (MS). Traditionally used in Chinese medicine, cupping therapy involved glass cups and fire and was primarily used to treat chronic disease.¹ Another type of cupping therapy that evolved from Chinese medicine is known as bloodletting. This technique includes clinicians making small incisions on the patient's skin before placing the cup over the incisions. The suction from the cup then induces bleeding in the area. Cupping therapy use in more recent years has expanded to treat a wide range of illnesses and muscular issues, and a new method of cupping emerged. The new method of cupping therapy involves plastic cups and a hand pump to generate suction that creates a vacuum within the cup.² This method is both easier to use and is generally considered safer because the treatment does not involve fire or incisions. For these reasons, a newer method of cupping therapy has grown in popularity; though, research regarding the therapeutic benefits has yet to be conducted using validated objective measurements. This study adds to the current literature by exploring the benefits of cupping therapy on MS, dorsiflexion (DF), and perceived pain.

Historically, cupping therapy was used to treat different chronic diseases, such as herpes zoster and arthritis¹. Recently, the treatment has been employed to treat musculoskeletal problems. Prior studies have investigated cupping therapy as a treatment for low back pain, shoulder, and neck pain^{3,4}. In these studies participants reported fewer symptoms and decreased pain after the cupping treatment was administered. These results

helped shift the indication of use for cupping therapy to pain relief and soreness reduction.

The use of cupping therapy to treat pain gained significant popularity during the 2016 Olympics. Athletes in the Olympic Games were seen with the signature cupping “kiss” on their skin and claimed this treatment was helping them reduce soreness and stiffness⁵. After that time cupping therapy was used on other professional athletes and college athletes. Athletes began using cupping therapy as another method to help with recovery. As with many popular trends, a trickle-down effect has been observed with cupping therapy. Once only seen on elite athletes, cupping has become a common treatment for the general population for pain control and muscle soreness. As the popularity of cupping therapy emerged, it is important for clinical research to determine if the use of this treatment is effective for recovery from stiffness, soreness, and pain.

One way to examine the therapeutic benefits of cupping therapy is its effect on muscle stiffness measured by shear wave elastography (SWE). SWE is a type of ultrasound that calculates the velocity of the shear wave through the tissue to get an objective measure of tissue stiffness.⁶ The stiffness of muscle tissue plays a role in performance and injury prevention. Athletes want to have a level of stiffness that does not lead to injury or pain but that allows for optimal performance. This method of ultrasound elastography displays results in a color coded elastogram and calculates a numerical value of shear wave velocity. SWE is used to measure muscle properties because it is considered more objective than other types of ultrasound elastography.⁶ This technique has been used in previous studies to measure the stiffness of the medial gastrocnemius after bouts of stretching and a massage treatment.^{7,8,9} In prior research,

pain and ROM have been measured in multiple ways. The visual analog scale (VAS) to measure pain and an inclinometer to measure ROM have become common tools used in modern research. Several studies explain these tools as relatively easy to use while still producing a reliable measurement.^{10,11,12,13} Using these measures, previous literature have found that cupping therapy treatments have improved pain and ROM.^{3,4,14}

Many studies involving cupping therapy have used subjective measures to evaluate the success of the treatment which produce poor clinical evidence for the effectiveness of cupping therapy.^{3,4,14} There is a need for studies utilizing more objective measures to gauge the effect of the treatment such as using SWE. With very little research on cupping therapy, more research is needed to justify the use of this treatment.

Aim of the Study

The aim of this study was to examine the effects of a single session of cupping therapy on MS, active DF, and perceived pain. Cupping therapy has become a common treatment for muscle stiffness and soreness due to the relative ease of application of treatment and the inexpensive equipment. Cupping therapy shares indications and benefits similar to massage, such as increased blood flow, increased temperature, and lymphatic drainage, but since there is little evidence about cupping therapy there could be more shared benefits that have not been explored. A possible benefit would be a decrease in MS, especially in physically active people with exercise induced soreness. More research should also investigate the connection between decreased MS, decreased pain, and increased ROM. As the popularity and use of cupping therapy increases, the effects of the treatment on the body should be explored further.

Hypothesis

This study examined the immediate effect of one cupping treatment on the stiffness of the medial gastrocnemius. This study also explored the effect of cupping therapy on active DF and perceived soreness and pain. It was hypothesized that a single session of cupping treatment would reduce MS, decrease perceived pain, and increase DF in the medial gastrocnemius. The null hypothesis is that a single cupping treatment will not reduce the MS in the medial gastrocnemius.

Significance

Currently there is a lack of prior studies that utilize objective measures of the effect of cupping therapy as a method to reduce MS. There is also a lack of prior studies that investigate the effects of a cupping treatment on MS, DF, and perceived pain and soreness. Evidence for the use of cupping therapy is needed due to the increased popularity of this treatment. There are clinical findings that support the hypothesis that a cupping treatment can be used for pain relief by a trained clinician. This study hoped to add to these findings by including evidence for the indication of use for MS.

Operational Definitions

For the purpose of this study, physically active is defined as meeting the physical activity guidelines for an active adult.¹⁵ This guideline suggests 150 to 300 minutes of moderate activity for adults in a week.¹⁵ The age range for participants in this study is 18-40 years of age. This age range represents the majority of students found at the university of study.¹⁶ MS was defined as spastic muscles that resist passive lengthening which is a common definition for stiffness used in the literature.¹⁷

CHAPTER TWO: LITERATURE REVIEW

Cupping therapy has been used in Eastern countries for many years but has recently become a therapeutic treatment on the World stage for athletes. As a result, there was an increase in popularity for cupping therapy among the general population. Since more people have been using cupping therapy as a treatment for pain and sore muscles, this study aims to provide evidence that cupping therapy is beneficial for MS, DF, and perceived pain. This literature review examines the effectiveness of cupping therapy as a modality for ROM, pain, and MS. This review also explores the use of shear wave elastography as a measurement of MS using shear wave velocity.⁶ Additionally, the reliability and validity of the measurement tools for ROM and pain will be reviewed.

Cupping Therapy

Cupping therapy is a treatment that has been used in Eastern medicine for many years. Traditionally this treatment has been used to treat “blocked Qi” which can cause muscle imbalances.² The use of cupping therapy has become more widespread and is now popular in Western medicine. Clinicians use this treatment for pain control and to address many musculoskeletal issues. Cupping therapy procedures have also evolved over time. The traditional method of cupping uses fire to create a vacuum under a glass cup. Another traditional form of cupping therapy is called wet cupping, or bloodletting. This type of cupping consists of the clinician creating small incisions on the patients’ skin where the cup will be placed. The goal of this treatment is to create bleeding to remove toxins from the body.¹⁸ Although traditional methods are still commonly used, an

alternative method has emerged. A newer method uses a hand-held device and plastic cups to create a vacuum.² In addition to being easier to perform, this method does not have risks associated with the use of fire or piercing the skin.

According to ACE Massage Institute (Asheville, NC), there are many proposed benefits a patient may receive from cupping therapy. Some of the observed benefits include an increase of blood flow and lymphatic drainage in the area being treated.¹⁹ Other potential benefits of cupping therapy are to loosen adhesions, move stagnant blood, and release deep tissue adhesion by increasing elasticity.¹⁹ After a treatment, patients usually notice a discolored mark on their skin, referred to as a “cup kiss”.¹⁹ The color of this mark depends on the duration of the treatment and the amount of stagnation in the tissue. It is recommended that a cupping treatment be between 5-20 minutes in duration. It is important to start a patient with a short duration treatment and then gradually increase the time. Due to the wide range of benefits, cupping therapy has been used as treatment for a variety of conditions.^{3,4}

Cupping Therapy for Specific Conditions

A common treatment area for cupping therapy is on the back. A study conducted by Teut et al²⁰ examined the effectiveness of cupping therapy as a treatment for low back pain. Subjects in the study either received eight cupping treatments or were given only Paracetamol, a pain medication, as needed. Subjects reported on their low back function, quality of life, and on pain level for four weeks and again at twelve weeks using the visual analog pain scale and SF-36 questionnaire.²⁰ At both measurement points, the treatment group reported a significant reduction in pain. Subjects also reported their quality of life had improved with the treatment as improved scores were reported on the

SF-36 questionnaire. The control group in this study observed no significant change in pain level or quality of life at both the four- and twelve-week follow-up. This study provided some evidence that cupping therapy may be another viable option instead of medication for pain relief for low back pain.²⁰ There is an opportunity to further research this comparison as well as other chronic conditions.

Other researchers have investigated cupping therapy on treating chronic neck pain.²¹ The inclusion criteria for this study was neck pain multiple days a week for at least three months. Fifty subjects were randomly assigned into the treatment group or control group. Subjects in the treatment group received five cupping treatments over the course of two weeks, once every three to four days while the control group received no treatment.²¹ To report symptoms, each subject completed a functional, pain, and quality of life survey before and after treatments. The results of this study reported a significant decrease in pain among the treatment group compared to the control group. The pain diaries of the subjects in the treatment group also found that multiple treatments were better at relieving pain than one treatment.²¹ Another study by Chi et al³ observed that a ten-minute cupping treatment on the shoulder and neck resulted in reduced pain. This study found that a reduction in pain was experienced after a single treatment based on the improved scores of the visual analog scale.³ Though cupping therapy has often been used on larger areas of the body, such as the back and neck, it can be used elsewhere such as the feet.

Another area of the body that can benefit from the use of cupping therapy is the foot. A common condition of the foot is plantar fasciitis. This condition is very common among adults and a proposed treatment for this disorder is cupping therapy.¹⁴ The authors

conducted a study to compare the effects of cupping therapy on plantar fasciitis versus the more commonly used modality of electric stimulation. Electrical stimulation was chosen due to its common use for pain control by clinicians. Subjects were randomly divided into the electrical stimulation group or the cupping group, and both groups received eight treatments over the course of four weeks. The authors used the visual analog pain scale and two lower leg functional questionnaires to test the effectiveness of the treatment.¹⁴ Although there was no significant difference between the two treatments, subjects reported a decrease in pain and an improvement in walking and mobility in both groups. These results provide support for the use of cupping therapy in place of electrical stimulation.

Cupping therapy has expanded beyond the clinical setting and into sports as a preferred treatment by athletes and clinicians. Cupping therapy is now being used to promote soft tissue recovery. This treatment can be used after exercise to reduce soreness or to promote healing of an injury by bring blood flow to the area. To expand the research on cupping therapy Kim et al²² investigated how cupping affected range of motion and muscle activity. The authors used the hamstring muscle group to compare the effects of cupping therapy to static stretching. Subjects received either a five-minute cupping treatment using three cups along the hamstring muscle or completed nine, ten-second duration hamstring static stretches.²² An electric goniometer and EMG test were used to measure range of motion and muscle activity. The results of this study demonstrated an increase in range of motion in both groups from baseline measurements, but there was no significant difference observed between groups.²² These results provide some evidence that cupping therapy may be as effective as static stretching when addressing muscular

issues that restrict range of motion. In addition to cupping therapy as a potential alternative treatment, cupping therapy can also be used in combination with other types of treatments such as medication, exercises, and acupuncture.

Combining Treatments

Typically, when pain exists after an injury a clinician will prescribe medication, rehabilitative exercises, or the use of different therapeutic modalities. Markowski et al⁴ analyzed the effectiveness of cupping therapy treatment in conjunction with rehabilitative exercises. Subjects with low back pain were observed to measure the change in pain, range of motion, and function after the combination of treatments. This study used an inclinometer to measure low back range of motion, and a digital force gauge to measure pain-pressure thresholds.⁴ The Oswestry disability questionnaire was also implemented to assess the subjects' low back function. All subjects did a combination of low back rehabilitation exercises and a ten-minute cupping treatment. The treatment consisted of the researcher placing four cups on the low back for the duration of treatment.⁴ A significant decrease in pain and an improvement in range of motion was reported after the cupping treatment.⁴ The observed increase in range of motion could be due to a reduction of pain and stiffness from the cupping treatment, which allowed rehabilitative exercises to be performed more often. This study demonstrated that cupping therapy can be used in conjunction with rehabilitative exercises to reduce pain and increase range of motion.⁴

Cai et al²³ studied the use of cupping therapy as an adjunct treatment to a modified Taiyi Miraculous moxa roll²³, a type of acupuncture. This style of acupuncture uses the heat of a burning moxa roll on acupuncture points. This study examined the combination of treatment effect on pain for a diagnosed lumbar disc herniation. A control

group received acupuncture five times in ten days and a treatment group received a combination of treatments every three days.²³ Results were measured using a simplified pain and symptom scale and identified a significant difference between the two groups. Although there was an improvement in symptoms for both groups, the combination treatment group had 11 subjects improve over 75% to baseline compared to the control group with 2 subjects improving over 75% to baseline.²³ This study also demonstrates that cupping therapy can be an effective treatment for pain when it is combined with another modality.²³

Muscle Stiffness Measurement Tool: Shear Wave Elastography

Muscle stiffness is explained throughout the literature with multiple definitions. One common definition for muscle stiffness is the “measurement of stretch resistance without muscle activation”.¹⁷ The amount a stiffness a muscle has can be an important factor in performance and wellbeing. When a muscle has too much stiffness, it is at risk of becoming injured. A newer method to measure muscle stiffness that has emerged is ultrasound elastography. Elastography is used to measure tissue mechanics by examining the stress placed on the tissue.⁶ A few different types of elastography exist, but the two most commonly used are strain and shear wave elastography.⁶ Strain elastography measures the relative strain between two different areas of the target tissue.⁶ This type of ultrasound uses very low frequency compression, which causes axial tissue displacement.⁶

The other commonly used method is shear wave elastography. This type of elastography uses sound waves to measure tissue displacement. Shear waves are created and propagate perpendicular in the target tissue.⁶ These waves travel much faster than the

waves used in strain elastography. The velocity of the shear wave is calculated and used to compute the stiffness of the target tissue.⁶ This stiffness measurement is displayed using a quantitative map and a qualitative elastogram.⁶ Shear wave elastography ultrasound is more objective than strain elastography due to the lack of compression of the tissue.⁶ Shear wave elastography has become a common measure for stiffness in recent years.

Stiffness can be caused by several different factors but delayed onset muscle soreness (DOMS) is one of the most common causes in athletes. Perceived pain, muscle stiffness, and reduced range of motion have been associated with DOMS.²⁴ DOMS is caused by exercise or activity that is unfamiliar, such as eccentric exercise. Intentional causation of DOMS was used by Agten et al²⁵ in a study to evaluate muscle stiffness in the brachialis. Subject completed an eccentric biceps curl that included a 3-5 second eccentric phase.²⁵ The authors used shear wave elastography to evaluate any change in muscle stiffness after the exercise protocol. The study found that there was a brief increase in muscle stiffness after the eccentric exercise protocol.²⁵

Shear wave elastography has also been used in studies that analyze the effect of static stretching on muscle stiffness. Taniguchi et al⁹ conducted a study to examine the acute effect of muscle stiffness after stretching. Subjects held a standing wall stretch for five minutes to stretch their gastrocnemius. Muscle stiffness was measured with shear wave elastography in five-minute increments up until twenty minutes after the stretch. Range of motion was also measured immediately after the stretch and at twenty minutes post stretch. This variable was not measured as frequently to avoid confounding effects from additional stretch on the gastrocnemius.⁹ The researchers found there was a

decrease in muscle stiffness after stretching up to fifteen minutes post treatment. There was also an inverse relationship between range of motion and muscle stiffness, such that as muscle stiffness decreased, range of motion increased. Immediately after the intervention, range of motion increased by 3.9° and muscle stiffness decreased by 14.2%.⁹ A negative relationship was reported up to fifteen minutes post-stretching but was no longer observed twenty minutes post-stretching. Although a change in muscle stiffness did occur after stretching, it was not a lasting change.

Hirata and colleagues⁸ conducted a study using shear wave elastography for measurement and examined stiffness of the gastrocnemius. This study used a five-minute static stretch hold as the intervention and measured muscle stiffness post-intervention with the shear wave elastography. To ensure a consistent spot for measurement, the researchers used a method that allowed them to identify and test the muscle belly of the medial gastrocnemius. A measurement from the popliteal fossa to the lateral malleolus was taken. Using this measurement, the researchers calculated the proximal 30% of the medial gastrocnemius and made a mark.⁸ This mark was the position of the muscle stiffness measurement for the duration of the study. The results of this study reported a decrease in muscle stiffness of the medial gastrocnemius and a reduction in passive plantarflexion torque after static stretching.⁸

The effects of therapeutic massage on muscle stiffness has also been measured using shear wave elastography. Massage is a frequently used manual therapy with similar effects to cupping therapy. Massage is used to release deep tissue tightness, knots, and increase blood flow and lymphatic drainage.⁷ These are similar to the proposed benefits of cupping therapy. The delivery of treatment is also similar in that both massage and

cupping use stroke techniques on the skin to break up adhesions and increase blood flow. The biggest difference is that cupping therapy uses instruments to assist with manual therapy. Eriksson Crommert et al⁷ had an interest in examining the effects of massage on muscle stiffness. The authors used a seven-minute massage that encompassed effleurage, petrissage, and circular friction techniques on the lower leg.⁷ Shear wave elastography measurements were taken before, immediately after treatment, and three-minutes after treatment. A decrease in muscle stiffness was observed immediately after the massage treatment but was no longer observed at the three-minute measurement⁷. Although this study reported a similar change in muscle stiffness to previous studies, the observed decrease did not last as long after massage when compared to static stretching.^{8,9} Because massage and cupping therapy have comparable benefits, it is possible that benefits from a cupping treatment may not last as long as stretching.

Range of Motion Measurement Tools

There are multiple ways for a clinician to measure range of motion of a joint such as using a goniometer or digital inclinometer. A goniometer can be a reliable tool to measure range of motion but would require the clinician to have plenty of practice to improve their accuracy.¹⁰ Using a goniometer, the clinician must be able to identify multiple landmarks on the body to get an accurate measurement. An inclinometer can be an easier method to measure range of motion because it only requires the clinician to identify one landmark on the body.¹³ A digital inclinometer includes a digital reading of the measurement which can also reduce errors.¹³ In a study comparing different range of motion techniques, the results showed that an inclinometer had a reliability of ICC = 0.96

when compared to using a goniometer with a reliability of $ICC = 0.85$.¹² This study found that even a novice clinician can obtain reliable results using a digital inclinometer.¹³

Perceived Pain

Most of the current studies investigating cupping therapy use pain as a measurement. Pain is a complex sensation that can be divided into three different categories: nociceptive pain, inflammatory pain, and pathological pain.²⁶ Nociceptive pain protects the body from a dangerous stimulus. This type of pain needs an intense stimulus to meet the threshold to be activated. Another type of pain is called inflammatory pain. This type of pain inhibits movement to protect injured tissue by enhancing sensory sensitivity.²⁶ The last type of pain is called pathological pain. This type of pain occurs when there is a dysfunction of the nervous system.²⁶

One theory that can help explain pain control is the Gate Control Theory. This theory states that a nonpainful stimulus can over run the nociceptive stimulus at the spinal cord.²⁷ This nonpainful stimulus is carried through A-beta fibers which are larger than the nociceptive stimulus. At the spinal cord, the “gate” will open or close depending on the input received. A flood of the larger, nonpainful stimulus will inhibit the nociceptive stimulus to get through the gate. This leads to a reduction in perceived pain.²⁷

A common way to measure perceived pain is with the visual analog pain scale. This method of measurement uses a 10 cm horizontal line with no marks. On either end of the line are two extremes; “no pain” on one end and “worst imaginable pain” on the other end.¹¹ A subject places a vertical line to indicate where their pain fell on the scale. A study conducted by Kersten et al¹¹ sought to investigate the validity of using this measuring tool. Using the Rasch measurement model, the study found that the visual

analog scale had internal validity.¹² Using a group of patients from an emergency department, another study found that the visual analog scale was a reliable tool to measure acute pain with an ICC= 0.95.¹¹ Both of these studies provide support for the use of the visual analog scale as a tool for measuring perceived pain.

Limitations of Previous Studies

Although previous studies have provided support for the effectiveness and use of cupping therapy for various pain conditions and restricted ROM, as well as the use of SWE for measurement of MS, they are not without limitations. One limitation that was present in many of the studies was the use of a small and/or convenient sample. A small or convenient sample limits the ability of the results to be generalized to a larger group. With the possibility of a wide range of uses for cupping therapy, it is imperative to provide support for the safe and beneficial use of the treatment to the general public. Another limitation for a few studies was that they only tested the effect of cupping therapy after a single treatment instead of multiple treatments. Results from a single treatment can provide an indication of the benefits of cupping therapy but do not provide a larger picture of long-term benefits. Additionally, most modalities are used as a series of treatments, not a single treatment. The largest limitation of studies involving cupping therapy is the inability to blind the study. A cupping therapy treatment can result in small, bruise-like marks on the skin. This mark is noticeable to the researcher and the subjects which makes it impossible to blind the researcher or subject to the treatment and control group.

The most significant limitation for the use of SWE is the need for a certain tissue depth to create sound waves. This could impact the results of very superficial structures if

there is not enough ultrasound gel between the transducer and the skin. On thinner subjects, a larger amount of ultrasound gel may be needed to reduce the likelihood of this occurring. Most of the measurement tools used to evaluate MS and ROM take practice and knowledge to ensure reliable measurements. This can be a time-consuming process. Without the necessary experience, the researcher could have a difficult time getting consistent results.

Conclusion

The goal of this study is to fill a gap in the current literature. Studies exist that use pain scales, ROM, and functional questionnaires to measure different aspects of cupping, but a gap exists in testing the effects of cupping therapy on MS. Using SWE as an objective measure is needed in the current research to provide quantitative data on the level of stiffness before and after a cupping treatment. If a decrease in stiffness is found after a cupping treatment, the results can have implications for the use of this treatment in a variety of settings. Based on the findings of Taniguchi et al⁹, a decrease in muscle stiffness correlated to an increase in range of motion. Cupping therapy has the potential to be a viable option for treatment of injured tissue with the purpose of restoring ROM and decreasing pain associated with MS.

CHAPTER THREE: MANUSCRIPT

Methods

Introduction

Cupping therapy has become a common treatment for muscle stiffness and soreness due to the relative ease of application of treatment and the inexpensive equipment. As the popularity and use of cupping therapy increases, the effects of the treatment on the body should be explored further. The aim of this study was to examine the effects of a single session of cupping therapy on MS, active DF, and perceived pain. It was hypothesized that a single session of cupping treatment would reduce MS, decrease perceived pain, and increase DF in the medial gastrocnemius. There are clinical findings that support the hypothesis that a cupping treatment can be used for pain relief and improved ROM by a trained clinician. This study hoped to add to these findings by including evidence for the indication of use for MS.

Participants

Twenty volunteer physically active participants were recruited for this study. Participants were recruited from the university of study using both paper and email advertisements. Potential participants completed the exclusion form (Appendix A) and the NASM Par-Q. The participant exclusion form informed the researcher if the subjects had met the criteria for the study. The NASM Par-Q was used to determine if the participant was physically active and able to perform the exercise protocol. All

participants that met the criteria were contacted by the researcher and set up a time to sign the consent form before beginning the study.

Exclusion criteria for this study included any current or previous injury to the lower leg within the last year. To eliminate potential preconceived expectations or physical changes, subjects could not have a history of cupping therapy in the previous year. Since this study did not examine the effect of cupping therapy on injured tissue, the subject could not have any recent injury to their lower legs. Injury can cause reduced range of motion and joint restriction which could have affected the results of the study. Likewise, participants were excluded that had diabetes or were on blood thinners, as these are contraindications for the cupping therapy treatment.

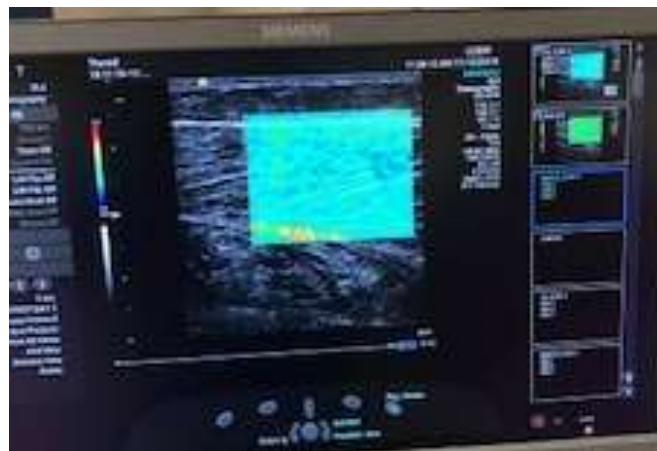
Inclusion criteria for this study were that the subjects reported participating in physically activity for at least 150-300 minutes per week, which is considered physically active.¹⁵ People that are physically active tend to experience tight or sore muscles as a result of their activities. Subjects between the ages of 18-40 were recruited for the study. All participants received a participant number that was used in place of any identifiable information. The only identifiable information was on the participant exclusion form, which was not used for data analysis. All participants that completed the participant exclusion form, the NASM Par-Q and met the inclusion requirements were contacted to set up an initial meeting with the researcher. At the start of the first session, the participant was given a consent form. Once the consent form was signed, the participant started the study.

Table 3.1 Subject Demographics Mean

Gender	N	Age (years)
Male	10	23.6
Female	10	22.3

Measurements

MS, active DF, and perceived pain were measured in this study. MS was measured using SWE (Siemens Acuson S2000, Malvern, PA, USA) with a 9hz linear musculoskeletal probe. SWE directs sound waves into the target tissue and the speed of returned shear wave was calculated which determined the stiffness of the muscle.⁶ Resulting data were represented in two ways: a color-coded elastogram and a numerical value of stiffness (in cm/s) as shown in Picture 3. The color-coded elastogram has a color range from blue to red, with a high stiffness value correlating to the color red. Previous studies have found that SWE can be used as a valid measure of muscle stiffness with a high correlation (97% accuracy) to other forms of ultrasound.^{6,28} The researcher had a reliability of ICC=0.96.



Picture 3.1 Shear wave elastography color-coded elastogram.

The second measurement was active DF. For this study, a digital inclinometer (Vizari: Paramount, CA, USA) was used. Inclinometers have demonstrated good reliability and low measurement error.¹³ To eliminate any additional movement or stretching of the gastrocnemius, the participant laid prone for all dorsiflexion measurements. Krause et al²⁹ found there was good intra-rater reliability when measurements were taken laying prone with the knee extended (ICC=0.81). The researcher identified the base of the fifth metatarsal and placed the digital inclinometer along it. When in place, the researcher instructed the participant to actively dorsiflex and took the measurement (Picture 3.2). The end point was determined when participants actively dorsiflexed as far as possible. The researcher then stabilized the ankle in this position to take the measurement. The participant performed this motion three times and the average was recorded. The researcher had a reliability of ICC=0.93.



Picture 3.2 DF measurement position

The third measurement in this study was perception of pain. Participants self-reported their soreness or pain at each time point during the study using a visual analog

scale (Appendix B). This scale includes a 10 cm line with “no pain” and “worse pain imaginable” at either end.¹¹ To measure the pain or soreness experienced, the researcher measured from the “no pain” end of the line to the line made by the participant. At each time point during the study, the participant marked their level of soreness of each leg on the visual analog scale. Participants were laying prone and not moving their lower limbs while taking this measurement.

Procedures

Data collection for this study took place over the fall semester and was conducted at the Center for Orthopaedic and Biomechanics Research lab at the university of study. Twenty (N=20) participants were recruited for this study. The dominant leg was used for the experimental leg and the non-dominant leg as the control leg for all participants. The experimental leg received the 5-minute cupping therapy treatment and the control leg rested for 5 minutes. Each subject attended two consecutive days of testing.

The first day started with the participant reading and signing the informed consent form. Any activity prior to the time of testing could have an impact on the stiffness of the muscle being tested, therefore, all subjects agreed to refrain from participating in any physical activity until after the second day of testing. After the consent form was signed, the participant completed baseline measures for all three measurements. Perception of pain was measured first. Subjects were instructed on how the visual analog scale worked and how to report soreness and pain at that time. Participants completed a pain scale for the experimental and control legs. Participants were lying prone and not moving their

lower legs when completing this measurement. The order the measurements were taken were not randomized.

The second measurement taken was muscle stiffness using SWE. Subjects laid prone on the treatment table with both feet hanging off the edge to assure a relaxed gastrocnemius. This was the position in which participants remained throughout the measurements. The origin of the medial gastrocnemius was identified using the ultrasound and a mark was placed on the skin. The researcher then used a tape measure to measure the length of the medial gastrocnemius. One edge of the tape measure was placed on the mark and the other end was placed on the lateral malleolus (Picture 3.3).⁸ This length was used to calculate the proximal 30% of the muscle and marked on the skin. This location was used to indicate the muscle belly and was used throughout the data collection to mark the spot of the muscle stiffness measurement. The mark was made in permanent ink and used for both days of testing. This process was used on both the experimental and control leg. Once the spot was marked for both legs, a MS measurement was taken.



Picture 3.3 US measurement location

The last baseline measurement taken was active DF. While participants were laying prone on the table, the researcher placed the ankle in a neutral position. The digital inclinometer was placed along the bottom of the foot in line with the fifth metatarsal. When the researcher was ready, participants actively dorsiflexed their foot. Subjects did this three times on each side. The average of the three measurements was recorded. Once the three baseline measurements were finished, participants started the exercise protocol.

Since this study measured muscle stiffness before and after a cupping treatment on healthy individuals, this protocol was used to induce DOMS. Creating muscle stiffness allows the researcher to observe the effects cupping therapy has on this condition. The exercise protocol for this study consisted of multiple sets of weighted calf raises. The protocol was previously used in a study by Kellermann et al.²⁴

Participants were fitted with a weight vest set at 20% of their body weight before starting the warm-up. Warm-up consisted of two sets of 15 calf raises on a step with a 20 second rest in between sets. After the warm-up, the protocol began. Participants attempted to complete five sets of 30 eccentric calf raises on a step with 10 seconds of rest in between sets. The exercise was completed on a step to make sure participants could achieve full range of motion with every calf raise. Participants were instructed to do a maximal contraction to raise their heel for one second then have a three second eccentric heel lowering back to the starting position.²⁴ If participants were unable to complete a heel raise with a full ROM, they were instructed to remove the weight vest. If the participant could not complete a full eccentric calf raise without the weight vest, they were instructed to stop, and the protocol was finished. There was one participant that was

not able to finish all 5 sets. Once five sets of calf raises were finished, the exercise protocol was completed, and participants had completed the first day of testing.

The second day of testing consisted of participants completing all three measurements at three distinct time points. Measurements were taken pre-treatment, immediately post treatment, and 5 minutes post treatment. These time points were chosen to examine if cupping therapy not only had an immediate effect but one that lasted at least 5 minutes. The second testing day was scheduled 24 hours after the first testing day. Participants started the second testing day with the pre-treatment measurements. Participants first completed the visual analog scale, then the MS measurement followed by active DF. The researcher followed the same steps for each measurement as the first testing day. After the first measurement set, the researcher performed the cupping therapy treatment.

The cupping equipment used in this study were plastic cups and a hand pump (Picture 3.4). With this type of equipment, the hand pump attaches to the top of the plastic cup to create a vacuum between the cup and the skin.¹⁹ In order to create the vacuum, the lever on the hand pump is squeezed. With every subsequent pump, the pressure under the cup increases. When the clinician is finished with the treatment, an air valve at the top of the cup is released. This releases a vacuum that was created under the cup.



Picture 3.4 Hand pump and plastic cups used.

All participants received a 5-minute cupping treatment on the medial gastrocnemius of the experimental leg. To start the treatment, the researcher performed 10 vertical passes along the medial gastrocnemius. After the 10 passes, the researcher placed three cups on the medial gastrocnemius for the remainder of the treatment (Picture 3.5). To have a consistent method of measuring pressure under the cups, the researcher created suction until participants' skin lifted to the first line of the cup (1.3 cm). This method was used for every cup. After a total of 5 minutes, the valve at the top of the cups was released and the treatment was finished.



Picture 3.5 Placement of the cups

Once the cupping treatment was completed participants did the second set of measurements immediately post-treatment. A final set of measurements were taken 5 minutes post-treatment. This completed the second testing day. All data for this study was collected by the researcher.

The researcher was trained extensively how to operate the shear wave SWE unit and had practiced using this system. The researcher was also certified in cupping therapy through ACE Institute and has over three years of experience administering this treatment in a clinical setting.

Data Analysis

For statistical analysis, MS, active DF, and perceived pain measurements were analyzed. For this analysis, active DF, MS, and perceived pain were the dependent variables and time and condition were the independent variables. The average was taken for active DF and MS at each time point. The researcher conducted pilot data with these measures and had a reliability of ICC=0.96 for MS and ICC=0.93 for DF. The mean of these variables at the individual time points were then submitted for analysis. A repeated measure ANOVA with a Bonferroni adjustment was used to test the effects of time and condition on the three dependent variables and the interaction between them. IBM SPSS version 26 (IBM: Armonk, NY, USA) software was used for the analysis. All statistical analysis had an alpha value of $P < 0.05$. Each DF and MS measurement was determined by the average of the three measurements taken at each time point. To measure the effect size, Cohen's d was calculated. Data was also analyzed for sex differences. There was no significant interaction between sexes on any of the variables. For additional data on sex differences see table C.1.

Results

The sample of participants consisted of 10 males (23.6 yr) and 10 females (22.3 yr) that participated in physical activity for an average of 6.65 hours per week. Three DF ROM measurements were taken at each time point. These measurements were averaged and submitted for analysis. The mean (SD) DF in the experimental leg and the control leg are displayed in Table 3.2.

Table 3.2 Mean (SD) active DF between conditions in degrees.

Time	Conditions	Conditions	Cohen's d
	Treatment	Control	
Baseline	20.5 (4.81)	19.3 (4.5)	0.25
Pre-Treatment	15.1 (4.5) ¹	14.1 (3.9) ¹	0.23
Post Treatment	16.8 (4.7) ^{*1}	14.0 (4.3) ¹	0.6
5-min Post Treatment	17.4 (4.5) ^{*1}	15.0 (4.8) ¹	1.2

*Significantly different from control group ($p < 0.05$). ¹Significantly different from baseline measurement ($p < 0.05$).

The analysis showed that there was a significant interaction between the conditions and time on active DF. DF was significantly different from baseline to pre-treatment ($F=13.8$, $p < 0.001$, Cohen's $d = 1.16$), post treatment ($F=13.8$, $p < 0.001$, Cohen's $d = 0.78$), and 5-minute post treatment ($F=13.8$, $p = 0.01$, Cohen's $d = 0.67$). Although both conditions show an increase from post treatment to 5 minutes post treatment, only the experimental leg showed a significant increase. DF was also significantly different between pre-treatment and 5-minute post treatment ($p = 0.05$, Cohen's $d = 0.5$). Figure 3.1 demonstrates the interaction between the conditions. Both conditions follow the same trend by decreasing at pre-treatment (Cohen's $d = 0.23$) compared to baseline (Cohen's

$d=0.25$). This trend differs between conditions at post treatment and 5-minutes post treatment. At post treatment, the experimental leg is significantly higher than the control leg (Cohen's $d=0.6$). The experimental leg is also significantly higher at 5-minutes post treatment (Cohen's $d=1.2$).

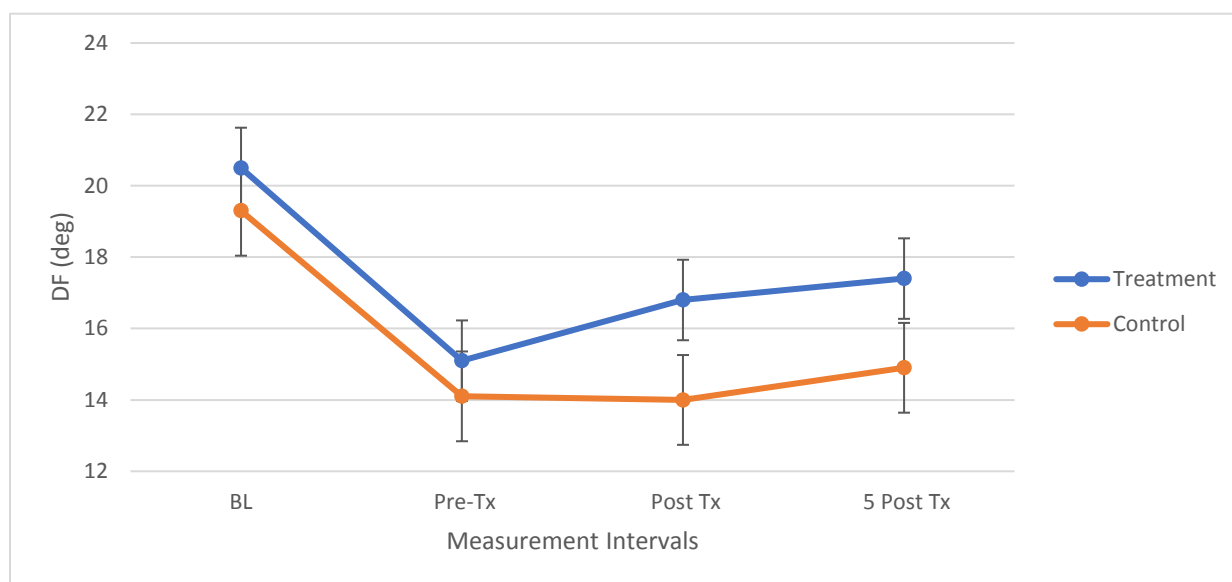


Figure 3.1 Mean DF between control and experimental legs

Mean (SD) MS for the experimental leg and the control leg are shown in Table 3.3. MS was not significant at any of the time points ($F=0.984$, $df=3$, $p=0.398$) or between conditions ($F=2.37$, $df=1$, $p=0.140$). Although there was no significance between conditions, Figure 3.2 shows a decreasing trend of the experimental leg at post treatment (Cohen's $d=0.65$).

Table 3.3 Mean muscle stiffness (SD) between the control and experimental legs in m/s.

Time	Conditions	Conditions	Cohen's d
	Treatment	Control	
Baseline	1.986 (0.163)	1.99 (0.132)	0.03
Pre-Treatment	2.011 (0.212)	2.033 (0.158)	0.12
Post Treatment	1.921 (0.141)	2.028 (0.181)	0.66
5-min Post Treatment	1.976 (0.192)	2.027 (0.147)	0.3

*Significantly different from control group ($p < 0.05$). ¹Significantly different from baseline measurement ($p < 0.05$).

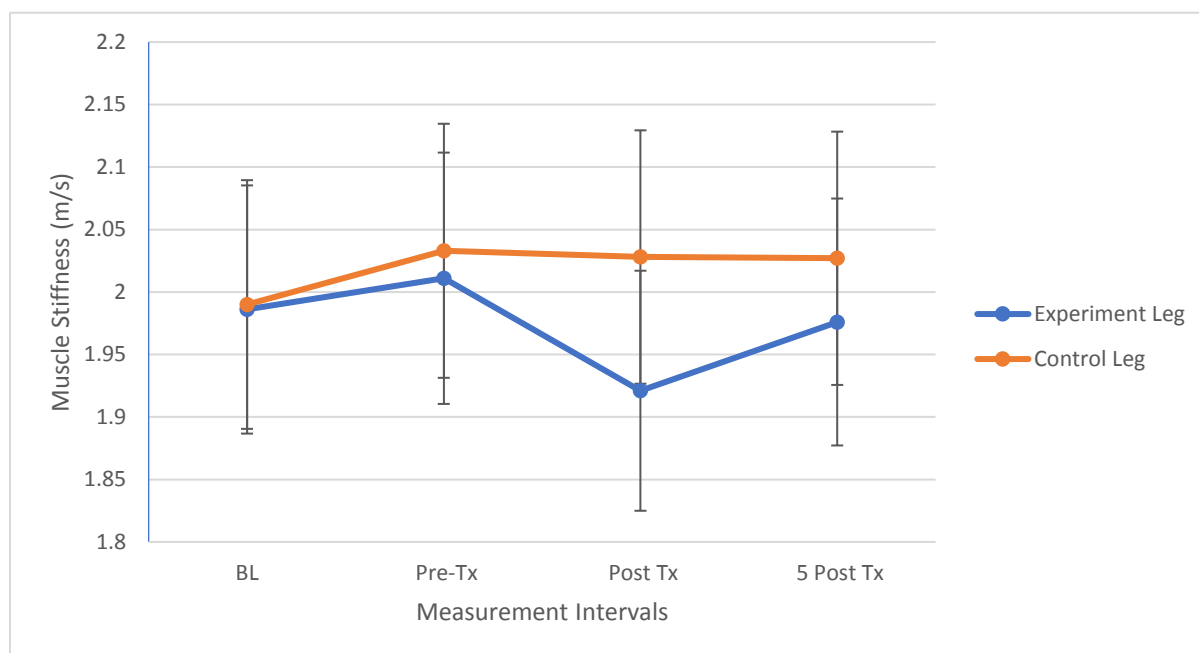


Figure 3.2 Mean MS measurements between conditions.

Mean (SD) VAS scores for the experimental leg and the control leg are shown in Table 3.4. Results showed a significant interaction between conditions and time on

perceived pain. The experimental leg was significantly different than the control leg ($F=19.37$, $df=1$, $p<0.001$) as shown in Table 3.4. The analysis also showed differences between time on perceived pain. Pre-treatment perceived pain was significantly different than post treatment ($p=0.09$, Cohen's $d=0.97$) and 5-minute post treatment ($p<0.001$, Cohen's $d=1.3$). Post treatment was also significantly different than 5-minute post treatment ($p=0.007$, Cohen's $d=0.38$). Figure 3.3 shows the interaction between conditions. Both conditions follow the same trend from baseline (Cohen's $d=0.12$) to pre-treatment (Cohen's $d=0.08$). The trend differs at post treatment and 5-minutes post treatment. At post treatment, the experimental leg is significantly lower than the control group (Cohen's $d=0.8$). At 5-minutes post treatment, the experimental leg is significantly lower than the control leg (Cohen's $d=1.23$).

Table 3.4 VAS mean (SD) scores in cm between conditions.

Time	Conditions	Conditions	Cohen's d
	Treatment	Control	
Baseline	0.27 (0.31)	0.23 (0.28)	0.12
Pre-Treatment	4.87 (1.68) ¹	4.75 (1.73)	0.08
Post Treatment	3.39 (1.36) ^{*1}	4.98 (1.74) ¹	0.8
5-min Post Treatment	2.88 (1.33) ^{*1}	4.82 (1.79) ¹	1.23

^{*}Significantly different from control group ($p<0.05$). ¹Significantly different from baseline measurement ($p<0.05$).

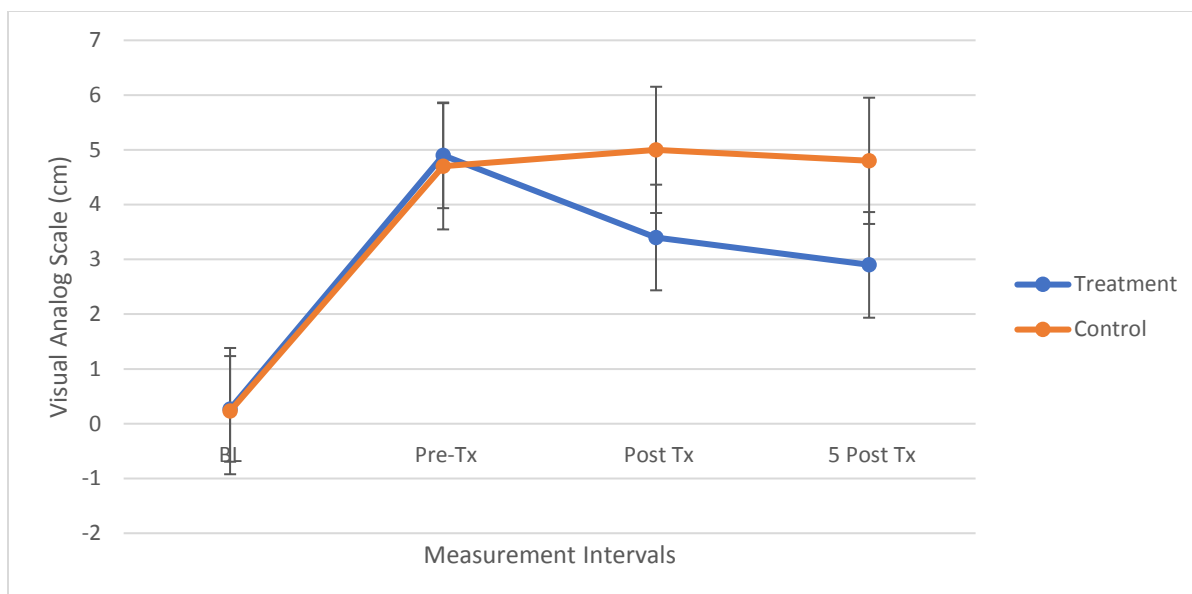


Figure 3.3 Mean perceived pain for the control and experimental leg.

Discussion

The purpose of this study was to examine the effects of cupping therapy on MS, active DF, and perceived pain. To make sure the healthy participants had MS, an exercise protocol was utilized to induce DOMS. The researcher took measurements before and after the exercise protocol to ensure participants experienced DOMS. Participants had decreased DF ($p < 0.001$) and increased pain ($p < 0.001$) 24 hours after the exercise protocol. Although active DF and perceived pain were both significantly different from baseline, MS was not.

Range of Motion

Results showed a significant difference in active DF from baseline to pre-treatment for both conditions. This result is one indicator that the exercise protocol was successful in inducing DOMS in participants in both legs. One product of DOMS is a reduction in ROM. DF was also significantly decreased in post treatment and 5-minute post treatment. This indicates that regardless of the condition, active DF did not return to

baseline measurements. There was an increase from pre-treatment to post treatment in the experimental leg compared to the control. This difference in DF was also observed from post treatment to 5-minute post treatment between conditions. This result indicates that the 5-minute cupping treatment did have a positive effect on active DF. The results of this study are similar to a study conducted by Kim et al²² and Markowski et al.⁴ Both studies found that subjects had a significant increase in ROM following a cupping therapy treatment, however these studies used a different muscle.

These results could be explained by the increase in blood flow to the muscle. As blood flow increases temperature around the treatment area can lead to an increase in ROM. The reduction of perceived pain could also help explain why an increase in DF was observed. Perceived pain can play a factor in participants willingness to actively move. When participants perceive that their pain has decreased in their gastrocnemius, they are more willing to actively DF their ankle to their normal limit.

Perceived Pain

Results of this study found that perceived pain in both conditions significantly increased following the exercise protocol. This is also an indication that the protocol was successful in achieving DOMS. Another result of DOMS is muscle soreness, which is often perceived as pain. The post treatment and 5-minute post treatment measurements are where conditions differ. Immediately following the cupping treatment, participants felt relief in the experimental leg whereas the control leg stayed about the same. A slight decrease in the experimental leg was noted from post treatment to 5-minute post treatment, however these were not significantly different from each other. The results of this study are similar to previous literature.^{3,20,21} Previous studies have also found that

subjects experienced a significant decrease in pain following a cupping therapy treatment using VAS. Although these studies used a different duration and amount of cupping sessions, similar results were found.

The reduction in perceived pain can be explained by the Gate Control Theory. A cupping treatment could be used to stimulate A-beta fibers. The stimulus from the cupping treatment is larger than the nociceptive stimulus and can inhibit the nociceptive stimulus at the spinal cord. This inhibition is the “gate” closing on the pain stimulus which results in reduced pain. Another possible explanation for the decrease in pain would be the increase of blood flow after a cupping treatment. Increased blood in the treatment area has been shown to increase the skin temperature around the treatment area.³ This increase in blood flow and temperature could play a factor in the perception of decreased pain at the treatment area. These results show that the cupping treatment was successful in significantly decreasing participants pain after 5 minutes.

Muscle Stiffness

This study did not find any significant difference in MS between conditions. Although not significant, MS was slightly increased after the exercise protocol in both conditions. MS in the experimental leg did show a slight decrease from pre-treatment (2.011 cm/s^2) to post treatment (1.92 cm/s^2). There was also a slight difference between conditions at the post treatment time point (Cohen’s $d= 0.66$). Based on the results, a single cupping treatment had no effect on muscle stiffness.

These results contradict the changes observed in DF but could be explained by a few factors. The study measured dorsiflexion of the ankle, which is a measurement of multiple muscles. The MS measurement was only taken at one point of the medial

gastrocnemius. The observed change in range of motion could be the result of the activation of multiple muscles instead of only the medial gastrocnemius. Although the muscle belly of the medial gastrocnemius was used to measure muscle stiffness, it is possible that this spot does not effectively represent the whole muscle. The cupping treatment for this study was also only 5-minutes in duration. It is possible a longer treatment could produce a significant decrease in muscle stiffness. Eriksson Crommet et al⁷ found changes in muscles stiffness after a 7-minute treatment. Another potential explanation could be that cupping therapy was only treating the fascia, but not the specific muscle. Since SWE measures the stiffness in the muscle, changes to the fascia would not be detected.

Conclusion

The purpose of this study was to examine the effects cupping therapy has on MS, active DF, and perceived pain. Results showed that active DF and perceived pain improved following the treatment of the medial gastrocnemius. After the cupping treatment, MS was not changed. This could be due to the smaller number of participants. It could also be due to only measuring a small area of the muscle and a short treatment duration. The decrease in pain could be responsible for the improvement in active DF since participants were more willing to reach their limit when they perceived it to be less painful. Further research needs to study the effect that a longer treatment would have on MS, as well as the effects of multiple cupping treatments.

CHAPTER FOUR: CONCLUSION

The goal of this study was to examine the effects of a single cupping therapy treatment on muscle stiffness (MS), active dorsiflexion (DF), and perceived pain. Twenty healthy, physically active participants were recruited to achieve this goal. After completing an exercise protocol, SWE was used to measure MS, a digital inclinometer was used to measure DF, and the VAS was used to measure perceived pain. Measurements were taken by the researcher before the exercise protocol, pre-treatment, post treatment, and 5 minutes post treatment.

Key Findings

After the cupping therapy treatment, significance was found in active DF and perceived pain. DF was significantly increased, and perceived pain was reduced but MS showed no change. DF of the experimental leg was significantly different from the control leg at post treatment and 5 minutes post treatment. Perceived pain was also significantly different in the experimental leg at post treatment and 5 minutes post treatment. There was no difference reported in MS between groups.

Significance

These findings support the use of cupping therapy for athletes or patients with restricted ROM and pain but not for decreased muscles stiffness. Cupping therapy is a modality that has become common in physical rehabilitation clinics and in the world of sports medicine. Evidence of the effectiveness of this treatment can be used to justify the

increased usage. The findings in this study can be used to provide an alternative method to achieve pain control and improve ROM in a relatively safe and non-invasive manner.

Limitations

Although this study's purpose was to help fill a gap in current literature, it is not without limitations. Using SWE to measure MS is a newer, more objective assessment tool; however, it can be user dependent. For example, the amount of pressure the clinician puts on the transducer can increase the recorded measurement. In addition, the measurement can also be influenced by the depth of penetration of shear waves. A depth of about 1.2 mm is required for a proper calculation of the shear waves in the target tissue⁵. Another limitation to the study was the size and sampling method used. Subjects were recruited using a convenience sampling method. Although flyers were placed in a public gathering space on campus, participants may not represent an accurate representation of all students on campus, which reduce the ability to generalize the results to the general population.

This study focused on one specific muscle in the body. This small area of focus limited the scope of the study from examining other benefits of cupping therapy such as performance improvement. The medial gastrocnemius only represents a small portion of the musculature of the gastrocnemius that is responsible for lower body performance. With only one portion of the calf musculature treated with cupping, a change in ROM may not be clearly observed. The population of this study included healthy, physically active adults. Eligibility criteria include participation in activities outside of daily living activities which can cause stiffness in muscles. This study provided a starting point for research on cupping therapy and its relation to muscle stiffness. Further studies should

address the effects cupping therapy on performance in athletes by using the entire musculature of the gastrocnemius or leg.

Future Research

The current study focused on cupping therapy's effects on MS, DF, and pain. One area for future research would be to analyze effects of cupping therapy after a longer treatment duration, as well as after multiple treatment sessions. Future research should also focus the affect cupping therapy has on performance. Cupping therapy has been commonly used in the world of sport, support for the use this modality is needed regarding performance affects. Another area in need of further research is the possible effect on fascia. This is an area of cupping therapy research that has not been fully explored.

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APPENDIX A

Participation Exclusion Form

Name: _____

Age: _____

Email: _____

Phone: _____

Dominate Leg: _____

Injury History:

Have you had an injury to your lower leg, ankle, or foot in the last year?

Have you received a cupping treatment for the lower leg in the last year?

Medical History:

Do you have a history of diabetes?

Are you currently taking blood thinners?

Do you have any allergies to lotion or any ingredients in lotion?

Activity History:

How many days a week do you participate in a recreational activity?

How long do you participate in a recreational activity per week? (in hours)

APPENDIX B

Visual Analog Scale for Pain

No pain

Worst imaginable pain

APPENDIX C

Sex Analysis

There were 20 healthy individuals recruited for this study. There were 10 male participants and 10 female participants that were included. To analyze for a potential effect of sex on the three variables, a repeated measures ANOVA was used. The results of this analysis showed no significant difference between sexes on MS, active DF, and perceived pain. Details of the analysis are included in table C.1. Chino and Takahasi³⁰ found similar results in MS of the medial gastrocnemius. In their study, no significant difference in MS was found between sex in DF.³⁰

Table C.1 Sex Data Analysis

	Time	Experimental leg		Control Leg	
		Male	Female	Male	Female
DF	Baseline	18.9 (5.65)	22.1 (3.4)	17.6 (4.82)	21.1 (3.59)
	Pre-treatment	15.7 (4.21)	14.5 (4.93)	14.3 (3.27)	14 (4.55)
	Post Treatment	16.9 (4.19)	16.6 (5.49)	14.2 (4.15)	13.8 (4.61)
MS	5 min Post Treatment	17.7 (3.82)	17 (5.37)	15.8 (4.32)	14.1 (5.09)
	Baseline	1.93 (0.15)	2.04 (0.17)	1.95 (0.15)	2.03 (0.11)
	Pre-treatment	1.95 (0.19)	2.07 (0.22)	2.07 (0.17)	2.00 (0.15)
Pain	Post Treatment	1.90 (0.14)	1.94 (0.14)	2.07 (0.21)	1.99 (0.14)
	5 min Post Treatment	1.96 (0.14)	2.00 (0.24)	2.06 (0.21)	2.00 (0.09)
	Baseline	0.2 (0.30)	0.3 (0.33)	0.2 (0.23)	0.3 (0.28)
Pain	Pre-treatment	4.4 (1.93)	5.4 (1.30)	4.3 (1.88)	5.2 (1.55)
	Post Treatment	3.0 (1.17)	3.8 (1.46)	4.6 (1.89)	5.4 (1.56)
	5 min Post Treatment	2.6 (1.45)	3.1 (1.23)	4.5 (2.00)	4.8 (1.79)

*Significantly difference (p<0.05)