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ORIGINAL RESEARCH

EFFECT OF TOPICAL MENTHOL ON IPSILATERAL AND CONTRALATERAL SUPERFICIAL BLOOD FLOW FOLLOWING A BOUT OF MAXIMUM VOLUNTARY MUSCLE CONTRACTION

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ABSTRACT

Purpose/Background: Various doses of topical menthol are commonly applied prior to, during, and after exercise to relieve pain although there is limited empirical evidence examining the physiological effects of this treatment. The purpose of this study was to examine the effects of two different doses of menthol (3.5% and 10%) on blood flow and arterial diameter before and after an acute bout of three isokinetic maximum voluntary muscular contraction (MVMC) of the quadriceps and hamstrings.

Methods: Blood flow and arterial diameter of the right and left popliteal arteries were measured with an ultrasound Doppler prior to and after subjects completed 1 set of 3 MVMC isokinetic knee extension/flexion exercises. Immediately following this exercise one of three different treatment conditions was randomly applied to the right thigh only; 3.5% menthol gel, 10% menthol wipe, or a control condition. Five minutes following the treatment application blood flow through both right and left popliteal arteries was reassessed. This procedure was completed once per week until each of the 16 subjects was exposed to each treatment condition.

Results: Repeated measures ANOVA with post hoc analysis indicated that both menthol dosages resulted in significant decreases in popliteal blood flow on the right (–19.60 to –8.39%) and left sides (–14.72 to –5.4%) while the control condition demonstrated an increase in blood flow bilaterally (+26.40 to +15.19%) as a result of the MVMC exercise. The right popliteal arterial diameter was also significantly reduced as a result of both menthol dosages (–5.73 to –6.73%) and increased under the control condition (+6.67%).

Conclusion: These results indicate that topical menthol has a rapid effect on reducing ipsilateral and contralateral arterial blood flow as well as ipsilateral arterial diameter.

Levels of Evidence: 2a

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INTRODUCTION

The active ingredient in many over the counter pain topical gels is menthol. Menthol is a terpene compound that when applied to the skin in varying doses causes anti-nociceptive and counterirritant sensations, resulting in a soothing sensation for the discomfort of burns, muscle soreness, and joint pain.[1-3] Menthol also generates the sensation of cold secondary to thermoreceptor excitation[4-9] of the transient receptor potential (TRP) family of ion channels. TRP's are located throughout the body and TRP melastatin 8 (TRPM8) thermosensitive neurons have been found to be particularly sensitive to menthol stimulation.[10-15] Previous authors have reported that stimulating thermoreceptors by lowering the temperature of the surrounding tissues results in vasoconstriction and a reduction in blood flow to the surrounding tissues. Hodges et al reported that stimulating thermoreceptors through localized cooling inhibited the release of nitric oxide synthase (NOS) from the endothelial cells that line the vascular smooth muscle (VSM), resulting in vascular constriction.[16] Stimulating thermoreceptors through localized cooling has also been reported to activate the α_{2c} adrenergic receptors via sympathetic reflex of the muscle afferents, creating arterial vasoconstriction.[17-20] Localized cooling of the skin that stimulates thermoreceptors elicits both local and generalized vasoconstriction. The magnitude of both of these vasoconstriction responses is, in part, dependent on declines in core temperature resulting from cooling of the periphery.[21]

Although menthol stimulates thermoreceptors that have been found to result in vasoconstriction with localized cooling, a limited number of studies have examined the vaso-reactive responses stimulated by topically applied menthol. Olive et al.[22] demonstrated that the topical application of 3.5% menthol gel to the forearm had a similar effect as applying ½ kg of crushed ice on reducing blood flow by an average of 24% through the brachial artery approximately 10 cm proximal to the treatment for up to 10 minutes following application. Another previous study reported that a 3.5% menthol gel or ½ kg of crushed ice applied to the forearm resulted in decreases in radial arterial blood flow distal to the treatment application.[23] Radial arterial blood flow decreased within the first 5 minutes of applying the menthol and slowly returned to pretreatment levels over 20 minutes.[23] The ice treatment in this trial resulted in a decrease in blood flow at 15 minutes following the application. These results suggest topical menthol and ice can significantly decrease blood flow with menthol resulting in a fast acting but short lived reduction in blood flow while the application of ice requires a longer application to achieve a similar vasoconstrictive effect.

High intensity-short duration exercise results increases blood flow to the involved tissues.[24] Topical menthol has been found to relieve pain[25] and is commonly applied prior to, during, and following exercise. No study has examined the vasoreactive effects of different doses of topical menthol applied immediately following a bout of high intensity (100% effort), short duration isokinetic muscle flexion/extension exercise (3 maximum repetitions). The purpose of this study was to examine the effects of two different doses of menthol (3.5% and 10%) on blood flow and arterial diameter before and after an acute bout of three isokinetic maximum voluntary muscular contraction (MVMC) of the quadriceps and hamstrings. It was hypothesized that the application of either 3.5% or 10% menthol to one of the thighs would result in a decrease in blood flow and arterial diameter in the ipsi- and contra-lateral limb popliteal artery following a bout of three maximum voluntary muscular contractions (MVMC).

METHODS

This research protocol was submitted to, and approved by the Institution's Human Subjects Review Board. A convenience sample of sixteen subjects, 8 males and 8 females (Table 1) with no pre-existing cardiovascular or peripheral vascular disorders provided written consent prior to participating in four 1-hour sessions in the laboratory. Each session was separated by approximately 1 week to allow a 'wash-out' period between intervention exposures and data collection sessions. Session 1 oriented the subjects to the testing procedures. During this first session subjects were introduced to the maximum voluntary muscle leg extension and flexion exercise (MVMC procedure) using the Biodex System 3 (Shirley, NY) dynamometer and the popliteal blood flow data collection protocol using the Philips HDI 5000 Ultrasound Doppler (Seattle, WA). When performing the

Table 1. *Subject demographics*				
	Minimum	Maximum	Mean	Std. Deviation
Age (years)	22.00	35.00	24.19	2.97
Height (cm)	155.56	185.42	169.19	8.71
Weight (kg)	51.26	100.24	77.44	15.59
Resting Heart Rate (bpm)	47.00	92.00	66.50	13.20
Resting Systolic BP (mmHg)	105.00	143.00	121.75	12.58
Resting Diastolic BP (mmHg)	54.00	73.00	66.63	5.49
Body Fat Percentage (%)	5.90	28.00	17.94	6.28
Waist circumference (cm)	60.00	85.00	72.81	8.48
Hips circumference (cm)	91.00	110.00	98.06	6.18
Aerobic Exercise (min/wk)	.00	410.00	200.74	122.22
Resistance Exercise (min/wk)	.00	410.00	150.51	125.38

MVMC procedure subjects were instructed to exert a "maximum effort" when performing three repetitions of the exercise. Maximum voluntary muscle contraction was determined when the peak torque measures for leg extension and flexion varied by no more than 5%. Sessions 2 through 4 involved the random application of one of three treatment conditions and data collection taking place prior to and following each treatment condition.

During data collection sessions, one of three randomly selected treatment conditions was applied to the right thigh distally from the inguinal and gluteal folds to the superior margin of the patella and popliteal fossa. No treatment was administered to the left leg in order to provide a within subject control measure. Treatment conditions were randomly applied at each of the three data collection sessions. Each subject had blood flow data collected on both legs prior to and following each of the three treatment conditions. The treatment conditions included an application of 3.5% menthol gel (BioFreeze® gel, 1 ml of gel for every 200 cm^2 sq cm of right thigh surface area),[22-23] the application of two 10% menthol wipes (BioFreeze® wipes) and a control condition which consisted of no treatment. The amount of 3.5% menthol applied to a subject's right thigh averaged 7.0 $\pm$ 1.1 mL. Pilot studies determined that 2 wipes were sufficient to pass over all areas of the entire thigh one time which provided a similar coverage during this treatment condition as was achieved with the 3.5% menthol gel. The menthol wipes were administered from a pre-packaged commercially available packet consisting of a saturated cotton wipe measuring 15 cm x 15 cm.

During data collection subjects reported to the laboratory at approximately the same time of day and were placed in a seated position and told to rest quietly for 10 minutes prior to any data collection to allow a consistent baseline for data collection. Heart rate (b/min) and blood pressure (mm/Hg) were assessed by the same researcher at all data collections using an automated blood pressure cuff (OMRON, Kyoto, Japan) on the right arm immediately prior to all blood flow measures. Subjects were then instructed to lie in a prone position on a padded table which allowed visualization of the popliteal fossa of both legs. Blood flow assessments were taken from right to left so as to standardize data collection. While prone, the popliteal artery was palpated in the popliteal fossa medial to the biceps femoris and lateral to the semitendinosus muscle. The popliteal artery was selected for this study since it was immediately distal to the treatment application site. Once the vessel was palpated, the Doppler sensor head was positioned over the artery until the vessel could be accurately visualized on the device's video screen. The popliteal artery was imaged longitudinally, by B-mode ultrasound, using a 12-5 MHz linear array transducer using high-resolution ultrasound (Philips HDI 5000, Seattle, WA). A video file of the ultrasound was

collected over five pulsations (or heart beats) and were analyzed by the HDI 5000 internal software. This analysis yielded average vessel volume (ml/min) and maximum diameter (cm/beat) in the popliteal artery at baseline and the subsequent data collection points within each session. This method of estimating blood flow has been reported to have strong validity (r = .96–98) when calculating blood flow in different vessel compartments.[26] This same protocol was repeated on the left leg immediately following collection of blood flow and vessel diameter on the right side. This assessment of blood flow and vessel diameter of both the right and left popliteal arteries was completed again following the MVMC exercises at approximately 5 minutes following the application of each of the three treatment conditions. The same technician completed all data collection.

Immediately following the baseline blood flow data collection subjects were asked to perform three knee extension/flexion MVMC exercises on a Biodex System 3 isokinetic dynamometer. Subjects were seated upright with restraints applied to their waist, torso, and right thigh. The axis of rotation of the dynamometer was placed at the lateral condyle of the tibia with the rotational arm attached to a pad at the terminal end of the anterior tibia with a strap wrapping around the posterior of the limb. Each subject's passive knee extension and flexion range of motion was determined. Subjects were then allowed to familiarize themselves with three submaximum knee extension/flexion exercises through their passive range of motion at a rotation speed of 90 degrees per second using the isokinetic dynamometer. Subjects were then asked to complete 3 repeated maximum flexion and extensions of the knee at a rotation speed of 90 degrees per second which is a commonly employed protocol to asses isokinetic leg strength.[27] Following this exercise on the right the MCMV procedure was repeated on the left side. Immediately following the MVMC exercises one of the three treatment conditions were randomly applied to the subject's right thigh. Since MCMV was not assessed in both legs simultaneously there was a slight difference (approximately 2 minutes) in the duration between performing the exercise and measuring blood flow on the right and left side. This protocol was developed to simulate a high intensity-short duration exercise in order to stimulate blood flow to the thigh and distally through the popliteal artery.

STATISTICAL METHODS

Outcome data to address the study hypothesis were analyzed using SPSS statistical (version 18.1) software. Repeated measures analysis of variance (ANOVA) were used to determine the time (baseline vs. 5-minute post treatment), treatment (3.5% menthol, 10% menthol vs. control), and interaction of time and treatment on popliteal arterial diameter and blood flow. Significant main and interaction effects ($p < .05$) were further addressed by using Tukey's least significant differences (LSD) post hoc comparisons between means.

RESULTS

Analysis indicated a significant ($F = 15.43$, $p = .00$) time effect on heart rate, with no significant treatment or interaction effects. Post hoc analysis indicated that the 3.5% menthol and control conditions resulted in a significant drop in heart rate of 4.63 and 5.19 beats/min respectively while the 10% menthol condition did not affect heart rate over the course of the trial. Heart rate was not different between the three treatment conditions at baseline or at 5 minutes post treatment application. The analysis also indicates no significant time, treatment, or interaction effect of any of the three treatment conditions on diastolic or systolic blood pressure.

A significant interaction ($F = 11.48$, $p = .00$) was observed on blood flow in the right popliteal artery, the side receiving the treatments (See Table 2). Post hoc analysis revealed a decrease in blood flow during the 3.5% and the 10% menthol treatments, while blood flow during the control treatment significantly increased through the right popliteal artery. Blood flow through the right popliteal artery was similar at baseline before application between each of the three treatment conditions. At 5 minutes following application of the treatments the control condition resulted in significantly higher blood flow compared to either the 3.5% or the 10% menthol conditions. The blood flow in the right popliteal artery was not different under the two different menthol dosages at 5 minutes post treatment. Analysis of blood flow in the left popliteal artery also resulted in a significant interaction ($F = 4.75$, $p = .01$). The post hoc analy-

Table 2. *Blood flow in right popliteal artery by time and treatment condition*			
Condition	Baseline (ml/min)	5 Minutes Post Treatment (ml/min)	Raw and Percentage Change (ml/min, % change)
3.5% Menthol Gel	128.95 ± 43.22	103.67 ± 47.76*	-25.28 (19.6%)
10% Menthol Wipes	100.91 ± 39.17	92.52 ± 35.44*	-8.39 (8.31%)
Control	114.63 ± 40.30	141.03 ±46.99*	27.03 (26.40%)

* indicates a significant change within treatment condition over time
Note: Shading indicates a significant difference between treatment and control condition at the specific data collection point

Table 3. *Blood flow in left popliteal artery by time and treatment condition*			
Condition	Baseline (ml/min)	5 Minutes Post Treatment (ml/min)	Raw and Percentage Change (ml/min, % change)
3.5% Menthol Gel	121.56 ± 53.45	103.67 ± 55.17*	-17.89 (14.72%)
10% Menthol Wipes	104.96 ± 34.46	99.29 ± 34.58	- 5.67 (5.40%)
Control	107.70 ± 35.65	124.07 ± 40.86*	16.37 (15.19%)

* indicates a significant change within treatment condition over time
Note: Shading indicates a significant difference between treatment and control condition at the specific data collection point

sis indicated that the 3.5% menthol treatment applied on the right thigh resulted in a significant decline in left popliteal blood flow (See Table 3). The 10% menthol application to the right thigh resulted in no change in left popliteal blood flow while blood flow through this vessel increased significantly under the control condition. At 5 minutes post treatment both the 3.5% and 10% menthol treatment conditions resulted is significantly lower blood flow in the left popliteal artery when compared to the blood flow in the left popliteal artery under the control condition.

The treatments also had a significant interaction effect on the right popliteal arterial diameter ($F = 8.06$, $p = .00$). Post hoc analysis indicated that both the 3.5 and 10% menthol treatment conditions resulted in a significant decline in right popliteal arterial diameter while the control condition resulted in a significant increase in right popliteal artery diameter (See Table 4). Right popliteal artery diameter under the control condition was also significantly greater at 5 minutes post treatment compared to this vessel's diameter under the 10% menthol treatment condition. At 5 minutes post treatment there was no difference in right popliteal artery diameter between the control and 3.5% menthol condition. There was no time, treatment or interaction effect detected in the left side popliteal artery diameter (Table 5).

DISCUSSION

The results of this study appear to support the hypothesis that the application of either 3.5% menthol gel or 10% menthol wipe to the thigh decreases blood flow in the ipsi- and contra-lateral popliteal arteries following a high intensity-short duration bout of exercise. The application of 3.5% menthol gel or a 10% menthol wipe reduced arterial popliteal diameter on the side receiving these treatments but not in the same vessel on the contra-lateral side.

Table 4. *Arterial diameter in right popliteal artery by time and treatment condition*			
Condition	Baseline (cm/beat)	5 Minutes Post Treatment (cm/beat)	Raw and Percentage Change (cm/beat, % change)
3.5% Menthol Gel	.751 ± .15	.708 ± .10*	- .043 (5.73%)
10% Menthol Wipes	.683 ± .08	.637 ± .08*	- .046 (6.73%)
Control	.688 ± .11	.734 ± .13*	.046 (6.67%)

* indicates a significant change within treatment condition over time
Note: Shading indicates a significant difference between treatment and control condition at the specific data collection point

Table 5. *Arterial diameter in left popliteal artery by time and treatment condition*			
Condition	Baseline (cm/beat)	5 Minutes Post Treatment (cm/beat)	Raw and Percentage Change (cm/beat, % change)
3.5% Menthol Gel	.741 ± .12	.726 ± .13	- .015 (2.02%)
10% Menthol Wipes	.693 ± .08	.668 ± .06	-.025 (3.60%)
Control	.702 ± .13	.720 ± .09	.018 (2.56%)

* indicates a significant change within treatment condition over time
Shading indicates a significant difference between treatment and control condition at the specific data collection point

This hypothesis is further supported in that the effect of the treatments on heart rate and blood pressure were equivocal. The changes in blood flow and arterial diameter observed under the treatment conditions do not appear to be attributed to changes in heart rate or blood pressure. Thus, the effect of the menthol treatments on decreasing blood flow and arterial diameter seem to be similar to the effect of more general tissue cooling, resulting in both local and generalized vasoconstriction as determined by high-resolution ultrasound.

The observed increases in blood flow and to a lesser extent arterial diameter during the control condition were consistent with results reported by previous authors who described increased arterial diameter and blood flow that occurs within muscles during and following exercise by those muscles.[28-29] This increase in arterial diameter and blood flow during and following exercise is primarily through shearing stress in the vessel resulting in the release of NOS and nitric oxide (NO) which in turn relaxes the adjacent vascular smooth muscle[30] increasing arterial diameter thereby allowing greater blood flow through the vessel. Following the MVMC subjects when receiving the control treatment demonstrated significant increases in popliteal artery blood flow (16-27% bilaterally) and a significant increase in popliteal artery diameter on the right side only. The anticipated increase in arterial diameter and blood flow following a bout of exercise appears to be dampened or reversed during the application of the 3.5% menthol gel and 10% menthol wipes. One explanation for this result is the increased adrenergic stimulation due to the sympathetic reflex which may be associated with menthol application stimulation of TRPM8 thermosensitive neurons similar to the effect of tissue cooling.[16-17, 19] This explanation is consistent with the findings of other investigators who have also reported that application of topical menthol resulted

in decreases in arterial blood flow and decreased arterial diameter.[16, 31-32] Topical menthol may also excite α_{2C} adrenergic receptors via sympathetic stimulation similar to tissue cooling[17, 19] which also leads to arterial vasoconstriction. Thus, both doses of menthol application appeared to decrease blood flow in the ipsi-lateral popliteal artery following an acute bout of exercise compared to the control condition which exhibited an increase in blood flow in this vessel following the exercise. This finding that topical menthol decreases local blood flow following acute exercise may be attributed to local inhibition of both NOS and NO and increased α_{2C} adrenergic tone.

This local vasoconstriction effect of topical menthol does not explain the reductions in blood flow observed in the contra-lateral limb which was not directly exposed to the topical menthol treatments. Topical menthol results in a cooling sensation[25, 33-34] detected by cutaneous cold receptors, which include A-delta and C fibers and has also been postulated to reduce blood flow through neuronal reflex mechanisms.[23] Menthol is believed to produce this cooling sensation by inhibiting calcium currents of the neuronal membrane[25] among transient receptor potential family of ion channels or TRP's. Haeseler et al[2] concluded that the application of topical menthol attenuates local increases in blood pressure during exercise likely through an increase in peripheral resistance at the muscle area[35-36] leading to a reduction in overall blood flow to the area. It is thought that the reduction in blood flow is due to the inhibition of the small-diameter sensory nerve fibers that are known as group III and IV afferents that synapse with spinoreticular tract neurons in the dorsal horn of the spinal cord.[35] This spinal reflex initiated through the application of topical menthol appears to have both a local and generalized vasoconstriction effect similar to tissue cooling[21] but does not rely on changes in tissue temperature. It is suggested by this author that this spinal reflex may account for the changes in blood flow in the contralateral popliteal artery following the application of topical menthol although the mechanism is unclear. This potential generalized vasoconstriction effect of topical menthol warrants further study.

The results appear to indicate that the 10% menthol wipe and the 3.5% menthol gel had a similar effect on blood flow in the ipsi- and contra-lateral popliteal arteries. Although statistically similar, the clinical impact may be different between these two doses of menthol. For example, blood flow was reduced by 19.60 – 17.89% under the 3.5% menthol gel and by 8.31 – 5.40% with the 10% menthol wipe in the ipsi- and contra-lateral popliteal arteries respectively. This finding is curious since it was anticipated a priori that a higher dose or a more concentrated application of menthol would result in a more pronounced effect on blood flow. Closer examination of the two commercially available products employed in this study may explain this outcome. The 3.5% menthol gel and the 10% menthol wipe are delivered through a different medium. The gel contains propylene glycol and glycerol that are not found in the wipes. Studies have shown that propylene glycol and glycerol facilitate absorption through increasing the skin's permeability to topical products.[37-39] Further, the high alcohol content of the 10% menthol wipe may have increased the rate of evaporation further limiting the absorption rate of the menthol under this condition.

These results must be interpreted cautiously due to a number of limitations with the study design. First, a single artery distal to the application to the two menthol treatments and not the blood flow within the tissues directly adjacent to the treatments was assessed. Thus, the effect of the menthol on the tissues directly adjacent to the treatments was not measured. Second, the local and spinal mechanisms resulting in alterations in blood flow were not directly studied and the possible effect of menthol on these mechanisms is conjectured based upon previous studies. Future studies may examine the possible physiological mechanisms explaining the significant effects of the interventions on the outcome variables. Finally, the sample consisted of a limited number of healthy young adults. This small sample likely limited the statistical power to detect a treatment effect on arterial diameter in the ipsi-lateral artery under the two menthol dosages. The small sample also limited examination of other potentially confounding variables including gender, physical fitness, and dominant versus non-dominant side comparisons. Further, the findings of this study may not be replicable among older populations with chronic health conditions such as diabetes or peripheral vascular disease which may affect blood flow.

CONCLUSION

The findings of this study indicate that topical applications of various doses of menthol to the thigh results in a significant decrease in local (ipsi-lateral popliteal artery) and generalized (contra-lateral popliteal artery) blood flow following a MVMC. These findings possibly indicate that menthol affects blood flow through inhibiting local NOS and NO, and increased increasing systemic α_{2C} adrenergic tone. Clinicians may apply the findings of this study to support using topical menthol gels or wipes to decrease local blood flow and attenuate the inflammation process following a soft tissue injury. Finally, the local and generalized effect of menthol on blood flow reported in this study warrants further investigation in order to identify the mechanisms of action and to determine the effect of topical menthol on muscle performance.

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