Vascular Conductance is Reduced After Menthol or Cold Application

Jennifer L. Olive, PhD, Brandon Hollis, MS,† Elizabeth Mattson, MS,‡ and Robert Topp, PhD§*

Objective: To compare the effects of commercially sold menthol (3.5%) ointment and cold application on blood flow in the forearm.

Design: Prospective counterbalanced design.

Setting: University research laboratory.

Participants: Twelve (6 men and 6 women) college-aged students.

Interventions: Each participant had blood flow measured in the brachial artery for 5 minutes before and 10 minutes after menthol ointment or cold application to the forearm.

Main Outcome Measures: Blood velocity, arterial diameter size, and blood pressure were recorded during testing procedures. Vascular conductance was calculated based on these measures and used to describe limb blood flow.

Results: We observed a significant reduction (35%; $P = 0.004$) in vascular conductance within 60 seconds of menthol and cold application to the forearm. Vascular conductance remained significantly reduced for 10 minutes by approximately 19% after both menthol and cold application [$F(2.313, 43.594) = 10.328$, $P < 0.0001$]. There was no significant difference between conditions [$F(1, 19) = 0.000$, $P = 0.945$].

Conclusions: The application of a 3.5% menthol ointment significantly reduces conductance in the brachial artery within 60 seconds of application, and this effect is maintained for at least 10 minutes after application. The overall decline in conductance is similar between menthol ointment and cold application.

Key Words: blood flow, menthol, vascular conductance, Doppler ultrasound, cryotherapy

(*Clin J Sport Med* 2010;20:372–376)

INTRODUCTION

Athletic trainers and physical therapists often use cryotherapy (the application of ice for therapeutic purposes) as a means to reduce inflammation and pain associated with athletic performance or injuries. Cryotherapy or cold application reduces inflammation by reducing blood flow to the area,[1] altering the inflammatory cascade,[2] and reducing the metabolic demand of injured tissues.[3] In specific regards to blood flow, cold application significantly reduces cutaneous[4,5] and intramuscular blood flow[3,6–8] within seconds after application. Cryotherapy has also been shown to reduce localized pain by inhibiting nerve conduction velocity.[9,10] In recent years, it has been documented that these physiological responses are multifactorial but are largely due to the activation of transient receptor potential (TRP) channels located in sensory neurons, which affect temperature perception and nociceptive signaling.[11,12]

Menthol products have been used for hundreds of years as a natural analgesic with a cooling sensation, but the mechanism by which they worked has not been well understood.[13,14] In recent years, menthol's effects as a natural analgesic compound have largely been shown to be due to the activation of some of the same TRP channels that are activated by cold, specifically the TRP melastatin-8 (TRPM8).[12,15] Interestingly, it has been found that the TRPM8 channels are also located in the arterial vascular smooth muscle and alter vasomotor tone.[16] Thus, it is possible that menthol may also have an effect on vascular tone and blood flow.

It has been fairly well documented that cold application reduces inflammation, pain, and blood flow after application[1,3,6–10]; however, little scientific research has been conducted investigating the effect of commercially sold menthol ointments on blood flow. Due to the fact that both cold application and menthol both can activate TRPM8 channels,[12] it raises the question whether a menthol ointment would have similar reduction in blood flow that acute cold application elicits. It was hypothesized that the application of commercially sold natural menthol (3.5%) ointment (Biofreeze; Performance Health, Inc, Akron, Ohio) would result in a reduction in arterial blood flow to the forearm in a manner that is similar to cold application.

METHODS

Participants

Twelve healthy men (n = 6) and women (n = 6) between 21 and 32 years (24 ± 4 years) of age were recruited to

Submitted for publication March 24, 2010; accepted June 29, 2010.
From the *Department of Exercise Science, University of Puget Sound, Tacoma, Washington; †Department of Exercise Science, Louisiana State University, Baton Rouge, Louisiana; and ‡Department of Health & Sport Sciences; and §School of Nursing; University of Louisville, Louisville, Kentucky.
The authors report no conflicts of interest.
Reprints: Jennifer L. Olive, PhD, 5804 66th Ave W, University Place, WA 98467 (e-mail: jenniferolive@yahoo.com).
Copyright © 2010 by Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

participate in the study. All subjects were either inactive or reported only light recreational activity (ie, walking, softball, and basketball 1 to 2 days a week). Individuals who reported exercising at moderate intensity more than 2 days a week were not included in the study. No subject had a history of chronic disease, had smoked, or was presently taking any medications or dietary supplements that may have an impact on vascular function. Subjects were asked to refrain from eating or drinking any substances that would be stimulatory (ie, caffeine) or foods that have been shown to have a negative impact on vascular function (ie, high-fat foods[17]). All subjects signed a written informed consent approved by the Institutional Review Board at the University of Louisville.

Procedures

A 15-minute measurement of resting, supine, forearm arterial blood flow was ascertained in the preliminary data collection period on a subset of subjects (n = 10) (Figure 1) representing a baseline control. All testing measurements were performed in a dimly lit, quiet, temperature-controlled room (22°C). There was no significant change in blood flow across this baseline period indicating that blood flow does not vary under resting supine condition, [$F(1, 9) = 1.290$, $P = 0.298$].

For experimental testing conditions, subjects reported to the laboratory on 2 separate days during the morning hours. On experimental test day 1, subjects were randomly assigned a test condition [menthol ointment (Biofreeze) or cold application]. On day 2, the opposite test condition was applied. All testing procedures were performed in an identical situation as the preliminary baseline control data.

On day 1, subjects had their height (171.2 ± 9.9 cm), mass (71.7 ± 15.1 kg), and their forearm surface area (553 ± 84 cm^2) measured. A single investigator measured forearm surface area by wrapping a premeasured cloth (5 × 40 cm) around the right forearm in the following manner. First, the person's forearm was carefully marked from the elbow to the wrist every 5 cm. The cloth was then placed at each mark and wrapped snugly, being careful not to pinch or compress the skin, around the person's arm. The length of the cloth that was needed to encircle the arm was determined. This process was repeated down the entire length of the forearm. The surface area of the cloth was calculated at each placement and summed together to determine total surface area of the forearm.

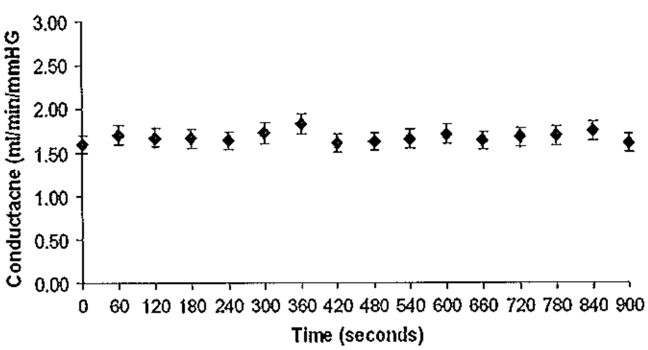

FIGURE 1. Conductance (milliliters/minute/millimeter of mercury) ± standard error of the mean. Conductance determined for 15 minutes with no treatment (control condition). There was no change in conductance during this period.

Once all measurements were taken, a blood pressure monitor (Omron Healthcare, Inc, Bannockburn, Illinois) was attached to the subject's left arm. Blood pressures were taken every 2 minutes throughout the entire testing period. The subject was then placed in a supine position to rest quietly for 10 minutes. After the 10-minute rest period, the experimental conditions began. Blood flow velocity was measured for a total of 15 minutes during which 5 minutes of baseline blood flow data was gathered, followed by 10 minutes in which blood flow data was measured during one of the two experimental conditions.

Experimental Conditions

Menthol Application

Based on the measured surface area, a standardized amount (1 mL per 200 cm^2) of commercially available menthol (3.5%) ointment (Biofreeze) was applied to the entire forearm. Using this ratio, an average of 2.77 ± 0.42 mL menthol ointment was applied to each person's forearm. The ratio for the amount of menthol ointment per surface area was based on previous pilot testing and what we found was typically applied in several athletic training facilities.

Cold Application

Ice was applied without compression to the same surface area of the forearm that the menthol ointment covered. The ice was collected from a commercial ice machine which produced small cubes. The ice was bagged and placed directly on the skin. The average ice temperature was −0.4 ± 0.1°C. There were no adverse responses to either treatment during testing.

Blood Flow Determination

Brachial artery blood velocities were measured in the right arm noninvasively using high-resolution ultrasound (Philips HDI 5000; Seattle, Washington) by a single investigator. The brachial artery was imaged longitudinally, 2 cm above the antecubital fossa by B-mode ultrasound, using a 12-5 mHz linear array transducer throughout the entire testing period. A video file (ULead Video Studio 7; Taipei, Taiwan) of the ultrasound data was collected throughout the testing procedure to allow for post-test data analysis.

A single investigator, who was blinded to condition, used a custom-made automated software program (LabView version 7.1; National Instruments, Austin, Texas) to measure brachial artery diameter after each heartbeat (during diastole) throughout the test. The analysis program also gathered all velocity measurements from the screen using an optical character recognition program. Velocity data are provided for each heartbeat, thereby allowing beat-by-beat data collection. Blood flow (milliliters/minute) was calculated as the product of the time average maximum velocity (centimeters/second) and the vessel cross-sectional area (square centimeter).

Mean arterial pressure (MAP) was measured at baseline and throughout the entire testing period (Omron Healthcare, Inc) to control for alterations in blood pressure. Cold treatment has been found to increase MAP and sympathetic responses[8,18]; thus, blood flow will be reported as conductance to control for alterations in blood pressure. Conductance was

© 2010 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

calculated by dividing the maximal blood flow (milliliters/minute) by the MAP (millimeters of mercury). Mean arterial pressure did vary throughout the testing period in some individuals (menthol, MAP range = 80–83 mm Hg; $t(10) = -2.006$; $P = 0.06$; cold application, MAP range = 81–85 mm Hg; $t(11) = -4.436$; $P = 0.04$).

Statistical Analysis

Conductance data that was available on a beat-by-beat basis was averaged over time to provide 6 time measurements for simplification of data reporting. Because there was no significant change in conductance over the baseline period, all data collected during the 5-minute baseline period were averaged to provide a baseline conductance value. During the experimental conditions, conductance was averaged to provide blood flow measurements at 60, 120, 180, 240, and 600 seconds.

A mixed model repeated measures analysis of variance using SPSS 16.0 (SPSS, Inc, Chicago, Illinois) was used to test for time, treatment, and interaction effects of the cold application and menthol ointment. Significant main effects were further evaluated by calculating Tukey least significant post hoc differences. For all analyses, differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Conductance was determined at baseline and then throughout a 10-minute period after the application of menthol ointment and ice (Figure 2). There was a significant time effect [$F(2.313, 43.594) = 10.328$, $P < 0.0001$]. However, there was no significant difference between the conditions [$F(1, 19) = 0.000$, $P = 0.945$]. Finally, there was no interaction effect [$F(2.313, 43.594) = 0.709$, $P = 0.517$]. Post hoc analyses were conducted to determine where the differences existed across time. It was found that baseline blood flow was significantly higher than blood flow measures recorded at 60 ($P = 0.004$; $d = 0.66$), 120 ($P = 0.008$; $d = 0.42$), 180 ($P = 0.006$; $d = 0.39$), 240 ($P = 0.029$; $d = 0.37$), and 600 ($P = 0.03$; $d = 0.36$) seconds. Furthermore, the blood flow at 60 seconds was reduced (≈35%) to a greater extent than at 120, 180, 240, and 600 seconds (≈19%) ($P \leq 0.039$) compared with baseline. There was no significant difference between any time points after 120 seconds. Blood flow was reduced by approximately 19% from baseline over the entire testing period for both conditions (Figure 3).

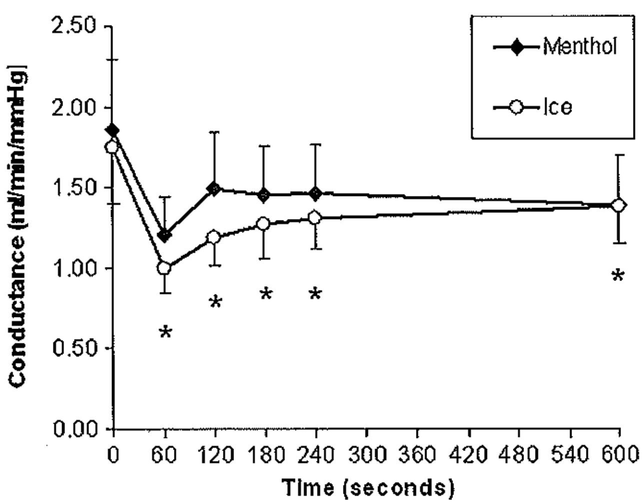

FIGURE 2. Conductance (milliliters/minute/millimeter of mercury) ± standard error of the mean. Conductance determined at baseline and for 10 minutes after the application of menthol ointment and ice. *Time point significantly different than baseline ($P < 0.03$).

DISCUSSION

The main finding of this study was that brachial artery conductance was significantly reduced for up to 10 minutes after the application of menthol ointment to the forearm. Due to the fact that TRPM8 channels are stimulated by both cold and menthol[11,12,15] and that they are also located in the arterial smooth muscle,[16] it was hypothesized that the application of menthol ointment would significantly reduce blood flow to the forearm after its application. We also found that the reduction in conductance after menthol ointment application was similar in magnitude to that of cold application.

Cold application has been used for many years to reduce pain and reduce edema after exercise or injury, but the exact mechanism for how cold is felt or how it works has not been clear. Studies have just recently begun to elucidate the physiological effects of cold and how cold is perceived.[11,12] Recent work has identified specific ion channels known as TRP channels that are located in afferent nerves. It is the increased activation of these TRP channels at temperatures that range from 30 to 15°C that is considered critical for our perception of cold sensation.[11,12] Interestingly, these TRP channels may also have an effect on the vasculature, as their activation results in an increase in sympathetic activity resulting in a release of norepinephrine and arterial vasoconstriction in response to cold.[5,8,16,18]

Research studies investigating the roles of cold application on intramuscular blood flow have shown that 10 minutes of local cold application via ice packs resulted in a 49% reduction in intramuscular blood flow.[19] Other research using magnetic resonance imaging has shown that cooling via ice or cooling pads reduces intramuscular blood volume[3,6,7] and decreases blood oxygenation.[3] In our study, we found that conductance was significantly reduced by cold application via ice by approximately 19% over the 10-minute period.

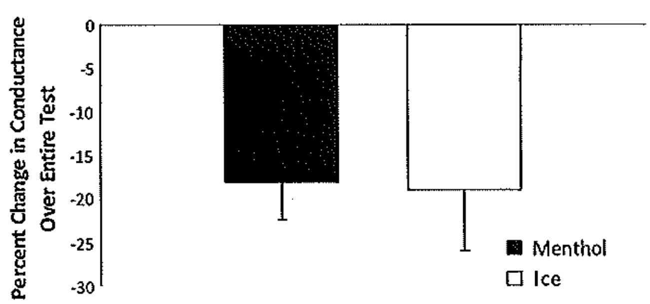

FIGURE 3. Percent change in conductance (±standard error of the mean) from baseline over the entire test. There is no significant difference between menthol or ice conditions ($P = 0.945$).

© 2010 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Interestingly, the TRPM8 channel that responds to cold also responds to menthol[12] and is responsible for the cold sensation that accompanies menthol ointments.[11] The effects of topically applied menthol ointments on blood flow are sparse. We found in our study that brachial artery conductance was reduced after menthol ointment application in a manner that was similar in the response and magnitude as cold application.

Two studies have documented an increase in cutaneous blood flow after the application of eucalyptamint (eucalyptus oil, lanolin, and 15% natural menthol)[20] and a 40% l-menthol balm.[21] The increase in cutaneous blood flow is not consistent with our findings. The differences in our findings may be due to different substances or concentrations used. Both of these studies use balms that contained menthol in much higher concentrations than what was used in this study or what is typically used in athletic clinical settings (2%–10% menthol). Another possibility may be that blood flow is increased directly to the skin, an area we did not measure, whereas blood flow to the whole limb and the muscle are decreased. The design of this study did not allow us to determine what portions of the vasculature network were affected by cold application or menthol ointment treatment. We calculated the conductance in the brachial artery, which is the main conduit artery for the forearm. Based on our measurements, it is impossible to ascertain if blood flow was significantly reduced both to the superficial and deeper vasculature. Thus, future research needs to investigate how blood flow is regulated to different tissues after the use of such substances.

Other research has shown findings that would be more consistent with our research. Two animal studies have shown that the pressor response to exercise is attenuated after the application of analgesic balms that include menthol[22] or methyl salicylate and capsaicin.[23] The authors of this research thought that the attenuation in blood pressure was most likely due to an increase in total peripheral resistance at the muscle area[22,23] and therefore a reduction in overall blood flow to the area.

From a clinical perspective, menthol ointments are routinely used to reduce pain both before and after exercise. Athletic trainers report that athletes often use menthol ointments to aid in the warm-up period. Our finding of a reduction in conductance after menthol application brings to question whether menthol application that is applied before exercise has the potential to negatively impact exercise performance due to limited blood flow to the exercising muscles. This would be of particular interest in the lower limb where the conduit vessels are deeper than those in the arm, and thus, menthol effects may potentially be attenuated due to the depth of the vessels. Thus, future research of interest would be to investigate the role of menthol ointment on exercise performance and to determine if menthol has similar effects in other limbs.

There are several limitations to our study. First, our population is young and homogeneous. Our study does not allow us to extrapolate our results to other populations that may be older and/or diseased. Second, we did not measure cutaneous blood flow response; thus, we are unable to determine how blood flow was distributed across the limb. We did measure blood flow in the brachial artery, which is the main conduit for blood flow to the forearm and hand and would distribute blood to both the superficial and deeper vasculature. But we were unable to determine how blood flow was regulated. A third limitation is that we do not measure how long the reduction in blood flow would last after the application of menthol. We found that conductance remained reduced for at least 10 minutes, but due to testing constraints, we were unable to continue measuring conductance for a longer period. Future research of interest would be to determine if the conductance response is similar in other populations and limbs and to determine how long conductance is reduced after the application of a menthol ointment.

In conclusion, this study found that the application of a 3.5% menthol ointment significantly reduces conductance in the brachial artery within 60 seconds of application and that this effect is maintained for at least 10 minutes after application. The overall decline in conductance was similar between menthol ointment and cold application.

REFERENCES

1. Deal DN, Tipton J, Rosencrance E, et al. Ice reduces edema. A study of microvascular permeability in rats. *J Bone Joint Surg Am.* 2002;84-A: 1573–1578.
2. Qing M, Nimmesgern A, Heinrich PC, et al. Intrahepatic synthesis of tumor necrosis factor-alpha related to cardiac surgery is inhibited by interleukin-10 via the Janus kinase (Jak)/signal transducers and activator of transcription (STAT) pathway. *Crit Care Med.* 2003;31:2769–2775.
3. Yanagisawa O, Homma T, Okuwaki T, et al. Effects of cooling on human skin and skeletal muscle. *Eur J Appl Physiol.* 2007;100:737–745.
4. Nagashima Y, Yada Y, Suzuki T, et al. Evaluation of the use of an integration-type laser-Doppler flowmeter with a temperature-loading instrument for measuring skin blood flow in elderly subjects during cooling load: comparison with younger subjects. *Int J Biometeorol.* 2003;47:139–147.
5. Johnson JM, Yen TC, Zhao K, et al. Sympathetic, sensory, and nonneuronal contributions to the cutaneous vasoconstrictor response to local cooling. *Am J Physiol Heart Circ Physiol.* 2005;288:H1573–H1579.
6. Yanagisawa O, Kudo H, Takahashi N, et al. Magnetic resonance imaging evaluation of cooling on blood flow and oedema in skeletal muscles after exercise. *Eur J Appl Physiol.* 2004;91:737–740.
7. Yanagisawa O, Shimao D, Maruyama K, et al. Evaluation of exercised or cooled skeletal muscle on the basis of diffusion-weighted magnetic resonance imaging. *Eur J Appl Physiol.* 2009;105:723–729.
8. Sendowski I, Savourey G, Launay JC, et al. Sympathetic stimulation induced by hand cooling alters cold-induced vasodilatation in humans. *Eur J Appl Physiol.* 2000;81:303–309.
9. Algafly AA, George KP. The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. *Br J Sports Med.* 2007;41: 365–369.
10. Chesterton LS, Foster NE, Ross L. Skin temperature response to cryotherapy. *Arch Phys Med Rehabil.* 2002;83:543–549.
11. McKemy DD. How cold is it? TRPM8 and TRPA1 in the molecular logic of cold sensation. *Mol Pain.* 2005;1:16.
12. Reid G. ThermoTRP channels and cold sensing: what are they really up to? *Pflugers Arch.* 2005;451:250–263.
13. Galeotti N, Di Cesare Mannelli L, Mazzanti G, et al. Menthol: a natural analgesic compound. *Neurosci Lett.* 2002;322:145–148.
14. Eccles R. Menthol and related cooling compounds. *J Pharm Pharmacol.* 1994;46:618–630.
15. Proudfoot CJ, Garry EM, Cottrell DF, et al. Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. *Curr Biol.* 2006;16: 1591–1605.
16. Johnson CD, Melanaphy D, Purse A, et al. Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. *Am J Physiol Heart Circ Physiol.* 2009;296:H1868–H1877.
17. Ballard K, Miller J, Robinson J, et al. Aerobic capacity and postprandial flow mediated dilation. *Int J Exerc Sci.* 2008;1:163–176.

© 2010 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

18. Bailey SR, Eid AH, Mitra S, et al. Rho kinase mediates cold-induced constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation. *Circ Res.* 2004;94:1367–1374.
19. Thorsson O, Lilja B, Ahlgren L, et al. The effect of local cold application on intramuscular blood flow at rest and after running. *Med Sci Sports Exerc.* 1985;17:710–713.
20. Hong CZ, Shellock FG. Effects of a topically applied counterirritant (Eucalyptamint) on cutaneous blood flow and on skin and muscle temperatures. A placebo-controlled study. *Am J Phys Med Rehabil.* 1991;70:29–33.
21. Wasner G, Schattschneider J, Binder A, et al. Topical menthol—a human model for cold pain by activation and sensitization of C nociceptors. *Brain.* 2004;127:1159–1171.
22. Ragan BG, Nelson AJ, Foreman JH, et al. Effects of a menthol-based analgesic balm on pressor responses evoked from muscle afferents in cats. *Am J Vet Res.* 2004;65:1204–1210.
23. Ichiyama RM, Ragan BG, Bell GW, et al. Effects of topical analgesics on the pressor response evoked by muscle afferents. *Med Sci Sports Exerc.* 2002;34:1440–1445.

© 2010 Lippincott Williams & Wilkins

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.