Effects of Menthol-Based Counterirritant on Quadriceps Motoneuron-Pool Excitability

Daniel H. Huffman, Brian G. Pietrosimone, Terry L. Grindstaff, Joseph M. Hart, Susan A. Saliba, and Christopher D. Ingersoll

Context: Motoneuron-pool facilitation after cryotherapy may be mediated by stimulation of thermoreceptors surrounding a joint. It is unknown whether menthol counterirritants, which also stimulate thermoreceptors, have the same effect on motoneuron-pool excitability (MNPE). Objective: To compare quadriceps MNPE after a menthol-counterirritant application to the anterior knee, a sham counterirritant application, and a control treatment in healthy subjects. Design: A blinded, randomized controlled laboratory study. Setting: Laboratory. Participants: Thirty healthy subjects (16 m, 14 f; 24.1 ± 3.9 y, 170.6 ± 11.4 cm, 72.1 ± 15.6 kg) with no history of lower extremity surgery volunteered for this study. Intervention: Two milliliters of menthol or sham counterirritant was applied to the anterior knee; control subjects received no intervention. Main Outcome Measures: The average vastus medialis normalized Hoffmann reflex \( H_{\text{max}} : M_{\text{max}} \) ratio was used to measure MNPE. Measurements were recorded at 5, 15, 25, and 35 minutes postintervention and compared with baseline measures. Results: \( H_{\text{max}} : M_{\text{max}} \) ratios for all groups significantly decreased over time \( (F_{4,108} = 10.52, P < .001) \); menthol: baseline = .32 ± .20, 5 min = .29 ± .18, 15 min = .27 ± .18, 25 min = .28 ± .19, 35 min = .27 ± .18; sham: baseline = .46 ± .26, 5 min = .36 ± .20, 15 min = .35 ± .19, 25 min = .35 ± .20, 35 min = .34 ± .18; control: baseline = .48 ± .32, 5 min = .37 ± .27, 15 min = .37 ± .27, 25 min = .37 ± .29, 35 min = .35 ± .28). No significant Group \( \times \) Time interaction or group differences in \( H_{\text{max}} : M_{\text{max}} \) were found. Conclusions: Menthol did not affect quadriceps MNPE in healthy subjects.

Keywords: knee, disinhibitory modalities, arthrogenic

Decreased motoneuron-pool excitability (MNPE) has been linked to arthrogenic muscle inhibition, a clinical impairment causing inhibition of an uninjured muscle surrounding an injured joint. Quadriceps activation has been reported to decrease after various joint injuries, and arthrogenic muscle inhibition has been

Huffman is with the Dept of Kinesiology, Texas A&M University, Corpus Christi, TX. Pietrosimone is with the Dept of Kinesiology, University of Toledo, Toledo, OH. Grindstaff, Hart, and Saliba are with the Dept of Human Services, University of Virginia, Charlottesville, VA. Ingersoll is with the Herbert H. and Grace A. Dow College of Health Professions, Central Michigan University, Mt Pleasant, MI.
suggested to be a limiting factor in regaining proper muscle function after injury. It has been suggested that increasing MNPE before therapeutic exercise may help enforce more normalized motor patterns during rehabilitation.

Focal knee-joint cooling has been reported to facilitate or increase quadriceps MNPE above baseline levels in healthy subjects with artificially induced knee-joint effusions, which are used to simulate arthrogenic muscle inhibition. Likewise, soleus MNPE is facilitated after focal ankle-joint cryotherapy in healthy subjects. It should be noted that these protocols specifically cool the targeted joint and not the muscle with the motoneuron pool of interest. Thus, it has been hypothesized that the increase in MNPE after focal joint cooling may be caused by an activation of supraspinal centers responsible for modulating neural inhibition by increasing afferent activity from mechanoreceptor stimulation. Increased quadriceps MNPE has been reported after transcutaneous electrical nerve stimulation to the knee, providing further evidence that sensory receptors may play a major role in altering MNPE. This may cause facilitation by decreasing the influence of the Ib-inhibitory interneuron responsible for decreased quadriceps MNPE.

Similar to cryotherapy, menthol stimulates transient receptor potential cation channels subfamily M, member 8 (TRPM8), to produce counterirritant sensory sensations. Both Aδ and C-fibers are sensitive to the TRPM8 ion channel, which is a protein expressed in sensory neurons resulting in the perception of cold and has been linked to cold-induced analgesia. Because of menthol’s ability to stimulate TRPM8 ion channels, it has been hypothesized that it could activate a supraspinal response similar to that of cryotherapy. However, menthol also stimulates receptors that appear to be specific only to menthol and not cold stimuli. The TRPM8 pathways on thermoreceptors are activated by both menthol and decreases in temperature, thus causing a sensation of cold. Because of TRPM8 sensitivity to menthol, applying a compound containing a menthol component may activate a similar supraspinal mechanism that will result in increased MNPE.

Menthol-based topical ointments are commonly used in sports medicine settings and are available in many commercial products. They have also been reported to be transdermally absorbed, with plasma concentrations rising for hours after application, typically with half-lives of 3 to 6 hours. There are empirical benefits with these sports creams and gels, such as decreased pain and improved flexibility, but there is little in the literature that substantiates their use. Menthol-based gels have the ability to activate cold thermoreceptors similar to those activated with cryotherapy application, yet it is not known whether the application of menthol to the knee joint will alter MNPE.

The purpose of this study was to compare quadriceps MNPE in healthy subjects after menthol-counterirritant application, sham treatment, or no treatment (control) in healthy subjects. We hypothesized that application of a menthol counterirritant would increase quadriceps MNPE compared with sham and control treatments.

Methods

This double-blinded, randomized control laboratory study had 2 independent variables including treatment group (menthol counterirritant [menthol], sham counterirritant [sham], and control [control]) and time (baseline and 5, 15, 25, and
35 min posttreatment application). A maximum Hoffmann reflex normalized to a maximum muscle response (H_{max}:M_{max} ratio) in the vastus medialis was the main outcome measure used to estimate quadriceps MNPE. Previous research has used the vastus medialis to determine the effects of other modalities and knee-joint effusions on the quadriceps. We chose to use the vastus medialis to be consistent with the previous literature. Subjects were randomly assigned to groups using a random-number generator.

**Subjects**

Thirty-eight subjects (Table 1) volunteered to participate in this study. Eight were excluded from participation after not displaying an obtainable Hoffmann reflex. Subjects included in the study were free from any discogenic pathology, cancer, spinal disease, vertebral fracture, neurological symptoms (decreased sensation or reflexes), history of low back or lower extremity surgery, chronic knee pain, or history of lower extremity injury during the 6 months before their participation in this study. All subjects voluntarily signed informed-consent forms, approved by the institutional review board, before participation.

**Instruments**

H_{max}:M_{max} ratio measurements were collected using surface electromyography (EMG; MP150, BIOPAC Systems, Inc, Santa Barbara, CA). Signals were acquired using disposable, 10-mm pregelled Ag-AgCl electrodes (EL503, BIOPAC Systems). Raw EMG signals were amplified (EMG100C, BIOPAC Systems), band-pass filtered from 10 to 500 Hz, and sampled at 1000 Hz. A stimulator module (STM150A, BIOPAC Systems) was used to deliver a stimulus (200-V maximum) via a 2-mm stimulating disc electrode (EL254S, BIOPAC Systems) to the femoral nerve. The 2-mm stimulating electrode and a 7-cm, round, carbon-impregnated dispersive pad covered with conducting gel and interfaced with a stimulus isolation adaptor (STMISOC, BIOPAC Systems) were positioned on the posterior thigh.

A commercially available menthol-based topical gel (Biofreeze, Performance Health, Inc, Export, PA) was used as the experimental counterirritant. This product contains 15% menthol as the active counterirritant and does not contain any other sensation-enhancing substances such as capsaicin or salicylates. Sham gel was

**Table 1  Subject Demographics, Mean (SD)**

<table>
<thead>
<tr>
<th></th>
<th>Menthol, n = 10</th>
<th>Sham, n = 10</th>
<th>Control, n = 10</th>
<th>Total, N = 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>23.7 (2.3)</td>
<td>24.5 (5.4)</td>
<td>24.0 (3.7)</td>
<td>24.1 (3.9)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172.7 (11.8)</td>
<td>168.9 (8.7)</td>
<td>170.1 (14.1)</td>
<td>170.6 (11.4)</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>76.4 (19.8)</td>
<td>67.7 (12.8)</td>
<td>72.3 (13.5)</td>
<td>72.1 (15.6)</td>
</tr>
</tbody>
</table>
custom-made specifically for this experiment (Performance Health) to be identical in composition, consistency, and scent but not contain any menthol.

**Subject Preparation**

Subjects were instructed to lie supine on a treatment plinth with arms at their sides and head in neutral position (Figure 1). The sites for the recording and reference electrodes were shaved, debrided, and cleaned with isopropyl alcohol before application of the EMG electrodes.8,23–25 Recording electrodes were placed 2 cm apart at approximately a 55° angle to the superior patellar pole parallel with the muscle fibers of the greatest bulk of the oblique portion of the vastus medialis muscle. The greatest bulk of the oblique portion of the vastus medialis oblique was determined by palpation during a submaximal isometric contraction. The reference recording electrode was placed over the ipsilateral medial malleolus. The dispersive pad was secured to the distal hamstring with an elastic wrap. The stimulating electrode was placed superficial to the femoral nerve located at the inguinal fold.

**H-Reflex and M-Wave Procedures**

During the testing procedures subjects were instructed to keep their heads still with their eyes open and focus on a spot on the ceiling.25 Testing took place in an isolated quiet laboratory with the lights dimmed. Stimuli used to elicit a Hoffmann reflex or muscle response were delivered at a minimum interval of 10 seconds26 apart to avoid postactivation depression. To obtain the H_{max}, a stimulus intensity of 3 V was first delivered to determine the status of the Hoffmann reflex. The stimulus intensity was increased by 1 V until a Hoffmann reflex appeared. Once a Hoffmann reflex was identified, increases in stimulus intensity were made in 0.2-V increments

![Figure 1](image_url) — Subject testing position. Subjects were instructed to lie supine with their arms at their sides, relaxed and focusing on a spot on the ceiling, keeping their eyes open during each measurement increment. Posttreatment, a towel was draped over the knee joint to blind the investigator as to which treatment had been applied.
until it began to decrease. Three consecutive stimuli using the voltage corresponding with the maximum peak-to-peak amplitudes of the Hoffmann reflexes were used to determine the $H_{max}$. To obtain the muscle response the stimulus intensity was increased in increments of 1 V until the muscle response with the maximum peak-to-peak voltage was obtained. Three consecutive $M_{max}$ signals were obtained.

**Testing Procedures**

All Hoffmann-reflex and muscle-response measurements were obtained by the same investigator. After obtaining a baseline Hoffmann-reflex and muscle-wave measurement, the initial investigator left the treatment room in order remained blinded to the intervention. At that time, a certified athletic trainer entered the room and randomly selected the intervention from an envelope that allocated the subject to 1 of 3 groups (menthol, sham, or control). The sham and menthol counterirritants were color coded, which enabled the clinician applying the treatment to remain blinded to which intervention he was applying. After group allocation, the secondary investigator applied the appropriate treatment and covered the knee with a towel. At this time, the initial investigator returned to the room. All subjects remained relaxed in the Hoffmann-reflex-testing position during treatment application and all posttest measurements.

**Treatments**

Subjects allocated to the menthol or sham groups had the gel applied using similar methods. Two milliliters of the respective treatment (menthol or sham) was lightly rubbed into the skin for approximately 1 minute, covering the anterior aspect of the knee joint, avoiding the muscle bellies of the distal quadriceps. The borders of application were from the lateral aspect of the biceps femoris tendon to the medial border of the semitendinosus tendon and from the superior aspect of the patella and to the tibial tuberosity. A control group that received no treatment intervention was also used. After treatment application or control, a cotton towel was placed over the test knee to blind the initial investigator performing Hoffmann-reflex measurements to treatment-group allocation. Subjects rested quietly for 5 minutes, at which time the postintervention maximum Hoffmann-reflex and muscle-response measurements were obtained at 10-minute intervals (5, 15, 25, and 35 min posttreatment).

**Statistical Analysis**

Sample size was estimated using an effect size of .90, which was obtained from means and standard deviations from a previous study examining the effect of ice on changes in Hoffmann-reflex amplitude. Based on these calculations it was estimated that 10 subjects per group would be necessary to have an 80% chance ($\beta$) of detecting a significant change in muscle activation with an a priori alpha level of $P \leq .05$.

Subject demographics (age, height, mass) were compared using a 1-way ANOVA. A $3 \times 5$ mixed-model ANOVA with repeated measures on time (pre-intervention and 5, 15, 25, and 35 min postintervention) was used to determine differences in quadriceps $H_{max}:M_{max}$ between intervention groups (menthol coun-
terirritant, sham counterirritant, and control). An a priori alpha level was set at $P \leq .05$, and post hoc $t$ tests were used as indicated. Standardized effect sizes and 95% confidence intervals were calculated from $H_{\text{max}}:M_{\text{max}}$ scores to determine the magnitude of the effect on each group compared with the baseline measure for that group. In addition, effect sizes and confidence intervals were calculated from $H_{\text{max}}:M_{\text{max}}$ change scores to determine the magnitude of the effect of the menthol and sham treatments compared with the control at all posttests. Statistical analyses were performed with SPSS Version 14.0 (SPSS Inc, Chicago, IL).

**Results**

Mean values and standard deviations for $H_{\text{max}}:M_{\text{max}}$ ratios are presented in Table 2. There were no significant differences over time for $M_{\text{max}}$ ($F_{8,108} = 0.40, P = .81, 1 - \beta = .14$). Because $M_{\text{max}}$ values did not significantly fluctuate, analysis of $H_{\text{max}}:M_{\text{max}}$ ratio was appropriate. There was no significant interaction between time and treatment group for $H_{\text{max}}:M_{\text{max}}$ ratio ($F_{8,108} = 0.65, P = .74, 1 - \beta = .29$). There was no significant difference in $H:M$ ratios between treatment groups ($F_{2, 27} = 0.62, P = .55, 1 - \beta = .14$). A significant difference in $H_{\text{max}}:M_{\text{max}}$ ratios was found over time for all treatment groups ($F_{4,108} = 10.52, P < .001$). Post hoc $t$ tests indicated that $H_{\text{max}}:M_{\text{max}}$ ratios were significantly higher at pretest than at 5 minutes ($P = .004$), 15 minutes ($P = .001$), 25 minutes ($P = .004$), and 35 minutes ($P = .001$) postintervention for all groups (Table 2). There were no significant differences ($P > .05$) between any of the subject-group demographics (Table 1). All effect sizes are presented in Table 3.

**Discussion**

The menthol counterirritant did not facilitate the quadriceps MNPE as reported in previous studies using cryotherapy.8,9,27 Previously, Hopkins et al8 suggested that cryotherapy-induced motoneuron-pool facilitation was likely caused by increased excitatory stimuli relayed to the central nervous system (CNS) from a combination of mechanoreceptors and thermoreceptors in the periphery, thus overriding inhibitory signals. Similar to cryotherapy, menthol stimulates cold-sensitive neurons (Aδ fibers and C fibers) and has been reported to activate TRPM8 ion channels on thermoreceptors even more rapidly than cryotherapy.11–13,16,18

<table>
<thead>
<tr>
<th></th>
<th>Menthol, n = 10</th>
<th>Sham, n = 10</th>
<th>Control, n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>.3218 (.20)</td>
<td>.4570 (.26)</td>
<td>.4785 (.32)</td>
</tr>
<tr>
<td>Post 5 min</td>
<td>.2864 (.18)</td>
<td>.3559 (.20)</td>
<td>.3654 (.27)</td>
</tr>
<tr>
<td>Post 15 min</td>
<td>.2694 (.18)</td>
<td>.3525 (.19)</td>
<td>.3736 (.27)</td>
</tr>
<tr>
<td>Post 25 min</td>
<td>.2776 (.19)</td>
<td>.3526 (.20)</td>
<td>.3683 (.29)</td>
</tr>
<tr>
<td>Post 35 min</td>
<td>.2678 (.18)</td>
<td>.3353 (.18)</td>
<td>.3499 (.28)</td>
</tr>
</tbody>
</table>
Table 3  Effect Sizes (95% Confidence Intervals) for Menthol, Sham, and Control Groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Menthol</td>
<td></td>
</tr>
<tr>
<td>compared with baseline</td>
<td>–0.16 (–1.03 to 0.73)</td>
</tr>
<tr>
<td>compared with control</td>
<td>0.68 (–0.25 to 1.55)</td>
</tr>
<tr>
<td>Sham</td>
<td></td>
</tr>
<tr>
<td>compared with baseline</td>
<td>–0.43 (–1.3 to 0.47)</td>
</tr>
<tr>
<td>compared with control</td>
<td>0.29 (–0.6 to 1.16)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>compared with baseline</td>
<td>–0.34 (–1.21 to 0.56)</td>
</tr>
</tbody>
</table>
Because of the lack of facilitation in MNPE found after the menthol-counterirritant application, it is reasonable to hypothesize that although menthol creates the sensation of cold, it may be interpreted differently by the CNS. Menthol has been reported to activate a subclass of C fibers called polymodal C fibers, and although similar painful sensations are felt with cryotherapy, it is likely that slightly different Aδ pathways are stimulated with menthol. It is possible that our menthol treatment stimulated fewer receptors than cryotherapy treatments in previous studies reporting increased MNPE. Previous researchers applied two 1.5-L ice bags to the anterior and posterior aspects of the knee compared with the 2 mL of menthol we applied to the anterior aspect of the knee. The decreased surface area stimulated by the counterirritant may have affected the intensity of excitatory stimulation, causing less of a response from the CNS.

Previously, cryotherapy treatments were applied to the knee joint to investigate the effects on the surrounding muscles. Cryotherapy was intentionally not applied to the surrounding muscles because muscle cooling was thought to be counterproductive to the goals of facilitation. Because menthol does not cause significant cooling, its application to the surrounding muscle may increase the surface area under stimulation and provide an increased excitatory effect on the CNS.

Small, negative within-group H_{max}/M_{max} effect sizes were found at all posttests for all 3 groups compared with their baseline values, suggesting that the magnitude of the decrease in the H_{max}/M_{max} values was clinically insignificant (Table 3). In addition, all confidence intervals for within-group effect sizes crossed zero, which makes it impossible to suggest that a definitive effect occurred in a positive or negative direction. One moderate effect size was found between menthol and control change scores at 5 minutes posttest, suggesting that the magnitude of the decrease in the control group was moderately larger than in the menthol group. All confidence intervals between group effect sizes for both the menthol and sham treatments crossed zero, making a definitive suggestion of the direction of the effect of either intervention impossible.

Krause et al. have suggested that the physical cooling of a joint may play a role in increased MNPE. Unlike menthol treatments that affect superficial receptors, cryotherapy has the ability to decrease intra-articular knee-joint temperatures by as much as 9.4°C, which may excite thermoreceptors in the knee joint. The ability to penetrate and stimulate deeper thermoreceptors may provide cryotherapy with an advantage for stimulating a greater number of sensory organs. Furthermore, there are thermoreceptors that respond only to decreases in temperature other than those stimulated by menthol, and they may play a vital role in the cryotherapy-induced increase in MNPE.

It should be noted that we found a significant decrease in MNPE across all groups over time. We are confident that the motoneuron pool being tested was not altered during the study because the muscle response did not change. We hypothesize that this decreased MNPE was caused by a systematic decrease in the excitability of the nervous system over the testing period. The effect of quietly lying supine for ~45 minutes likely caused the subjects to relax and, in the absence of an excitatory agent, decreased MNPE of the quadriceps. A similar inhibitory drift has been reported in quadriceps-activation measurements in patients with osteoarthritis who did not receive an excitatory stimulus.
Because the first posttest measurement was not conducted until 5 minutes after the intervention was applied, a limitation of this study is that an immediate effect (within the first 5 min) was not evaluated. Historically, cold modalities have not reached their full facilitatory potential immediately after MNPE but, rather, continued to climb until 45 minutes after the cryotherapy intervention. Another limitation of this study was that skin-temperature changes were not collected. Although previous authors report that skin temperatures only decrease approximately 1°C after menthol application, we were not able to determine the relationship between a possible skin-temperature change and MNPE. Cryotherapy has previously been reported to facilitate MNPE above baseline values, which allows researchers to study its effects in a healthy, noninhibited population. It is possible that menthol does not have the same facilitatory effect on MNPE, and therefore any disinhibitory effects were undetected in this noninhibited population. It would be interesting to determine whether menthol would have the same effects on an inhibited population. Because menthol has clinically been used for its analgesic qualities, it may also be interesting to determine how it may affect MNPE in subjects with knee pain compared with a population without pain. Although we did not evaluate the effects of focal knee-joint cooling in this study, previous research suggests that cryotherapy applied to the knee joint is effective in increasing quadriceps MNPE. Because we did not find a significant increase in quadriceps MNPE with this specific dose of menthol, we cannot clinically advocate the application of menthol for increasing quadriceps muscle activation.

**Conclusion**

The menthol counterirritant (15% menthol) did not facilitate quadriceps MNPE as hypothesized.

**Acknowledgments**

Performance Health, Inc, provided menthol counterirritant and sham products.

**References**


