Study Protocol and Statistical Analysis Plan

Title: The Influence of Topical Menthol Gel on Thermoregulatory and Perceptual Outcomes During Exercise Within the Heat

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Abstract

The purpose of this study is to examine the human thermoregulatory impact of applying a commercially available menthol gel (BioFreeze) to the skin prior to moderate intensity walking under heated conditions. Eligible participants will be aged 19-45 years, have no previously known adverse reactions to menthol (active ingredient in BioFreeze) based upon a self-report, no adverse reactions to BioFreeze as determined through gel application to a small forearm area, and cleared for physical activity via the 2022 Physical Activity Readiness Questionnaire (PARQ). There will be a total of 3 experimental visits to the laboratory. Visit 1 will test for adverse BioFreeze reaction by applying a small amount of gel to the forearm (~2 x 2 in). Anthropometric and body composition data will also be collected at this time. Visits 2 and 3 will consist of 30 min of treadmill walking at a moderate pace (3.5 mph, 5% grade) under hot conditions (38°C, 60%RH). Visits 2 and 3 will be randomized and counterbalanced for BioFreeze or a hypoallergenic gel (control) application. Gels will be applied to areas commonly exposed during outdoor activity in warm conditions (shoulder to wrist, mid-thigh to ankle). Accordingly, participants will wear shorts and a tank top shirt during exercise. Core temperature, skin temperature, galvanic skin response, laser doppler blood flow, and heart rate will be continuously recorded throughout the exercise bout via an integrated analog to digital converter. Sweat will be collected during exercise using commercially available absorbent patches. Thermal sensation will be assessed throughout exercise via the ASHRAE thermal sensation likert scale (cold to hot). Lastly, nude body weight will be recorded pre and post exercise for sweat rate determination. The BioFreeze and control trials will be separated by a 7 to 14 day washout period.

Purpose and Rationale

The purpose of this study is to examine the impact of applying a menthol-based gel to the skin on human thermoregulation and thermal perception during exercise in the heat.

Transient receptor potential (TRP) channels are a family of membrane proteins evolved to interpret environmental stimuli through ion channels. One of these channels, the TRP melastatin 8 (TRPM8), is responsible for the sensation of innocuous cool temperatures and it might be responsible for noxious cold temperatures [1,2]. When applied topically, menthol acts upon a cold transduction enzyme to decrease the thermal sensitivity of TRPM8. This decreased thermal sensitivity allows activation of TRPM8 at warmer temperatures, mimicking a cool sensation in warm environments. This cool menthol feeling is used widely in everyday commercial products, but most popular when used as an analgesic in medicated pain-relieving gels/creams.

Current recommendations suggest menthol can be used as an ergogenic aid resulting in an improved performance within heated environments [1]. By decreasing thermal sensitivity of TRPM8 receptors, menthol exerts a physiological response that is perceived as external cooling. The most salient response is an increase in heat conservation, resulting in a reduced exercise capacity due to fatigue associated with a rise in core body temperature [3]. It seems

the application and concentration strength of menthol application to the skin may be the most obvious influential factors. As such, researchers have used proprietary menthol solutions with varying intensities (0.05- 10%) [1], yet, to our knowledge, no one has investigated widely available, over-the- counter products. Indeed, the translation from proprietary formula to over-the-counter application is unknown. Furthermore, it is unclear if currently available menthol products may also aid in performance. This incongruence requires further investigation into the effect of menthol on exercise performance.

Participants

12-15 subjects, age 19 to 45 years, will be needed to complete the study. Nineteen is the age of majority at the study site. The 2022 physical activity readiness questionnaire (PARQ) requires that adults aged >45 who are not accustomed to regular physical activity seek professional consultation prior to beginning physical activity. Thereby, keeping an age range of 19-45 ensures that the participants will be of consenting age and cleared for physical activity participation.

Research Plan

Three experimental visits to the laboratory will be conducted. All visits will take place at the University of Nebraska at Omaha, Exercise Physiology Laboratory. Visit 1 will consist of the physical activity readiness questionnaire (PARQ), adverse reaction to BioFreeze, and body composition (hydrostatic weigh, bioelectrical impedance). Visits 2 and 3 will be separated by 7-14 days, randomized, and counterbalanced for experimental (BioFreeze) and control (hypoallergenic cream) trials. Both the experimental and control visits will follow the same protocol.

Participants will arrive following an overnight fast state while also refraining from strenuous activity, alcohol consumption, tobacco use, and recreational drugs for the previous 24-hour period. During these visit researchers will apply BioFreeze using a gloved hand from shoulder to wrist and mid-thigh to ankle (areas outside of the clothing). A hypoallergenic cream will be applied during the control visit.

Exercise will consist of 30 min of moderate intensity treadmill walking (3.5 mph at 5% grade) within a heated temperature/humidity-controlled chamber (38°C, 60% relative humidity).

Participants will be weighed before, and after completion of the exercise calculate sweat rate. Prior to the exercise session, participants will sit for 5 min in an ambient room temperature environment as a baseline. During baseline and during the exercise core temperature, skin temperature, laser doppler blood flow, galvanic skin response, and heart rate will be collected continuously. Sweat patches will be adhered to the forehead during exercise at minute 10 for collection of sweat composition over the next 10-20 minutes.

Thermal perception using the ASHRAE scale (cold to hot) will be assessed pre and during exercise.

VISIT 1

Visit 1 will take approximately 1 hour. Informed consent will be given. Participants will then complete the physical activity readiness questionnaire (PARQ) to be cleared for physical activity. Participants will have a small amount of the BioFreeze cream applied to a small area of the forearm (~2 x 2 in) to test for any adverse reaction over a period of 15 minutes. This initial skin reaction test is not a part of the research protocol. Then, resting blood pressure will be taken. Those without a reaction, and a resting blood pressure lower than 140/90, will have height, weight, and body fat composition measured. Height and weight will be measured using a medical scale and stadiometer, respectively. Body fat will be assessed with hydrostatic weighing using an electronic load cell-based system (Exertech, Dresbach, MN) correcting for residual lung volume or via a bio-electrical impedance analyzer (InBodyUSA, Cerritos, CA).

VISITS 2 AND 3

Visits 2 and 3 will take approximately 2 hours each. Upon arrival at the lab, a nude body weight will be collected with the subject in a private room. While still in the room, subjects will self-insert a rectal thermistor 12-15 cm beyond the anal sphincter and don a chest strap heart rate monitor. After dressing in their exercise clothing, subjects will exit the private room so that chest, forearm, and calf thermistors can be adhered to the skin with tape.

Laser doppler flow (forearm and or/finger) will be adhered with tape and galvanic skin response (fingers) will be adhered using Velcro straps to the skin. Measuring instruments will be recorded using an integrated digital to analog converter (ADInstruments, Colorado Springs, CO).

Following instrumentation, subjects will sit for 5 minutes as a baseline in a temperate environment. Immediately after, a researcher with gloved hands will topically apply BioFreeze or the control hypoallergenic cream from the shoulder to wrist and mid-thigh to ankle. Subjects will then enter the heat chamber (38°C, 60% relative humidity) and immediately begin a 30-minute walk on a treadmill (3.5 mph, 5% grade). Subjects will be intermittently asked to hold their arm steady for laser doppler flow collection (10, 20, 30 minutes). Subjects will also briefly pause at minute 10 in order to adhere an absorbent patch to their forehead for sweat collection over the following 10-20 minutes. Thermal perception using the ASHRAE scale (cold to hot) will be assessed pre and during exercise. After 30 minutes of walking, subjects will exit the chamber for removal of all skin and finger sensors. Subjects will then re-enter the private room, remove their own rectal thermistor with gloved hands, and then a final nude body weight will be recorded after toweling off.

The rectal thermistor will measure core temperature. The heart rate monitor will measure the beats of the heart. Skin thermistors will measure the temperature of the skin surfaces. Laser doppler flow measure the relative units of blood flow velocity.

Galvanic skin response measures skin conductivity changes as individuals begin to sweat. Sweat is collected for assessment of sweat composition. Thermal perception is assessed for

individual perception surrounding the temperature of the ambient environment. Nude body weight is measured to calculate sweat rate.

Statistical Analysis

Recruitment

Previous exercise research suggests that the difference in response of matched pairs is normally distributed with a standard deviation of 0.86. If the true difference of the mean response of matched pairs is 0.75, we will need to complete 12 experimental trials for each condition to be able to reject the null hypothesis that this response difference is zero with a power of 0.8. The Type I error probability associated with this test of the null hypothesis is 0.05.

Interventions

A repeated measures two-way ANOVA (Time x condition) will be used for the dependent variables. If the F-ratio values are found to be significant, a Fisher's Least Significant Difference post hoc analysis will be conducted. A type I error probability of < 5% will be considered significant ($p < 0.05$). All data will be analyzed using a combination of Microsoft Excel and the Statistical Package for the Social Sciences (SPSS).