Post-exercise Hot Water Immersion to Improve Overnight Blood Pressure

Protocol & Statistical Analysis Plan

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Human Subjects Procedures

a. Human Subjects Involvement, Characteristics, and Design

Study design: This study will examine heart rate variability (HRV), endothelial function (FMD), and overnight blood pressure in response to acute moderate aerobic exercise followed by leg heating in 42°C water (Ex + HT), aerobic exercise followed by a sham leg immersion in 33°C water (Ex), leg heating alone in 42°C water (HT), or control conditions (no exercise or heat exposure) in adults with elevated blood pressure. The study will consist of four trials, administered in a randomized, counterbalanced fashion, with all participants completing all treatments, with at least 5 days between each trial.

Subject population: The subject population will be adults with elevated blood pressure (defined as resting blood pressure >120/80 mmHg; not diagnosed hypertension). Inclusion criteria for all subjects will be age 18-50, BMI between 18-39.9, not taking any anti-hypertensive medications, no history of heat illness. Blood pressure will be measured at the screening visit and volunteers must have duplicate resting blood pressures >120/80 (taken after 15 minutes of quiet rest in the lab) in order to qualify for the study. These inclusion criteria, including age range, were selected to minimize the risk of acute cardiovascular events during exercise. All subjects must be capable of walking 30 min at a moderate intensity on a treadmill. An effort will be made to recruit men and women in equal numbers, and for the diversity of the subject pool to match the local community.

Research sites: This is a single-site study and all research activities will be conducted at Providence College.

b. Study Procedures, Materials, and Potential Risks

Volunteers will report to the lab for an introductory visit and screening 24 hours prior to their first study day. After a review of study procedures and resting blood pressure measurement, they will be instrumented with an ambulatory blood pressure cuff which will take hourly measurements for the 24 hours preceding their first study day for familiarization with the equipment.

Upon arrival for testing the following afternoon, weight and height will be assessed, and volunteers will be instrumented with a 3-lead ECG and a beat-by-beat blood pressure monitor before lying supine in a room-temperature lab for 15 minutes prior to baseline (pre-intervention) measures of heart rate, heart rate variability (HRV), blood pressure, and flow-mediated dilation (FMD) of the brachial artery (described below). After baseline measures, volunteers will complete the daily protocol, consisting of either exercise + heat (Ex + HT), exercise + sham immersion (Ex) or heat alone (HT). On the control day, only baseline measures will be taken and the volunteer will be instrumented with the ambulatory blood pressure cuff with instructions to refrain from exercise or heat exposure during the 24h measurement.

Blood Pressure measurement: Blood pressure will be measured regularly in the lab for safety monitoring using a clinical-grade automated blood pressure cuff. Ambulatory blood pressure will be measured for a 24 hour period following each of the four conditions: control (no exercise or heat), Exercise, Heat Therapy, and combined Exercise and Heat Therapy. The Welch-Allyn

ambulatory monitor inflates once per hour and stored blood pressure readings for download by the research staff.

Heart Rate Variability (HRV): HRV will be measured during supine rest on a padded exam table. Participants will be instrumented with three foam stickers on the shoulders and hip which connect to a device that records and analyses heart rhythm (Finapres Nova). They will be asked to breathe to a metronome (paced breathing) for a period of 10 minutes to ensure adequate data collection.

Flow-mediated dilation (FMD): While resting supine on a padded exam table, participants will be asked to extend their right arm so that we can obtain an ultrasound image of the brachial artery. We will also instrument the participant with an inflatable cuff placed just below the elbow. Once a quality image of the artery is obtained, we will ask the participant to stay still while we record a baseline measure of artery diameter and blood flow before inflating the cuff to 250mmHg for a period of 5 minutes. After five minutes have passed, the cuff pressure will be released, allowing for a rapid increase in blood flow through the brachial artery to reperfuse the forearm. We will continue to record blood vessel diameter and blood flow for 3 minutes after cuff release for analysis of % dilation of the brachial artery and changes in blood flow and shear patterns.

Exercise Intervention: During Exercise (Ex) or combined exercise and heat therapy trials (Ex + HT), the exercise session will consist of 30 minutes of treadmill walking at 55-60% of heart rate reserve (calculated based on resting heart rate measurement taken in the lab and age-predicted maximum heart rate). The treadmill will initially be set at 2% grade and 2.5 miles per hour, and adjusted in the first five minutes to achieve the target heart rate. Heart rate will be monitored continuously, and RPE will be assessed every five minutes. This exercise protocol was selected because it has been shown to produce reductions in systolic and diastolic blood pressure in hypertensive subjects while minimizing risk of adverse events during exercise. This exercise protocol is also supported by the American College of Sports Medicine guidelines for individuals with hypertension.

Leg heating: Following exercise, participants will be seated in a chair for 45 minutes with their legs immersed to mid-calf in either lukewarm (33°C; sham leg immersion in Ex trial) or hot (42°C, Ex + HT trial) while heart rate, blood pressure, and tympanic temperature are monitored every 5 minutes. For the standalone heat therapy trial (HT), participants will complete the 45 minute hot (42°C) leg immersion with no prior exercise. Heart rate, blood pressure, and tympanic temperature will be monitored every 5 minutes, and subjects will have free access to fluids.

Potential Risks to Participants: The proposed study will utilize cardiovascular monitoring techniques with minimal risks. The exercise session will be supervised within the Health & Human Performance lab with heart rate and perceived exertion continuously monitored by study staff. Exercise will be stopped in the event of difficulty breathing, chest pain, if heart rate exceeds 90% of age-predicted maximum, or if they feel they cannot continue. There is a risk of slight muscle soreness following exercise, but this is uncommon given the mode (walking) and duration (30 minutes).

Additional measures include blood pressure, tympanic temperature, blood flow, and heart rate variability (HRV). Insertion of the tympanic temperature sensor in the ear and inflation of the blood pressure cuff may cause mild discomfort (common). The inflation of the cuff on the forearm for flow-mediated dilation for 5 minutes may cause mild to moderate discomfort (sense of pressure

at the site of inflation and mild tingling in the fingers). There is also a chance of irritation of the skin where foam sticker electrodes are applied to measure heart rhythm (less common).
The immersion of the lower limbs in hot water may cause mild discomfort, including a warm sensation and initiation of the sweating response (common). During heating, an increase in heart rate, increase in core temperature, and small decrease in blood pressure are also likely (common). Heart rate, blood pressure, and temperature will be monitored throughout heating and the subject will be removed from the leg bath if heart rate increases more than 50 beats per minute, tympanic temperature exceeds 39.5°C, blood pressure falls more than 10 mmHg, or if the subject reports any heat-related symptoms (uncomfortably hot, light-headed, nauseated, heart racing). These potential risks are uncommon given the small amount of body surface area being heated. Water temperature will be continuously monitored and set at a level (40-42°C) well below the threshold for burning (47°C). Ice and fans will be on hand to initiate rapid cooling if needed.

There is a remote (uncommon) risk of breach of confidentiality. Maintaining research volunteers' privacy and keeping personal identifiers confidential is important to the study staff. All research activity including subject screening and data collection will be performed and stored at Providence College. Subject names, contact information, health history, and other information that can be traced back to the subject will be kept securely and separately from data collected for the study. Personal information will be kept in a locked office and away from data. Collected data will have the subject's identification code and some computer software used to collect data (LabChart) will have the date and time marked on the file. Protocol sheets used during data collection will only have the subject's ID. The tests will have a subject ID and may contain other pertinent information needed to clinically evaluate the test, such as age, gender, and blood pressure measurement. All data will be stored in a secure server and encrypted hard drives. All key personnel have completed education on the use of human subjects (CITI, Good Clinical Practices [GCP] training) in compliance with NIH regulations.

2. Adequacy of Protection Against Risks

a. Informed Consent and Assent

An initial screening and consent meeting will be scheduled with the PI over e-mail and will take place in person at the research lab. The potential participant will be sent a copy of the consent form to review in advance (at least 48 hours before the meeting). The details of the study & potential risks will be discussed in this meeting. The potential participant will have an opportunity to ask any questions, and will additionally be screened for participation (inclusion/exclusion criteria). If the person qualifies and agrees to participate after written and oral information are provided on the study, they will be given the opportunity to sign the informed consent document and enrolled in the study. They will also be informed that they can choose to withdraw at any time.

b. Protections Against Risk

Physical risks: All portions of research, including exercise and heating sessions, will be closely supervised by study staff. We have procedures and criteria in place to discontinue exercise or heating in the event of unusual heart rate or blood pressure responses, difficulty breathing, chest pain, elevated temperature, or any symptoms related to heat illness. We will also monitor subjective ratings every 5 minutes to assess physical comfort. During leg heating, water temperature will be continuously monitored and set at a level (42°C) well below the threshold for burning (47°C). Ice and fans will be on hand to initiate rapid cooling if needed.

Breach of confidentiality: All research activity including subject screening and data collection will be performed and stored at Providence College. Subject names, contact information, health history, and other information that can be traced back to the subject will be kept securely and separately from data collected for the study. Personal information will be kept in a locked office and away from data. Collected data will have the subject's identification code and some computer software used to collect data (LabChart) will have the date and time marked on the file. Protocol sheets used during data collection will only have the subject's ID. The tests will have a subject ID and may contain other pertinent information needed to clinically evaluate the test, such as age, gender, and blood pressure measurement. All data will be stored in a secure server and encrypted hard drives. All key personnel have completed education on the use of human subjects (CITI, Good Clinical Practices [GCP] training) in compliance with NIH regulations.
Medical emergencies: In the unlikely event of a medical emergency or adverse response, the Health Sciences Laboratory Medical Emergency Safety Plan will be followed. Adverse events will be handled by immediately stopping any testing and monitoring the subject (including signs, symptoms, and blood pressure) as necessary, with immediate medical assistance available through Providence College emergency procedures if necessary.
Incidental Findings: The cardiovascular assessments we make as part of this research will provide insight into the health of the participant. In the event that an individual has a result (very high blood pressure, abnormal ECG), no diagnosis will be made by study staff. However, the results will be shared with the participant and we will suggest they follow up with their primary care provider.

3. Potential Benefits of the Proposed Research to Research Participants and Others

There are no direct benefits from participating in this study. The interventions in this study (moderate exercise, heat exposure) have been shown to improve blood pressure and cardiovascular health with repeated use, but it is unlikely that a meaningful long-term change in fitness or health will occur with this acute protocol.

More broadly, the results of this study have important health implications in the treatment of hypertension using lifestyle interventions. The findings have the potential to improve non-pharmacological treatment of hypertension by developing an exercise and heat exposure protocol that substantially reduces blood pressure and improves cardiovascular risk profile for the >100 million Americans with hypertension. Each individual participant will have the option to review their results from their trials after completion of the study, and may share these results with their physician to discuss lifestyle interventions to prevent hypertension. This potential benefit outweighs the minimal risks (muscle soreness, mild discomfort during study procedures) associated with the study.

4. Importance of the Knowledge to be Gained

Nearly half of the U.S. population has elevated blood pressure, and 1 in 3 Americans has diagnosable hypertension. Progression to more severe disease states carries a significant health and financial burden, so low-cost, non-pharmacological interventions provide a critical avenue to prevent disease progression. The results of this work will provide insight into the possible complementary nature of exercise and heat exposure to reduce blood pressure, and the mechanistic underpinnings that may allow for a more targeted lifestyle approach to different types of hypertension (related to sympathetic overactivity, impaired blood vessel function, or both). Further, as nocturnal dipping (the decrease in blood pressure that occurs during sleep) is related to disease severity and adverse cardiovascular outcomes, the examination of overnight responses will provide insight into the efficacy of exercise, heat, or both to improve overnight

blood pressure. Given the health burden of hypertension on and individual and on society, the potential benefits outweigh the risks of individuals participating in this research.

Statistical Design and Power

This study investigates the potential for heat therapy to supplement or augment exercise for blood pressure reduction. To estimate expected effect sizes, we calculated standardized mean differences in our key outcomes using pilot data from our healthy participants: $d = (x_{baseline} - x_{intervention})/S$, where x represents the baseline (no intervention) and post-treatment (combined exercise and heat) values and S is the standard deviation. This effect size calculation reflects differences in outcome means anticipated for the proposed study. Taking into consideration the relatively low costs and low risks of hot water leg immersion, these differences are not only statistically significant, but also clinically meaningful at the individual and population levels.

Primary analyses: The primary outcome variable for Specific Aim 1 is mean nocturnal blood pressure. Using previous data from our lab in healthy men and women, a power analysis indicates that a minimum of 8 participants should be required to see changes between intervention and control treatments using a repeated-measures ANOVA design. For Specific Aim 2, our primary outcome variable is the time domain analysis of Heart Rate Variability (standard deviation of normal to normal R-R intervals; SDNN). Using pilot data from a study examining post-exercise hot or cold water immersion, a minimum of 10 participants is required to see differences in HRV between treatments using a repeated-measures ANOVA design. For Specific Aim 3, data from existing literature examining endothelial function (flow-mediated dilation) before and after a single bout of exercise in individuals with hypertension indicates that a minimum of 10 participants would be required to see significant differences in % dilation (the primary outcome of FMD analysis) between treatments. Given that there may be sex differences in the magnitude, timing, or mechanisms of blood pressure responses to exercise and heating, we plan to recruit sufficient numbers (10 men, 10 women) to allow for a statistical comparison between sexes. Significance for each test will be evaluated with a two-sided alpha, $p < .05$.

Projected effect size: Based on preliminary data from our pilot work, we project large within-subject effects across multiple outcomes. We used mean changes in the primary outcome variables, and estimated the standard deviation from the baseline data in our pilot participants or published values using exercise or acute heat exposure as accurate estimates. Effect sizes were calculated as mean change from baseline (control) to post-intervention as follows: Mean arterial pressure $d = 1.33$, SDNN (HRV) $d = 1.03$, FMD $d = 1.12$.

Results will be analyzed using a repeated measures ANOVA (each participant will complete all treatments in a randomized, counterbalanced design) with four trials (Control, Ex, ExHT, HT). Statistical significance will be set at $\alpha=0.05$ and power at 80%. Secondary analyses using a mixed-model ANOVA design may also be used to examine possible differences in magnitude of change between men and women for each variable. Descriptive statistics will be presented including mean, median, standard deviation, and range.