PennState

HRP-591 - Protocol for Human Subject Research

Protocol Title:

STUDY00005798: Effects of heating on exercise pressor reflex in peripheral artery disease: Exercise ability

Principal Investigator:

Name: Jian Cui
Department: HVI
Telephone: 717-531-1799
E-mail Address: jcui@hmc.psu.edu

Version Date:

12/18/24

Clinicaltrials.gov Registration #:

NCT03900832

Table of Contents

1.0 Objectives

2.0 Background

3.0 Inclusion and Exclusion Criteria

4.0 Recruitment Methods

5.0 Consent Process and Documentation

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

7.0 Study Design and Procedures

8.0 Subject Numbers and Statistical Plan

9.0 Confidentiality, Privacy and Data Management

10.0 Data and Safety Monitoring Plan

11.0 Risks

12.0 Potential Benefits to Subjects and Others

13.0 Sharing Results with Subjects

14.0 Subject Stipend (Compensation) and/or Travel Reimbursements

15.0 Economic Burden to Subjects

16.0 Resources Available

17.0 Other Approvals

18.0 Multi-Site Research

19.0 Adverse Event Reporting

20.0 Study Monitoring, Auditing and Inspecting

21.0 Future Undetermined Research: Data and Specimen Banking

22.0 References

1.0 Objectives

1.1 Study Objectives

The study objectives of this projects are to examine the hypothesis that the sympathetic and blood pressure (BP) responses to exercise will be attenuated during and after heat exposure in patients with peripheral artery disease (PAD), via altering the sensitivity of the muscle afferent receptors.

We will examine if a heat treatment will decrease the BP response to walking and increase the walking distance in PAD patients.

We hypothesize that after one warm bath, the walking distance/time for a PAD patient will be longer than that without a warm bath. We further hypothesize that after the warm bath, the magnitude of the BP response to walking will be lower than without the warm bath at the same stage/distance. We will also examine if warm water immersion of only lower limbs can induce similar effects.

We speculate that these effects are induced by the heat exposure, and not by the hydraulic pressure of water in the bath. Thus, we hypothesize that a neutral temperature bath will not induce similar effects to the warm bath, while whole body heat exposure using a heating suit will induce similar effects.

1.2 Primary Study Endpoints

The primary variables of interest are the walking ability and the responses in hemodynamic variables (BP, HR) during walking with and without a heat treatment in PAD patients

1.3 Secondary Study Endpoints

The comparison of the effects of heat treatment on BP, HR responses during walking between the PAD patients and healthy subjects is the second interest in this project. The comparison of the effects between warm bath and non-heating bath or between warm bath and heating suits are additional interest in this project.

2.0 Background

2.1 Scientific Background and Gaps

Peripheral arterial disease (PAD) affects 8-12 million Americans and its cardinal symptom is leg pain during exercise, termed “intermittent claudication”. The diagnosis of PAD involves measuring the ankle-brachial index (ABI, i.e. the systolic pressure in the ankle divided by the systolic pressure in the arm); values below 0.9 are indicative of large vessel obstruction. Intermittent claudication is the most common symptom of this disease and it regularly occurs during exercise/physical activity but is relieved promptly by rest. Previous studies suggest that as the exercise pressor reflex is activated in patients with PAD, BP and heart rate (HR) are exaggerated (2, 3, 21). The BP rises during walking in the PAD patients were significantly greater than that seen in healthy control subjects (2, 3). A recent human study from our group (23) further indicates that an early BP response occurred during plantar flexion exercise before claudication was noted by the subjects, which may suggest that the accentuated BP response was due to an augmented muscle mechanoreflex in PAD. Moreover, another recent study from our group (15) also showed that PAD patients have augmented renal vasoconstriction during plantar flexion exercise.

It has been shown that exercise has benefits for patients with PAD. However, exercise becomes limited due to the pain and fatigue associated with the disease. Moreover, the augmented pressor response to exercise in PAD may increase the risk for the end organ damage (e.g. brain and/or heart). Thus, finding alternate/complimentary interventions that modulate the cardiovascular system and autonomic nervous system, and can be tolerated by the patients, would be beneficial.

One possible intervention is heat exposure, since acute bouts of exercise and acute heat exposure have similar acute effects on both the autonomic and cardiovascular systems. For example, both exercise

and heat exposure increase body temperature (19, 28), increase HR (19, 26), increase cardiac output (CO) (19, 26), increase left ventricular ejection fraction (EF) (4, 31), and enhance myocardial function (6, 16). In the peripheral circulation, both exercise and heat exposure increase limb blood flow (19, 28), muscle blood flow (27) (22), and skin blood flow (28, 32). Moreover, recent studies suggest that whole body exposure improves the endothelium function in PAD patients (e.g. activates endothelial progenitor cells CD34+) (29).

However, the effect of heat treatment on the ability of walking of PAD patients has not been examined. Moreover, it is unknown if heating can normalize/decrease the accentuated the pressor responses to exercise in PAD patients.

2.2 Previous Data

We do not have any preliminary data using heat treatment in PAD patients. However, our pilot data from healthy middle aged and older subjects show that that 4 weeks of warm baths (39 - 41 °C water for 30 min, 5 times/week) may lead to: 1) reductions in resting MSNA and HR; 2) enhancement in forearm flow mediated dilation (FMD; an index of endothelial function); 3) increase in systolic myocardial velocity (an index of cardiac function); and 4) increase in systemic maximal oxygen consumption (VO_2max). Based on these pilot data in healthy subjects, we speculate that heat treatment may have beneficial effects on PAD patients.

2.3 Study Rationale

PAD is caused by progressive narrowing of the arteries in the lower extremities. Exercise triggers symptoms of leg pain, which limits the ability and/or the intention of exercise in the patients. Moreover, the augmented pressor response to exercise in PAD may increase the risk for the end organ damage. We will examine if heat treatment can be used as an alternate/complimentary intervention in this protocol. The possible mechanisms will be examined with another protocol (STUDY5856).

We speculate that vasodilation and increased blood flow induced by heat treatment may induce changes in the chemical milieu of the interstitial space, which also alters the exercise pressor reflex in PAD patients. Moreover, the vasodilatory effects could potentially improve skeletal muscle perfusion and oxygenation and thereby lessen the effects of intermittent claudication (i.e. leg pain while walking). Thus, the walking ability of the PAD patients might be improved after the heat treatment.

For PAD patients, we do not intend for heat treatment to replace existing medical and surgical therapy but rather to serve as an adjunct and additional intervention to improve their medical condition.

Besides examining the physiological mechanisms, a long term goal of this project is to develop a simple, convenient and efficient approach for heat treatment in PAD patients. We speculate that warm bath is the simplest and most efficient approach for these patients. First, most homes in the US have a bathtub, so patients do not need special facility (e.g. sonar bath). Second and more importantly, water has higher thermal conductivity (~ 26 folder greater) and much higher volumetric heat capacity (~ 3300 folder greater) compared to air. Thus, to achieve the same increases in body temperature and vasodilation, the heating time in a bathtub at a relatively lower temperature (e.g. ~ 40 °C) can be shorter than in a hot room/chamber with relatively higher temperature (e.g. ~ 60 °C). The efficiency of a heating suit/blanket is likely somewhere between a warm bathtub and in a hot room. Thus, we believe that warm water bath is a simple and efficient approach for patient treatment. Moreover, we will examine the effects of warm water immersion of only the lower limbs. If the warm water immersion of lower limbs can induce similar effects with those seen after a warm bath, the data will suggest that the effects of heat treatment on local muscles in the lower limbs (e.g. local vasodilation, etc.) play a key role in improving the walking ability. Otherwise, the data will suggest that the effects of whole body heating (e.g. whole body vasodilation, increase heart rate and cardiac output, etc.) are important for inducing the effects.

However, a warm bath may not necessarily be a “pure” approach for research. The hydraulic pressure of the water may induce fluid redistribution, which may alter autonomic regulation. Moreover, a water bath is not conducive for some measurements such as MSNA recording. To determine if the effects we observe are due to the heat exposure, and not hydraulic pressure, we will employ warm baths and heating

suits, (that we propose will induce similar effects), along with neutral water baths (that will measure the effects of hydraulic pressure alone, and we propose will not induce the same effects).

STUDY00005798 and STUDY00005856 have the same overall objectives, but focus on different aspects of the responses to the experimental conditions. The hypotheses in the two protocols can be examined with different subjects. In our experience, we found that it is much more difficult to recruit volunteers (especially a patient population) for a study with many visits than for a study with fewer visits. Therefore, these studies are divided into 2 IRB protocols to assist with recruitment.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

For patients with PAD and Control subjects

- Capable of giving informed consent
- Any race or ethnicity
- Men and women age 21- 85 years (inclusive)
- Fluent in written and spoken English

Patients with peripheral arterial disease (PAD)

- Diagnosed with PAD (i.e., ankle-brachial index below 0.9)
- Fontaine stage II or less – no pain while resting
- Satisfactory history and physical exam

Healthy subjects

- Satisfactory history and physical examination
- Free of acute medical conditions

3.2 Exclusion Criteria

For patients with PAD and Control subjects

- Age < 21 years
- Pregnant or nursing women
- Decisional impairment
- Prisoners
- Use of recreational drugs (excluding marijuana)
- Use of recreational or medicinal marijuana within 24 hours prior to study visits.
- Recent alcohol abuse history (less than 6 months) or is currently using or abusing excessive alcohol. Excessive alcohol will be defined as greater than 14 drinks per week.

For patients with PAD

- History of CAD with symptoms of unstable angina or myocardial infarction (<6 months)
- History of epilepsy or seizure disorders

For healthy subjects

- Any other chronic diseases (heart, lung, neuromuscular disease or diabetes)

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Participation in the study will be discontinued if pre-screening requirements are not met or the main variables could not be measured. If vital signs shift outside acceptable levels or if the subject does not tolerate an intervention, the intervention will be stopped. The subject may withdraw from the study at any time.

3.3.2 Follow-up for withdrawn subjects

Subjects withdrawn from the study will not return for any more visits. The research nurses will contact subjects after withdrawal to ensure there are no further problems.

4.0 Recruitment Methods

4.1 Identification of subjects

Dr. Faisal Aziz in the Penn State Heart and Vascular Institute will provide our research nurses with the names of new potential PAD patients. Our research nurses will also scan the HVI clinic lists to look for patients who meet inclusion criteria. PAD patients who have participated in some of our other studies, will be identified from our own subject pool that meet the inclusion/exclusion criteria and have previously agreed to be contacted for future studies.

Healthy subjects will be contacted from our database of volunteers who agreed to be contacted for future studies.

4.2 Recruitment process

New potential PAD patient subjects will be presented with a Invitation to participate Letter during a clinic visit to the Heart and Vascular Institute, or sent to their home after their visit. This letter allows the patient to provide contact information giving permission for our research nurses to contact them, or the patient may contact the research nurses directly using the contact information in the letter. If the patients respond to the letter, the nurses will provide the full details of the study using the phone script, and schedule a screening visit.

Healthy Control subjects will be recruited from our HVI Participant registry, subjects who expressed interest in participating in other studies. Potential subjects, who call to inquire about a study, will be informed about the details of the study using the phone script that leads to a series of eligibility questions pertaining to their health, and medical background. If they wish to participate in additional research studies, the participant will be informed about STUDY18959, the HVI Participant registry following that study's procedures. If they indicate that they are not interested in future studies, their information will be destroyed at the end of this study or at any future time when they request destruction. The information will not be disclosed to anyone outside of the HMC Cardiology Research team.

4.3 Recruitment materials

Invitation to participate letter
Phone script

4.4 Eligibility/screening of subjects

To determine the eligibility of new and returning PAD patients who are interested in participating in the study, our research nurses will screen their medical records and the eligibility checklist.

Healthy subjects will be screened from information on the eligibility checklist.

On the first visit, a brief history and physical will be performed in all subjects to confirm their health status.

5.0 Consent Process and Documentation

5.1 Consent Process

Obtaining Informed Consent

5.1.1.1 Timing and Location of Consent

Subjects will be contacted using the HRP-585 by email or telephone to obtain implied consent to hold caffeine for 12 hours and abstain from alcohol, recreational and/or medicinal marijuana ,over-the-counter medications, and extreme exercise for 24 hours. PAD patients will be asked to take their medications

according to their regular schedule, but at a similar time on the days of the study visits. Subjects do not need to be fasted for the study visits.
Written informed consent will be obtained by our research nurse from all research subjects before any testing is performed, using Penn State Hershey College of Medicine Institutional Review Board approved forms. Consent will be obtained after a member of the investigative team explains the study procedures in layman's terms and answers any questions the subject may have. Consent will be obtained in a private room and subjects will be given a copy of their signed consent form.

5.1.1.2 Coercion or Undue Influence during Consent

Voluntariness will be emphasized. The subjects will be provided with ample opportunity to have questions answered before participating in the study. All procedures will be explained to them in layman's terms and they will be given a copy of their signed consent form. The consent process is performed in a private room. Subjects will have the right to withdraw from a study at any time.

5.1.2 Waiver or alteration of the informed consent requirement

Waiver requested for recruitment. HVI Clinic lists will be accessed by our research nurses to determine if a patient should receive a Permission to Contact letter. If the patient is interested in participating in the study, the medical record will be accessed to determine if they meet eligibility criteria.

5.2 Consent Documentation

5.2.1 Written Documentation of Consent

Subjects will sign the IRB-approved consent form and it is documented with the ICF documentation form.

5.2.2 Waiver of Documentation of Consent (Implied consent, Verbal consent, etc.)

Waiver requested for recruitment screening and for the use of the HRP-585 Screening Consent.

HVI Clinic lists will be accessed by our research nurses to determine if a patient should receive an Invitation to Participate. If the patient is interested in participating in the study, the medical record will be accessed to determine if they meet eligibility criteria.

Subjects will be contacted using the HRP-585 Screening Consent to obtain verbal consent to refrain from caffeine for 12 hours and abstain from alcohol, recreational and/or medicinal marijuana over the counter medications, and extreme exercise for 24 hours before the first visit. These restrictions present no more than minimal risk of harm to the subjects, so verbal consent is appropriate. PAD patients will be instructed to take their medicines on their regular schedule according to guidance of their physicians, and take the medicines at same time (e.g. 7:00 AM) on each visit day. Since no medication is altered, verbal consent is appropriate.

Once the subjects arrive for the Screening visit, they will be given sufficient time to read the consent, have all their questions answered, and to sign the written consent.

5.3 Consent – Other Considerations

5.3.1 Non-English Speaking Subjects

Not planned at this time. We also do not plan to study subjects who cannot read or write because we would be concerned that they might not understand the procedures. Our studies are rather complex, and we want our subjects to understand what they are being asked to do.

5.3.2 Cognitively Impaired Adults

Not applicable

5.3.3 Subjects who are not yet adults (infants, children, teenagers)
Not applicable

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☒ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☒ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☒ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure

Information is included in the "Confidentiality, Privacy and Data Management" section of this protocol.

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers

We do not plan to destroy identifiers attached to data because we sometimes need to go back to older records to retrieve more data. In this case, we will access stored data with appropriate IRB approval.

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI

We must be able to access the medical records to determine eligibility and information related to the patients' condition. We will request verbal consent to record the health information the research nurses ask over the phone during recruitment, as well as requesting the subjects to fast before any study visits. We also need contact information and SSN to pay the subjects.

6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

The medical record and other health information is necessary to determine eligibility before consent. It is preferable for the subjects to be fasted before study visits to provide cleaner data.

6.3 Waiver or alteration of authorization statements of agreement

Waiver or alteration is requested to screen for eligibility.

Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.

The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

7.0 Study Design and Procedures

7.1 Study Design

In this protocol, we will examine if a heat treatment can improve the ability of walking and decrease the BP responses to walking in PAD patients.

Two groups of subjects, PAD patients, healthy subjects, will be examined. The heating trials and non-heating trials will be performed in a random order using a Latin Square design. Because the nature of the interventions (i.e. heating or non-heating), neither subjects nor investigators can be blinded to the interventions.

Five trials (no bath/ no heating, warm bath, neutral temperature bath, heating with heat suit, lower limb warm water immersion) will be performed on 5 separate visits. After some pilot data are obtained, the 5 visits will be performed in a random order using a Latin Square design. AFTER the heating and non-heating treatments, PAD patients will perform the Gardner protocol in each of the 5 visits to assess their walking ability. BP and HR will be measured during each stage of the Gardner protocol. A small subset of subjects may be asked return for another visit for a 1-leg warm water immersion visit (Visit 6). Without any pilot data, our plan is that half of the subjects will be asked to participate in the single leg immersion visit. Prior participants and future participants who seem like they would be amenable to participating in another visit will be asked first. After obtaining and analyzing this data, we may perform the single leg immersion visit on all subjects, or remove this visit for future participants.

We hypothesize that after one warm bath, the walking distance/time with Gardner protocol in PAD patient will be longer than without the warm bath. We further hypothesize that after the warm bath the BP response (i.e. changes) to the walking will be lower than during the walking at the same stage/distance without the warm bath. We also hypothesize that a neutral temperature bath will not induce the similar effects, while the whole body heat exposure with the heating suit will induce the effects seen with the heat treatments.

7.2 Study Procedures

Hemodynamic parameters

Heart rate will be continuously monitored using EKG patch electrodes and monitor (Cardiocap, Datex-Ohmeda, GE Healthcare). Blood pressure will be monitored using an automatic, non-invasive arm cuff (SureSigns VS3, Philips, Philips Medical Systems), and on the finger with a Finometer (Finapres Medical Systems). Baseline Finometer BP will be adjusted to match the arm cuff BP. A pneumograph belt will be placed around the abdomen to measure respiratory movement.

Ankle-Brachial Index: An Ankle-Brachial Index (ABI) measurement will be performed by inflating a BP cuff on an arm or leg and measuring the BP at which the pulse signal is audible (by Doppler). ABI index is the ratio of the ankle/higher brachial systolic pressure. PAD patients will have an ABI of <0.9. This will be performed at the start of each visit for all PAD patients and healthy subjects.

Echocardiographic measurements

Traditional echocardiographic and tissue Doppler imaging ultrasound techniques (GE Vivid 7 or iE33, Philips Medical, Bothell, WA) will be used to measure echocardiographic data, including stroke volume (SV), Cardiac Output (CO) and Ejection Fraction (EF), and right and left ventricular function.

Mean skin temperature:
Mean skin temperature (Tsk) will be measured from the weighted average temperature from thermocouples placed at 6 sites (chest, abdomen, upper back, lower back, thigh and calf) on the skin (30).

Local skin temperature:
Local skin temperatures will be monitored from thermocouples placed on the exercising and non-exercising limbs.

Internal temperature:
The internal temperature (Tcore) will be measured from the stomach/intestines via a telemetry pill swallowed by subjects (24). If the subject has a pacemaker/defibrillator or cannot take the pill, the internal temperature will be obtained from a thermocouple placed sublingually. Body temperature will be also measured using a tympanic or temporal thermometer. We have experience on using these methods to monitor internal temperature in healthy subjects and patients (e.g. heart failure) (7, 8).

Laser-Doppler flowmetry and skin temperature
Skin blood flow and skin temperature will be monitored non-invasively by laser-Doppler flowmetry and thermistors using special probes taped to the skin on both arms and legs (up to 8 probes mounted on a round disk). This disk may be moved to several locations on the arms and legs and the measurements will be recorded. The precise positions of the disk will be marked with a marker and possibly photos of just the disk placement. The disk holding the probes will be removed during the intervention (e.g. water bath or walking on the treadmill), and will be replaced back on the exact same positions after the intervention so the post measurements can be recorded.

Sweat rate
Sweat rate will be measured by capacitance hygrometry. A ventilated capsule (surface area = 2 cm^2) is attached to the skin. Nitrogen is passed through the capsule at a rate of 150 - 500 $ml \bullet min^{-1}$. Water from the sweat on the surface of the skin under the capsule evaporates into the nitrogen gas. Humidity of the effluent gas is measured via a highly sensitive capacitance hygrometer (Vaisalva). Sweat rate is calculated online via the data acquisition computer from the nitrogen flow rate and effluent gas humidity.

Near infrared spectroscopy (NIRS) system
This system is designed specifically for non-invasive measurements of muscle tissue. The system measures the tissue saturation of the investigated muscle, called the 'tissue saturation index' (TSI). In addition to TSI, the system also measures oxygenation changes in terms of oxy-hemoglobin, deoxy-hemoglobin, and total hemoglobin, which is an indication of the blood volume in the muscle.

Treadmill walking:
This test will be performed on PAD patients and the healthy subjects using the Gardner protocol (17) that begins at 2 mph and 0% grade for 2 minutes. The grade increases by 2% every 2 minutes (the speed remains 2 mph) until the PAD patient says they want to stop. They will tell or point to their exertion and pain rating on a chart every 1-2 minutes. BP will also be measured with an automated arm cuff every 2 minutes. During walking, the subjects will be allowed to gently hold onto the handrails but will be discouraged from leaning on the rails or squeezing their hands tightly.

The primary outcome measurements in this study are claudication onset time (i.e., when the subject first notices leg discomfort which is a “1” on the 0-4 discomfort scale) and peak walking time (i.e., the time it takes for the leg pain or discomfort to become too severe to continue which is a “4” on the 0-4 discomfort scale or a “10” on the 0-10 pain scale). According to the ACSM guidelines for exercise testing, 0=no discomfort, 1=onset of discomfort, 2=moderate discomfort, 3=severe discomfort but able to keep walking, 4=maximum discomfort and stop walking immediately. The current data in our lab show that the

mean walking time in PAD patients is ~ 8 min, although some patients could walk for up to 22 minutes maximum.

The healthy subjects will also perform the Gardner protocol. However, the final walking time/stage will be up to 22 minutes or until fatigue.

Warm bath/ neutral temperature bath

Before the bath, resting BP and HR will be recorded while the subjects rest in a chair. Then the subjects will sit in a warm bathtub with water up to their shoulders, while one arm will be out of water for BP and HR measurement. The water temperature will be measured and recorded using a Supco SL500XT temperature data logger throughout the bath. The internal temperature (Tcore) will be monitored via an ingestible pill telemetry system. BP using an automatic arm cuff set at a 3 min interval, and beat-to-beat BP and HR with a Finometer will be monitored and recorded thorough out the bath.

The initial water temperature will be start around 38-40°C and after the subject gets into the tub, warm water will be mixed in until the temperature reaches 41°C. During the bath, if the water temperature drops below 39°C, then warm water will be added to the tub until the temperature reaches 41°C. Subjects will remain in the tub for ≤ 30 minutes. If the HR increases more than 15 -20 bpm, or a significant BP drop occurs (e.g. ∆BP >20 mmHg), the subject will be removed from the bath even if the time is <30 min. We will ask subjects to avoid sudden standing after each bath and assist them to change position slowly as needed.

During the neutral temperature bath, the water temperature will remain at 35 - 37 °C throughout the 30 minute bath.

Lower limbs warm water immersion

Subjects will sit on a chair and put their lower legs and feet in a bucket with warm water. The water level will be just below the knees (depth of approximately 40 cm). The water temperature will be monitored and controlled at ~38 - 42 °C throughout the 30 minutes. BP, HR, Tcore, the skin temperature and skin blood flow (e.g. on a thigh and/or forearm) will be measured before, during and after the lower limbs warm water immersion. In some subjects, the water immersion may be performed on only one leg in a separate visit. The water volume in the lower leg immersion bath will contain less volume than the full bathtub and therefore the temperature may be more difficult to maintain. Therefore, we estimate needing a larger temperature range for the lower leg immersion bath. The maximum temperature of 42° for the lower limb bath is safe and has been used in prior studies.

Whole-body heating with suits

As described in our previous studies (7-9), the mean skin temperature Tsk will be controlled with a water-perfused suit worn by subjects. Whole-body heating will be accomplished by perfusing warm water (~ 45 – 50 ℃) through the suit to elevate the Tsk to ≤ 38 °C (100 ^{o}F). Warm water is through plastic tubes, and subjects' skin will not directly touch warm water. The heating will be continued until the ΔTcore reaches the same temperature seen during the 30 min warm bath (~ 0. 6 – 1.0 ℃). This may take approximately 60 - 90 min. We have experience using these methods to control body temperature in healthy subjects and heart failure patients (7, 8).

Consent for Research Screening Procedure

Subjects will be contacted by phone or email using the Consent for Research Screening Procedure. Subjects do not need to be fasted for this study, but are asked to refrain from caffeine for 12 hours and refrain from alcohol, recreational and/or medicinal marijuana, over-the-counter medications and extreme exercise for 24 hours prior to a visit. PAD patients will be asked to take their medications according to their regular schedule, but at a similar time on the days of study visits (e.g. ~ 7:00 am).

7.2.1 Screening visit and Visit 1: Walking ability without heat treatment

The screening tests may be performed at the beginning of the first visit. The screening visit will involve obtaining written informed consent, a brief history and physical examination. A resting 12-lead EKG will be conducted in PAD patients and may be conducted in healthy subjects. ABI will be measured. Female subjects of childbearing age will have a urine pregnancy test performed to ensure that they are not pregnant. An Ankle/Brachial Index (ABI) will be determined on both sides. If the investigators determine that the subject meets the enrollment criteria for this study, the subjects will be invited to continue the study and participate in activities of the first visit.

PAD patients will walk on a treadmill using the Gardner protocol until the patient says they want to stop. For the healthy subjects, the final walking time/stage will be up to 22 minutes or until fatigue. BP, HR and NIRS will be continuously measured during Gardner protocol. Skin blood flow, skin temperature and BP will be measured in the supine position before and after the Gardner protocol.

7.2.2 Visit 2: Walking ability after a warm bath.

Subjects will take a warm bath as described above in an HMC personal care room. BP, HR, Tcore will be measured during the bath. After the end of the bath, all subjects will be transported using a wheelchair from the HMC personal care room to the laboratory in the CRC. This is to reduce any possible influence of walking from the HMC personal care room to the laboratory on the results of the Gardner protocol. Then, subjects may have a break, and then the instrumentation for Gardner protocol will be placed. Approximately 40 – 60 min after the end of the bath, the Gardner protocol from Visit 1 will be repeated. Skin blood flow, skin temperature, ABI and BP will be measured in the supine position before and after the bath or Gardner protocol.

7.2.3 Visit 3: Walking ability after a neutral temperature bath.

Subjects will take a neutral temperature bath as described above in an HMC personal care room. BP, HR, Tcore will be measured during the bath. After the end of the bath, all subjects will be transported using a wheelchair from the HMC personal care room to the laboratory in the CRC. Approximately 40 – 60 min after the end of the bath, the Gardner protocol from Visit 1 will be repeated. Skin blood flow, skin temperature, ABI and BP will be measured in the supine position before and after the bath or Gardner protocol.

7.2.4 Visit 4: Walking ability after whole body heating with suit.

Whole body heating with the suit will be performed until the increase in ΔTcore is similar with that seen during warm bath. BP, HR, Tcore, Tsk, skin blood flow and sweat rate will be measured during the whole body heating. Approximately 40 – 60 min after the end of the bath, the procedures in Visit 1 will be repeated. Skin blood flow, skin temperature, ABI and BP will be measured in the supine position before and after the whole body heating or Gardner protocol.

7.2.5 Visit 5: Walking ability after lower limbs warm water immersion.

Lower limbs warm water immersion will be performed. BP, HR, Tcore, the skin temperature and skin blood flow will be measured before, during and after the lower limbs warm water immersion. Approximately 40 – 60 min after the end of the lower limbs water immersion, the Gardner protocol from Visit 1 will be repeated. Skin blood flow, skin temperature, ABI and BP will be measured in the supine position before and after the lower limbs water immersion or Gardner protocol.

In some subjects, this visit may be repeated with the water immersion on only one lower limb (e.g. the most affected leg in PAD patients) in a separate visit (Visit 6). Initially, only about half of the subjects will be asked to participate in this additional (optional) visit. Once the data are analyzed, we will either add the visit for all participants or remove this visit for future participants.

7.3 Duration of Participation

Each of visits may take about 2-3 hours.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

Total subjects = 39 (20 PAD patients and 19 healthy control subjects). Previous participants will be the first to be presented with the opportunity to participate in the lower limb immersion visits, so no additional subjects are requested at this time.

8.2 Sample size determination

This is a pilot study, and we have no prior data using heat treatment in PAD patients. In the publication by Julious, it is stated that a sample size of 12 is a good rule of thumb for a pilot study (20). We anticipate that many subjects will return for all 5-6 visits, but predict a least a 30% dropout. Therefore, we plan to recruit 16 unique PAD patients and 16 unique healthy control subjects for the studies to see if there is an effect and variability worthy of pursing further. The effects of the pandemic on recruitment and research limited the number of subjects visits for this study. As a result, we have incomplete data sets from several participants. Since we cannot bring back all of these participants for the additional visits because of changes in health status, we request to increase the number of subjects from 32 to 39.

8.3 Statistical methods

First, graphical displays of the mean values with standard deviation of the variables will be constructed to visually assess the results of the pilot data. Later, analysis of variance may be used to determine statistical significance, and its criterion level will be 0.05. One-way repeated measures ANOVA, two-way ANOVA or two-way repeated measures ANOVA will be used. The interaction between the two independent variables will be the key test. Post hoc analysis will use a Tukey test.

A one-way repeated measures ANOVA will be used to compare the walking time in PAD patients within the visits. A two-way ANOVA will be used to compare the BP and HR (i.e. absolute values or the changes from the resting baseline values) between each stage of the Gardner protocol (factor 1) and between the subject groups (factor 2).

9.0 Confidentiality, Privacy and Data Management

See the Research Data Plan Review Form

10.0 Data and Safety Monitoring Plan

10.1 Periodic evaluation of data

Safety is constantly monitored during a study and any concerns are immediately reviewed by the PI. Adverse events are not anticipated, but any occurring will be documented and reported according to HSPO policies and procedures. The PI will be solely responsible for data collection and verification, and review of cumulative adverse events.

10.2 Data that are reviewed

A research nurse or technician contacts the patient 24 hours after each study visit to check on their well-being and answer any questions. Any concerns are brought to the attention of the PI, and study physician immediately.

10.3 Method of collection of safety information

Paper documentation of the contact with the subject (listed above) is included in the subject's chart.

10.4 Frequency of data collection

Data collection starts at the screening visit and continues through the testing visit. Safety is constantly monitored during every visit and documented in the subject's chart.

10.5 Individuals reviewing the data

The research nurses and the PI will review the data.

10.6 Frequency of review of cumulative data

Subject data are analyzed by the PI after the testing visit. Summary data are reviewed after every few subjects to insure the protocol and measurements are appropriate. Safety is constantly monitored during visits and any concerns are brought to the attention of the PI immediately. We do not wait for a cumulative review to assess safety.

10.7 Statistical tests

Statistical tests are performed to determine significant differences (see Section 8.3). We do feel we need to run statistics to determine safety because any occurrences will be evaluated immediately on a case-by-case basis.

10.8 Suspension of research

If a serious adverse event occurred, we would assess the need to suspend the research.

11.0 Risks

Confidentiality: There is a risk of loss of confidentiality if medical information or identity are obtained by someone other than the investigators, but precautions will be taken to prevent this from occurring.

It is important that a fetus not be exposed to any unnecessary risks; therefore, pregnant women will not be studied. A pregnancy test will be administered at the beginning of all experimental visits to all women of childbearing potential to ensure that they are not pregnant.

There are no risks to refraining from caffeine, alcohol, recreational and or medicinal marijuana or exercise before a visit.

The risk of holding over-the-counter medication would be pain, if you are holding pain medication, or allergy symptoms if you are holding allergy medication. If there are any concerns about holding over-the-counter medications, we will discuss them with the study physician.

The placement of EKG electrodes may be involved with insignificant risk of skin irritation. There are no risks or discomforts associated with measuring blood pressure, heart rate, pneumograph, skin temperature, skin blood flow, muscle oxygenation using NIRS or sweat rate.

Internal temperature will be monitored throughout the experiment. Subjects should excrete the telemetry pill within 24-72 hours, and it is advised that they do not have any MRI procedures within 72 hours of swallowing this pill due to the metal contained in it. The internal temperature of patients with a pacemaker or defibrillator will be assessed via sublingual thermocouple.

The water temperature for warm bath will be under 41°C, and the duration will be $\leq$ 30 minutes per day. A hot tub bath was employed in previous studies [37.8-41.0˚C] (1, 5, 18, 25) and is safe. Whole body heating will increase body temperature, evoke sweating, induce peripheral vasodilation and increase HR. BP may (not always) drop slightly in some subjects during heating. The water temperature will be controlled, and the subject's body temperature, BP and HR will be monitored during the bath. We will ask subjects to avoid sudden standing after the bath. We have extensive experience on whole body heating studies (7, 9-14).

The maximum temperature of 42° for the lower limb immersion warm bath is safe and has been used in prior studies (33).

Whole body heating experiment with suits will be terminated if internal temperature exceeds 39.5 °C (103°F). Temperatures below this level are well tolerated by subjects and pose no risk to subjects.

Treadmill walking using Gardner protocol has a minimal risk of temporary lightheadedness, fainting, chest discomfort, leg cramps, occasional irregular heartbeats, shortness of breath or abnormal BP response. There should be no additional risk for healthy subjects participating in a maximum of 22 minutes of treadmill exercise.

12.0 Potential Benefits to Subjects and Others

12.1 Potential Benefits to Subjects

There are no benefits to the subjects

12.2 Potential Benefits to Others

Research scientists may better understand how autonomic control is altered in the PAD patients during rest and exercise. Importantly, the goal of this project is to examine an intervention that may improve conditions of PAD patients. If successful, the studies may suggest a potential therapeutic intervention and some related mechanisms. On the other hand, the data may provide insight regarding the effects of temperature on cardiovascular control during exercise in PAD, an important issue in physiology

13.0 Sharing Results with Subjects

There is a rare risk of an incidental finding. The testing performed during this study is intended solely for research purposes and will not be utilized to detect any medical condition. However, if we notice something unusual, we will consult one of the cardiologists on the research team to determine if it merits follow-up. If so, we will contact the subject within 48 hours and suggest that they follow up with their private medical provider. To facilitate follow-up care, a copy of the data may be provided upon request.

14.0 Subject Stipend (Compensation) and/or Travel Reimbursements

Subjects will receive $25 per hour as compensation for the study on each visit. They will need to provide their Social Security number and address and will receive payment with a Greenphire ClinCard.

15.0 Economic Burden to Subjects

15.1 Costs

The patient is not responsible for any charges incurred as a result of the study.

15.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

16.0 Resources Available

16.1 Facilities and locations

The research will be performed in the Clinical Research Center (CRC) at the Penn State Hershey Medical Center.

16.2 Feasibility of recruiting the required number of subjects

It may take some time to recruit enough PAD patients.

16.3 PI Time devoted to conducting the research

The PI has sufficient time to devote to this study.

16.4 Availability of medical or psychological resources

The studies being performed in the CRC have access to crash cart and other medical staff to assist in the event of an emergency. We also have the HMC ED if necessary.

16.5 Process for informing Study Team

The PI will review the protocol with all members of the research team and will be present during in the room during all study visits.

17.0 Other Approvals

17.1 Other Approvals from External Entities

Not applicable

17.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – Hershey only – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of HRP-902 - Human Tissue For Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ Animal Care and Use – All campuses – Human research involves animals and humans or the use of human tissues in animals

☐ Biosafety – All campuses – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☐ Clinical Laboratories – Hershey only – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes, but are no longer needed for clinical use. Upload a copy of HRP-901 - Human Body Fluids for Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☒ Clinical Research Center (CRC) Advisory Committee – All campuses – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – All campuses – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – Hershey only – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of HRP-903 - Radiation Review Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ IND/IDE Audit – All campuses – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☒ Scientific Review – Hershey only – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Hershey Cancer Institute Scientific Review Committee is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website at: http://www.pennstatehershey.org/web/irb/home/resources/investigator

18.0 Multi-Site Research

Not applicable

19.0 Adverse Event Reporting

19.1 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

20.0 Study Monitoring, Auditing and Inspecting

20.1 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the Penn State quality assurance program office(s), IRB, the sponsor, and government regulatory bodies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

21.0 Future Undetermined Research: Data and Specimen Banking

Not applicable

22.0 References

1. **Allison TG, Miller TD, Squires RW, and Gau GT**. Cardiovascular responses to immersion in a hot tub in comparison with exercise in male subjects with coronary artery disease. *Mayo Clin Proc* 68: 19-25, 1993.

2. **Baccelli G, Reggiani P, Mattioli A, Corbellini E, Garducci S, and Catalano M**. The exercise pressor reflex and changes in radial arterial pressure and heart rate during walking in patients with arteriosclerosis obliterans. *Angiology* 50: 361-374, 1999.

3. **Bakke EF, Hisdal J, Jorgensen JJ, Kroese A, and Stranden E**. Blood pressure in patients with intermittent claudication increases continuously during walking. *Eur J Vasc Endovasc Surg* 33: 20-25, 2007.

4. **Berger HJ, Johnstone DE, Sands JM, Gottschalk A, and Zaret BL**. Response of right ventricular ejection fraction to upright bicycle exercise in coronary artery disease. *Circulation* 60: 1292-1300, 1979.

5. **Boone T, Westendorf T, and Ayres P**. Cardiovascular responses to a hot tub bath. *J Altern Complement Med* 5: 301-304, 1999.

6. **Brothers RM, Bhella PS, Shibata S, Wingo JE, Levine BD, and Crandall CG**. Cardiac systolic and diastolic function during whole body heat stress. *American journal of physiology Heart and circulatory physiology* 296: H1150-H1156, 2009.

7. **Cui J, Arbab-Zadeh A, Prasad A, Durand S, Levine BD, and Crandall CG**. Effects of heat stress on thermoregulatory responses in congestive heart failure patients. *Circulation* 112: 2286-2292, 2005.

8. **Cui J, Boehmer JP, Blaha C, Lucking R, Kunselman AR, and Sinoway LI**. Chronic heart failure does not attenuate the total activity of sympathetic outflow to skin during whole-body heating. *Circ Heart Fail* 6: 271-278, 2013.

9. **Cui J, Shibasaki M, Davis SL, Low DA, Keller DM, and Crandall CG**. Whole body heat stress attenuates baroreflex control of muscle sympathetic nerve activity during postexercise muscle ischemia. *Journal of applied physiology* 106: 1125-1131, 2009.

10. **Cui J, Wilson TE, and Crandall CG**. Baroreflex modulation of sympathetic nerve activity to muscle in heat-stressed humans. *Am J Physiol Regul Integr Comp Physiol* 282: R252-R258, 2002.

11. **Cui J, Wilson TE, and Crandall CG**. Muscle sympathetic nerve activity during lower body negative pressure is accentuated in heat-stressed humans. *Journal of applied physiology* 96: 2103-2108, 2004.

12. **Cui J, Wilson TE, and Crandall CG**. Orthostatic challenge does not alter skin sympathetic nerve activity in heat-stressed humans. *Auton Neurosci* 116: 54-61, 2004.
13. **Cui J, Wilson TE, and Crandall CG**. Phenylephrine-induced elevations in arterial blood pressure are attenuated in heat-stressed humans. *Am J Physiol Regul Integr Comp Physiol* 283: R1221-R1226, 2002.
14. **Cui J, Zhang R, Wilson TE, and Crandall CG**. Spectral analysis of muscle sympathetic nerve activity in heat-stressed humans. *American journal of physiology Heart and circulatory physiology* 286: H1101-H1106, 2004.
15. **Drew RC, Muller MD, Blaha CA, Mast JL, Heffernan MJ, Estep LE, Cui J, Reed AB, and Sinoway LI**. Renal vasoconstriction is augmented during exercise in patients with peripheral arterial disease. *Physiol Rep* 1: e00154: 1-9, 2013.
16. **Ehsani AA, Ogawa T, Miller TR, Spina RJ, and Jilka SM**. Exercise training improves left ventricular systolic function in older men. *Circulation* 83: 96-103, 1991.
17. **Gardner AW, Skinner JS, Cantwell BW, and Smith LK**. Progressive vs single-stage treadmill tests for evaluation of claudication. *Med Sci Sports Exerc* 23: 402-408, 1991.
18. **Hooper PL**. Hot-tub therapy for type 2 diabetes mellitus. *N Engl J Med* 341: 924-925, 1999.
19. **Johnson JM, and Proppe DW**. Cardiovascular adjustments to heat stress. In: *Handbook of Physiology - Environmental Physiology*, edited by Fregly MJ, and Blatteis CM. New York: Oxford University Press, 1996, p. 215-243.
20. **Julious SA**. Sample size of 12 per group rule of thumb for a pilot study. *Pharm Stat* 4: 287-291, 2005.
21. **Lorentsen E**. Systemic arterial blood pressure during exercise in patients with atherosclerosis obliterans of the lower limbs. *Circulation* 46: 257-263, 1972.
22. **Minson CT, Wladkowski SL, Cardell AF, Pawelczyk JA, and Kenney WL**. Age alters the cardiovascular response to direct passive heating. *Journal of applied physiology* 84: 1323-1332, 1998.
23. **Muller MD, Drew RC, Blaha CA, Mast JL, Cui J, Reed AB, and Sinoway LI**. Oxidative stress contributes to the augmented exercise pressor reflex in peripheral arterial disease patients. *The Journal of physiology* 590: 6237-6246, 2012.
24. **O'Brien C, Hoyt RW, Buller MJ, Castellani JW, and Young AJ**. Telemetry pill measurement of core temperature in humans during active heating and cooling. *Med Sci Sports Exerc* 30: 468-472, 1998.
25. **Olah M, Koncz A, Feher J, Kalmanczhey J, Olah C, Nagy G, and Bender T**. The effect of balneotherapy on antioxidant, inflammatory, and metabolic indices in patients with cardiovascular risk factors (hypertension and obesity)--a randomised, controlled, follow-up study. *Contemp Clin Trials* 32: 793-801, 2011.
26. **Rowell LB**. Central circulatory adjustments to dynamic exercise. Chapter 7. In: *Human Cardiovascular Control*. New York: Oxford University Press, 1993, p. 255-301.
27. **Rowell LB**. Control of blood flow to dynamically active muscles. Chapter 5. In: *Human Cardiovascular Control*, edited by Rowell LB. New York: Oxford University Press, 1993, p. 162-203.
28. **Rowell LB**. Control of regional blood flow during dynamic exercise. Chapter 6. In: *Human Cardiovascular Control*, edited by Rowell LB. New York: Oxford University Press, 1993, p. 204-254.
29. **Shinsato T, Miyata M, Kubozono T, Ikeda Y, Fujita S, Kuwahata S, Akasaki Y, Hamasaki S, Fujiwara H, and Tei C**. Waon therapy mobilizes CD34+ cells and improves peripheral arterial disease. *J Cardiol* 56: 361-366, 2010.
30. **Taylor WF, Johnson JM, Kosiba WA, and Kwan CM**. Cutaneous vascular responses to isometric handgrip exercise. *Journal of applied physiology* 66: 1586-1592, 1989.
31. **Tei C, Horikiri Y, Park JC, Jeong JW, Chang KS, Toyama Y, and Tanaka N**. Acute hemodynamic improvement by thermal vasodilation in congestive heart failure. *Circulation* 91: 2582-2590, 1995.
32. **Wilson TE, Dyckman DJ, and Ray CA**. Determinants of skin sympathetic nerve responses to isometric exercise. *Journal of applied physiology* 100: 1043-1048, 2006.

33. Thomas KN, van Rij AM, Lucas SJ, and Cotter JD. Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls. *Am J Physiol Regul Integr Comp Physiol* 312: R281-R291, 2017.