NCT03557502

Heat Therapy Versus Exercise Training in Hypertension

Research Plan Document

Last Edited March 15, 2023

Heat Therapy versus Exercise Training in Hypertension

IRB # 05042018.006

RESEARCH SUMMARY

A. Introduction and Background

Protocol 1:

In the United States, hypertension (HTN) accounts for more cardiovascular disease (CVD) related deaths than any other modifiable CVD risk factor, second only to cigarette smoking as a preventable cause of death for any reason[1]. In fact, in 2010, high blood pressure was the leading cause of death and disability-adjusted life years worldwide[2,3]. According to the CDC, about 75 million American adults (32%) have high blood pressure, and about 1 in 3 American adults have pre-HTN. Based on very recently published new guidelines on the prevention and management of hypertension by the American College of Cardiology/American Heart Association Task Force on hypertension[4], the percent of people in the United States with HTN are even higher with the new classification. Lifestyle and exercise interventions are considered a first-line preventative against the development of HTN and in the treatment of diagnosed HTN. Unfortunately, the vast majority of people are reticent to initiate exercise training or have poor compliance. Additionally, some patient groups are not able to perform exercise due to a number of limitations or are not able to obtain the full benefits of exercise to lower CVD risk. Thus, alternative non-pharmacological options to lower blood pressure and improve CVD risk are critically needed. Heat therapy, in the form of hot bath or sauna, is an ancient practice that has recently regained attention in the prevention and treatment of CVD. A recent 20+ year prospective cohort study from Finland, in which sauna use is extremely common and part of the culture, has demonstrated that regular sauna use was associated with reduced risk of developing hypertension[5], which may have explained in part the reduced rates of all-cause cardiovascular deaths and reduced rates of Alzheimer's Disease and other memory-related disorders with regular sauna use previously reported in the larger cohort[6]. We recently demonstrated in young, normotensive sedentary subjects that heat therapy in the form of hot water immersion lowered resting blood pressure and greatly improved numerous biomarkers of cardiovascular health including endothelial function and arterial stiffness[7]. We have pilot data demonstrating that chronic heat therapy can improve blood pressure and numerous biomarkers of vascular function in people with HTN, potentially through a profound reduction in sympathetic neural outflow and improvements in arterial compliance. Furthermore, our preliminary data suggest improvements in blood pressure are greater than observed with the idealized standard of care, aerobic exercise training.

Protocol 2:

Protocol 2 will be performed within the experimental framework of Protocol 1 in a subset of study participants. Subjects are not able to undergo Protocol 2 without undergoing Protocol 1, as the heat therapy and exercise interventions are in Protocol 1. Subjects enrolled in Protocol 1 may elect to not participate in Protocol 2.

Aging is associated with a gradual decline in cognitive function, which can affect quality of life and predisposes individuals to age-related neurodegenerative diseases, including Alzheimer's disease and its related dementias (ADRD). As the population of middle-aged and older adults is increasing rapidly, there is increasing need to understand the mechanisms of cognitive aging. Aging exerts direct effects on the heart that increase our susceptibility of developing CVD[8], including HTN. However, the largest portion of the marked increase in CVD risk with advancing age is driven by two highly interactive factors: increases in systolic arterial blood pressure (SBP) and vascular dysfunction[9]. These events are

responsible for many of the changes observed with aging, are key risk factors for other major clinical disorders, and are increasingly implicated in cognitive declines with aging and risk of ADRD. Importantly, it has been reported that the frequency of sauna bathing was associated with lowered risks of dementia and Alzheimer's disease[10]. The results of these studies suggest that heat therapy may reduce the risk of hypertension and dementia either independently or through reductions in systolic blood pressure but are confounded by other factors such as greater sauna use by individuals with higher income, education and discretionary time. The effects of heat therapy on cardiovascular health and cognitive function are best determined by randomized clinical research trials. Notably, women were not included in previous studies, and we have substantial preliminary data demonstrating that women are showing all of the same benefits of heat therapy as men. Heat therapy represents a possible lifestyle intervention that, as our preliminary findings suggest, may be more effective in lowering systolic blood pressure and reducing arterial stiffness than aerobic exercise training, which is known to slow/delay the development of normal cognitive aging. Therefore, the purpose of Protocol 2 is to determine the potential of heat therapy in middle-life individuals with hypertension to improve cognitive function and MRI-based markers of ADRD risk.

B. Specific Aims/Study Objectives

Protocol 1:

The overarching goal of this research is to perform a clinical trial to determine whether heat therapy improves blood pressure and vascular health in HTN.

Specific Aim 1: Utilizing a prospective randomized clinical trial study design, we will determine whether heat therapy improves blood pressure (both in clinic and ambulatory) better than dynamic aerobic exercise, the gold-standard non-pharmacological treatment of essential HTN. Specifically, we hypothesize that 30 sessions of passive heat therapy using hot water immersion over 8-10 weeks will improve blood pressure in HTN individuals to a greater extent than 30 sessions of exercise training over the same time period.

Specific Aim 2: We will determine whether heat therapy will improve biomarkers of arterial health and compare responses to exercise training. We hypothesize that 30 sessions of heat therapy will improve arterial stiffness in HTN to a greater extent than exercise. Furthermore, we hypothesize key clinical biomarkers of systemic inflammation and oxidative stress will be improved following heat therapy.

Specific Aim 3: In an exploratory aim, we will determine whether the acute decreases in blood pressure following an initial heat therapy session or exercise peak test predict the sustained reduction in resting blood pressures following heat therapy treatment or exercise training in HTN. These findings would provide significant predictive information beyond baseline resting blood pressures. We hypothesize that the reduction in blood pressure following acute stressors will be predictive of treatment effects on resting blood pressure.

Protocol 2:

The overarching goal of this research is to determine whether 30 sessions of heat therapy or exercise training (performed by subjects in Protocol 1) improves cognitive function (using the NIH Toolbox: http://www.nihtoolbox.org) and MRI-based markers of aging and Alzheimer's risk (using specific MRI-based biomarkers and tests).

Subjects enrolled in Protocol 1 will be given the opportunity to participate in an additional protocol in which they will undergo biomarker testing for risk of ADRD before and after heat therapy or exercise training. Subjects will not be able to enroll in Protocol 2 without enrolling and participating in Protocol 1; this is required as Protocol 2 only adds some additional measurements for cognitive function and ADRD risk performed before and following the heat therapy and exercise interventions detailed in Protocol 1. Protocol 2 will include completing the NIH Toolbox for cognitive function, Pittsburgh Sleep Quality Index (PSQI), and MRI scans at the Lewis Center for Neuroimaging (LCNI).

Specific Aim 1: These studies in mid-life adults will determine if heat therapy can be used to improve cognitive aging and dementia risk, with potentially important clinical implications for the prevention of ADRD.

Demonstrating that heat therapy can be used as a novel treatment for essential hypertension is timely and important as there is a clear need for alternatives to exercise training and pharmacological approaches. As HTN is linked to ADRD risk, these studies will allow us to link outcome measures in Protocol 1 (focused on CVD risk) with Protocol 2 (focused on ADRD risk) before and after subjects undergo heat therapy or exercise intervention in Protocol 1. The proposed studies are innovative and include an intention-to-treat randomized clinical trial and detailed high-resolution phenotyping to better understand the improvements in blood pressure regulation following heat therapy. *Overall, this work advances a novel treatment option for those with HTN and in the prevention of CVD and ADRD.*

No devices are being studied for safety or effectiveness. Instruments are used for data collection purposes only.

C. Methods, Materials and Analysis

Protocol 1:

We will address our aims in a randomized clinical trial comparing the effects of heat therapy versus aerobic exercise training. This is a well-controlled, clinically relevant approach, as we will be comparing our novel heat therapy intervention directly to the current idealized standard of care, aerobic exercise. We will specifically study individuals with Elevated Blood Pressure and Stage 1 or 2 HTN, in the absence of cardiovascular events such as heart attacks or strokes, diabetes, or chronic kidney disease, as current guidelines[4] do not mandate prescribing medications to these individuals. They are the ideal target group for the proposed intervention, as it has the potential to obviate the need for life-long pharmacological therapy.

RECRUIT · Inactive to moderately active men and women with HTN
PRE-SCREEN · Pre-screen consent and questions
SCREEN · Day 1: Protocol 1 consent process, medical history, height, weight, blood pressure (x2), finger stick blood sample · Day 2: Protocol 2 consent process, blood pressure (x2)
PRE-TESTS · Hemodynamic tests · Biomarkers · 24-h Ambulatory blood pressure · 24-h Urine collection · VO_2Peak Test
ASSIGN · Randomized to intervention group
INTERVENTION · First 15 sessions
INTERIM-TESTS · Hemodynamic tests · 24-h Ambulatory blood pressure · 24-h Urine collection · Biomarkers
INTERVENTION · Last 15 sessions

General workflow for the protocol. The table at the right depicts the workflow for this clinical trial protocol, which is as follows:

POST-TESTS
· Hemodynamic tests
· Biomarkers
· 24-h Ambulatory blood pressure
· 24-h Urine collection
· VO_2Peak Test

RECRUIT: The trial will be conducted in a cohort of physically inactive to moderately active men and women with high blood pressure (Elevated and Stage 1 or 2 Hypertension, based on current guidelines)[4] from the local Eugene and Springfield, OR communities (details under section D). Potential subjects will be pre-screened by phone to determine eligibility.

PRE-SCREEN: Candidates will complete a pre-screening consent and answer pre-screening questions online and over the phone. If candidates appear to qualify, they will be further screened for eligibility over the phone using the IPAQ.

SCREEN: Subjects will need to come to the laboratory on two separate occasions as part of the initial screening process. Seated blood pressure will be measured on two separate screening visits to determine resting blood pressure. During each visit, blood pressure will be measured twice, performed according to clinical guidelines for assessing hypertension[4]. The average blood pressure of these four readings obtained over two assessments must fall within the inclusion criteria range to qualify for inclusion in the study. If the average blood pressure of these four readings (two obtained during Screening Day 1 and two obtained during Screening Day 2) does not fall within the allowable limits for this study, the subject will be excluded from the study. During Screening Day 1, the informed consent process will be completed for Protocol 1. We will also assess medical history, BMI, fasting glucose, hemoglobin A1c, and lipid profile to verify eligibility. If the subject is interested in participating in Protocol 2, the informed consent process for Protocol 2 will be completed on Screening Day 2 and the subject will be pre-screened using the MRI PRE-SCREENING FORM, LCNI MRI SAFETY SCREENING QUESTIONNAIRE and MMSE. Subjects who undergo screening and satisfy all inclusion criteria and no exclusion criteria will be enrolled in the study and progress to a battery of pre-intervention tests (PRE-TESTS).

PRE-TESTS: Before and after the INTERVENTION, we will conduct high-resolution phenotyping of subjects which will include: lab-based hemodynamic tests of vascular function; blood-based biomarkers related to cardiovascular health; 24-h urine collection; 24-h food/beverage log; 24-h ambulatory blood pressure recording; VO_2peak fitness test. These are detailed below *Specific Experimental Approaches*.

ASSIGN: Subjects will be randomized to either the heat therapy group or exercise training group, then undergo 30 sessions of the specific intervention at a rate of 3-4 per week over an 8 to 10-week period. Randomization will be stratified by age and sex to ensure equal numbers within each stratum. Further stratification, for example by BMI or initial blood pressure, would be difficult given the small sample size. (More details of the randomization process are under *Randomization*). After the first 15 sessions, subjects will be re-tested for hemodynamic and biomarker adaptations to the intervention (INTERIM-TEST). After 30 sessions, subjects will undergo final POST-TESTS.

INTERVENTION: Subjects in the **heat therapy** group will report to the lab to undergo heat therapy sessions 3-4 times per week, with 45 minutes of hot water immersion per session. The goal of heat therapy is to maintain core temperature above 38.5°C for at least 30 min. This target has previously been shown to be the threshold temperature and time exposure to optimize adaptations to passive heat exposure[11]. Subjects in our lab, including sedentary, hypertensive, obese, and spinal cord-injured subjects, have tolerated this heating protocol very well. Subjects will be checked for euhydration prior to entering the tub and will be monitored closely throughout heating for heart rate and blood pressure during all sessions. In addition, core temperature ($T_{core,}$; FDA approved ingestible core temp sensing pill; Accutemp Temperature Monitoring System by HQ Inc.) will be monitored during the first 5 and final sessions whereas aural temperature may be monitored in all sessions. Subjects will be removed from the hot bath if T_{core} exceeds 39.5°C. Water immersion is the preferred method of heating since it is capable of increasing core temperature and heart rate at a rate similar to moderate-intensity exercise[12]. Subjects in the **exercise training** group will report to the lab for a supervised exercise training session 3-4 times per week. We will use heart rate

responses determined during baseline $VO_{2\ peak}$ testing to assign workloads during each session. Exercise will consist of a 5-minute warm-up of seated upright cycling at 30% of the subject's $VO_{2\ peak}$, followed by 40 minutes of cycling at 60% $VO_{2\ peak}$, then a 5-minute cool down at 30%. In addition, core temperature will be monitored during the first and final sessions. On the 1st heat therapy or exercise training session, the subject will lay supine for 20 min of rest followed by three measures of blood pressure before the heat therapy or exercise training session. At the end of this 1st session, the subject will lay down for 1 hour of recovery, during which, we will continue to monitor core temperature, heart rate, and blood pressure every 5 min.

NOTE: Subjects with contraindications for the ingestible core temp sensing pill will be encouraged to use a self-inserted rectal thermistor. If subject is contraindicated for the core temp pill and the rectal thermistor, their temperature will be monitored with a digital aural or oral thermometer.

INTERIM-TESTS: After half the intervention sessions have been completed, we will repeat our assessment of hemodynamic tests, 24-h urine collection and food/beverage log, 24-h ambulatory blood pressure and sleep/wake/activity log, and blood-based biomarkers related to cardiovascular health.

POST-TESTS: After the INTERVENTION, we will repeat high-resolution phenotyping of subjects which will include all the measures from the PRE-TESTS.

The following table summarizes the time commitment for participants

Visit	Activities	Time commitment
Visit 1	Screening Visit 1	1 hour
Visit 2	Screening Visit 2	1 hour
Visit 3	Pre-Test Visit 1. Pick up 24-hour monitoring supplies	30 minutes
At home	Pre-24-hour urine collection + food/beverage log and 24-hour BP + sleep/wake/activity log	2½ hours
Visit 4	Pre-Test Visit 2. Return 24-hour monitoring supplies/samples to lab + blood draw	30 minutes
Visit 5	Pre-Test Visit 3. Vascular studies + Exercise testing	3 hours
Visit 6	1st Heat Therapy or Exercise Training Session	2 ½ hours
Visits 7-20	14 Heat Therapy or Exercise Training Sessions	1 hour each session
Visit 21	Interim-Test Visit 1. Pick up 24-hour monitoring supplies	30 minutes
At home	Interim-24-hour urine collection + food/beverage log and 24-hour BP + sleep/wake/activity log	2½ hours
Visit 22	Interim-Test Visit 2. Return 24-hour monitoring supplies/samples to lab + blood draw	30 minutes
Visit 23	Interim-Test Visit 3. Vascular studies	2 hours
Visits 24-38	15 Heat Therapy or Exercise Training Sessions	1 hour each session
Visit 39	Post-Test Visit 1. Pick up 24-hour monitoring supplies	30 minutes
At home	Post-24-hour urine collection + food/beverage log and 24-hour BP + sleep/wake/activity log	2½ hours
Visit 40	Post-Test Visit 2. Return 24-hour monitoring supplies/samples to lab + blood draw	30 minutes
Visit 41	Post-Test Visit 3. Vascular studies + Exercise testing	3 hours
Totals	***Activities on 41 days over about 12 weeks***	***2 hours of screening; 9.5 hours of testing; 31 ½ hours of therapy or training; 9 hours sample collection/logging***

Subjects may elect to complete the Vascular Studies + Exercise Testing when they return the 24-hour monitoring supplies/samples to the lab (visit 4, 22, and 40). This would eliminate 3 visits to the lab (visit 5, 23, and 41). Blood draws occur immediately following the last void of the 24-hour urine collection before the vascular studies and exercise test during Pre-, Interim-, and Post-tests. Subjects may elect to complete the Vascular Studies and Exercise Testing on separate days. This would add 2 visits to the lab.

General Methodological Considerations. For the VASCULAR STUDIES, subjects will be studied in the morning after an overnight fast, with subjects having abstained from caffeine and medications or supplements (except oral contraceptives) for 12 h, alcohol for 24 h, and exercise or heat therapy for 48 h prior to testing. For the EXERCISE TESTING, subjects will abstain from food for 3 h, caffeine for 6 h, alcohol and medications or supplements (except oral contraceptives) for 12 h, and exercise or heat therapy for 24 h prior to testing. These abstentions are required before all PRE-TESTS, INTERIM-TESTS, and POST-TESTS. For the first HEAT THERAPY or TRAINING SESSION, subjects will abstain from food for 2 h, caffeine for 6 h, alcohol and medications or supplements (except oral contraceptives) for 12 h, and exercise or heat therapy for 12 h prior to testing. For the 24-H AMBULATORY BLOOD PRESSURE, subjects will abstain from prolonged periods in a motorized vehicle and exercise or heat stress.

All persons of childbearing potential will be required to have a negative urinary pregnancy test on the initial screening day and prior to all PRE-TESTS, INTERIM-TESTS, and POST-TESTS. In addition, a negative urinary pregnancy test will be required on the first session per week during the hot water immersion or exercise training sessions in all persons of childbearing potential. The volunteer will be asked to collect a sample of their urine in a urine specimen cup in a private restroom in the physiology lab. An investigator will test the urine by placing the cotton tip of the pregnancy test in the urine, then watch for the color changes associated with a negative (not pregnant) or positive (pregnant) test. These tests have been determined to have >99% accuracy. If the test is positive, the volunteer will not be allowed to participate and will be encouraged to see their physician or schedule a visit to the Student Health Center (if the subject is a UO student). Volunteers with a negative urine pregnancy result will be allowed to participate in the study. Further, persons who are pregnant, nursing, or desiring to become pregnant will be excluded (see section D).

Volunteers will complete a 3-page MEDICAL HISTORY FORM which includes personal identifying information for use in emergencies (see Emergency Procedures section below). On each study day that involves procedures which are considered greater than minimal risk, the subjects will be asked if the MEDICAL HISTORY FORM needs to be updated. If so, the original will be replaced with the updated form, and the original will be disposed of as described below. This document will be retained in a locked file cabinet in the physiology lab for up to a week after the end of a subject's participation in the project. One week after the volunteers complete the study, the MEDICAL HISTORY FORM will be placed in a locked confidential documents disposal bin which is emptied by a secure shredding service.

During the in-person screening, volunteers will complete the CORE TEMPERATURE PILL CONTRAINDICATIONS QUESTIANNAIRE to identify contraindications. If a contraindication is identified, rectal thermistors will be used instead of core temperature pills.

Randomization. As indicated above, subjects will be randomized to either the heat therapy group or exercise training group. While subjects will not be blind to their treatment group, preliminary analysis of data and data entry will be by a blinded researcher (i.e., this will be a partially blind/observer-blind trial). The randomization and blinding process will be coordinated by Dr. Jodi Lapidus (a biostatistician at OHSU who is supporting this project). In brief, the Research Coordinator will enter age, sex, and subject ID number into a web-enabled randomization database via REDCap and receive the randomized group assignment. As indicated above, randomization will be stratified by age and sex to ensure equal numbers within each stratum. Further stratification, for example by BMI or initial blood pressure, would be difficult given the small sample size.

Specific Experimental Approaches. The experiments combine our core reseach methods which are as follows:

Screening Activities. Potential subjects will be pre-screened by phone or a combination of online and by phone to determine eligibility. For those that are eligible following the pre-screening, they will be scheduled to come into the laboratory on two separate days for the screening process. On each of these visits, seated blood pressure will be measured to determine resting blood pressure, and in each visit, blood pressure will be measured twice, performed according to clinical guidelines for assessing hypertension[4]. The average blood pressure of these four readings obtained over two assessments must fall within the inclusion criteria range to qualify for inclusion in the study. During the first initial screening in the laboratory, we will obtain a small sample of blood (<1 mL) via finger stick using aseptic technique. From this sample we will measure fasting glucose, hemoglobin A1c, and lipid profile to verify eligibility. In addition, we will assess the subject's medical history, screen for core temperature pill contraindications, measure arm circumference, and perform measurements of height and weight to calculate BMI. Persons of childbearing potential will be required to have a negative urinary pregnancy test. Additionally, subjects who want to participate in Protocol 2 will be screened for MRI eligibility. Subjects who undergo the screenings and satisfy all inclusion criteria and no exclusion criteria will progress to a battery of pre-intervention tests (PRE-TESTS), to be carried out on three separate visits.

Hemodynamic Tests. To assess hemodynamic changes underlying adaptation to heat therapy and exercise training, we will measure resting heart rate, blood pressure, cardiac output, total peripheral resistance, and arterial stiffness as follows. These are all well-established methods in our research program[7–9].

Heart rate and **blood pressure** will be monitored by an automated auscultatory device with integrated 3-lead ECG.

Cardiac output will be estimated using an open-circuit acetylene washin method that has been validated in humans versus the direct Fick approach[15] and used extensively by the investigators[14]. Subjects will breathe a C_2H_2-He-O_2-N_2 gas mixture while breath-by-breath C_2H_2 and He uptake are measured by mass spectrometer.

Arterial stiffness indices. We will measure **pulse wave velocity**, as done previously[7]. Tonometry pressure tracings will be obtained from the common carotid and ipsilateral common femoral artery (all recordings will be performed on the left side of the body). Time delays between pressure tracings will be calculated using the upswing of the pressure tracing[22].

Pulse wave velocity[23] will be calculated as the direct distance between the probe and the thigh cuff divided by the time delay.

Biomarkers. Methodology for blood-based biomarkers is well-established in our research program. All venous blood samples will be collected into appropriate vacutainers, centrifuged, and serum/plasma separated. For purpose of screening, hemoglobin A1C, glucose, and lipids will be analyzed immediately in the investigator's lab (Afinion II, Cholestech LDX). All other samples will be stored at -80°C until delivery by courier to the Oregon Clinical and Translational Research Institute (OCTRI) at Oregon Health and Science University. OCTRI will analyze samples for fasted glucose and insulin (for the calculation of the homeostatic model assessment of insulin resistance (HOMA-IR). Samples collected for measurement of circulating biomarkers of cardiovascular health (IL-6, C-reactive protein, oxidized LDL, ET-1) and renal function (angiotensin II, plasma renin activity, aldosterone) will also be measured at OCTRI. A reduction in oxidized LDL will indicate reduced oxidative stress. Reduced C-reactive protein will indicate reduced systemic and vascular inflammation. HOMA-IR will be calculated using the available on-line calculator from the Oxford Center for Diabetes. Three 40ml blood draws will occur throughout the study immediately following the last void of the 24-hour urine collection during the Pre, Interim, and Post Tests.

24-hour Urine Collection. 24-hour urine collections are the gold standard for measuring basal kidney function (i.e., creatinine clearance, urinary albumin excretion, urinary sodium excretion). Subjects will be given two urine collection containers to take home. In our experience, subjects typically only need to use one container in a 24-hour period. Therefore, the second collection

container serves as a backup if needed. Additionally, subjects will be offered a specimen container pan to assist in collecting urine samples. Subjects will be instructed to completely void their bladder immediately prior to starting the 24-hour urine collection, after which they will be instructed that all subsequent urine voids are collected in the provided containers. Upon completion of the 24-hour collection, subject will return the urine collection containers to the lab where sample volume and specific gravity will be measured. A blood sample will be collected immediately following the last void of the 24-hour urine collection. Urine samples will be analyzed at a later time for osmolality and urine biomarkers.

Food/Beverage Log. We are not specifically controlling the subjects' dietary patterns with the exception of asking them to abstain from caffeine for 12 hours and alcohol for 24 hours prior to the start of the VASCULAR STUDY visits. However, because of the importance of sodium intake and kidney function, subjects will be instructed to document their food and beverage intakes for the 24-hours immediately preceding the pre-, interim-, and post-VASCULAR STUDY visits. These logs will be analyzed to determine total intakes for energy (kilocalories), fat, protein, carbohydrate, sugar, dietary fiber, sodium, caffeine, and fluid volume. Pre-, interim-, and post-VASCULAR STUDY intakes will be used to compare changes in 24-hour kidney function.

24-h Ambulatory Blood Pressure. Subjects will wear an ambulatory blood pressure monitor for 24 hours. Ambulatory pressure will be monitored using a commercially available unit set to record pressure every 20 minutes during the daytime and every hour at night. This provides a means to study the "real world" impact of chronic exercise on arterial pressure and may be a better predictor of cardiovascular morbidity and mortality. Studies have shown that the prognostic significance of ambulatory blood pressure is in general superior to that of office blood pressure in healthy populations as well as in untreated and treated hypertensive individuals[10,11]. Subjects will be given a set of instructions and also be asked to complete an activity log during ambulatory blood pressure monitoring and record activities performed during each hour of the day, any unusual physical or emotional events, and sleep and wake times. This deidentified activity log will be returned to the investigator.

VO_2 Peak Test. Exercise testing is well-established in our research program[9,12,13]. Subjects will have their peak aerobic power (VO_2Peak) and maximal heart rate determined with a graded maximal cycle ergometer test. This test will be used to assess changes in aerobic fitness in both groups and will be used to determine exercise prescriptions for the aerobic exercise training sessions. VO_2 will be measured using a mixing chamber and mass spectrometer with subjects on an electromagnetically braked cycle ergometer. Heart rate will be measured by a Polar Heart Rate Monitor.

Heat Therapy. Passive heating is well-established in our research program[7,14]. For Heat Therapy sessions (Heat Therapy group only), subjects will be immersed in hot water for up to 45 mins on 30 occasions. On each day a subject will undergo Heat Therapy, we will obtain a urine sample first to determine whether the subject is dehydrated (determined by urine specific gravity reading >1.024). We will also measure nude body weight before and after the heat exposure. When possible, this will be performed by lab personnel of the same sex as the subject. The scale will be inside a restroom with the readout on the outside of the restroom to maximize subject privacy and comfort. During the first five and final session of Heat Therapy for each subject, core temperature will be continuously monitored and recorded using a wireless, disposable core temperature sensing pill. After the first 5 sessions, once an individual subject's sensitivity to heating has been established, subsequent sessions may be monitored with an infrared aural temperature monitor. There are some risks associated with heat exposure, including: fatigue, light-headedness, muscle cramps, dehydration, and neurological detriments (i.e., heat stroke). However, these symptoms do not typically occur until core temperature rises above 40°C. Subjects will be removed from the hot bath immediately if either core temperature reaches 39.5°C or the subjects experience any symptoms of heat-related illness. All symptoms subside upon lowering core temperature. Ice packs will be on hand if rapid cooling is necessary. Heart rate will also be continuously monitored and recorded throughout heating. If heart rate increases >60 beats/min above resting or increases >20 beats/min with a 5-min time period,

subjects will be moved to a seated position if they were previously fully submerged or removed from the hot tub if they were already sitting up.

Exercise Training. For Exercise Training sessions (Exercise Training group only), subjects will report to the lab for a supervised exercise training session 3-4 times per week. Subjects will be instrumented with a heart rate monitor chest strap and heart rate will be constantly monitored during training sessions. During the first and final session of Exercise Training for each subject, core temperature will be continuously monitored and recorded using a wireless, disposable core temperature sensing pill. Exercise will consist of a 5-minute warm-up of seated upright cycling at 30% of the subject's $VO_{2\ peak,}$ followed by 40 minutes of cycling at 60% $VO_{2\ peak}$, then a 5-minute cool down at 30%. There are some risks associated with exercise, including: light-headedness, fatigue, muscle cramps, and shortness of breath. Procedures will be terminated immediately if any subject feels light-headed, nauseated, or experiences any other adverse signs or symptoms.

1st Heat Therapy or Exercise Training Session. During the 1st heat therapy or exercise training session, we will measure the acute post-heating/exercise hypotension response. This information will be used to test the predictive power of acute post-heat/exercise hypotension on the individual response to the interventions. The subject will lay supine for 20 min of rest followed by three measures of blood pressure before the heat therapy or exercise training session. At the end of this 1st session, the subject will lay down for 1 hour of recovery, during which we will continue to monitor core temperature, heart rate, and blood pressure every 5 min.

Protocol 2:

Upon consenting to Protocol 1, a subset of subjects will be offered the opportunity to participate in additional study activities (Protocol 2). We will enroll up to 40 subjects total for Protocol 2; consistent with the 1 year of additional funding provided by NIH. This is a supplemental protocol that only adds measures of cognitive function and ADRD risk. Subjects are not able to enroll in Protocol 2 without enrolling and participating in Protocol 1, in which the interventions (heat therapy and exercise training) are detailed. Subjects who consent to participate in Protocol 2 will undergo two additional experimental sessions (additional to those in Protocol 1) at LCNI. The first session will be conducted at baseline, before the onset of 30 sessions of heat therapy or exercise training over 8-10 weeks (Protocol 1), and will include the PSQI, a short cognitive assessment battery, and the MRI session. The second session will take place approximately 8-10 weeks later with the same measures from session one.

MRI scans will be acquired at LCNI and uses the standard operating procedures for use of the 3-Tesla Siemens Skyra. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are approved by the US Food and Drug Administration (FDA) to look at structure and function of the brain and body.

The informed consent for Protocol 2 will occur during Screening Day 2 after it has been determined that the subject qualifies for Protocol 1. If the subject agrees to participate in Protocol 2, their first visit to LCNI will consist of completing the PSQI, a 20-minute cognitive assessment, and a 1-hour MRI scan. Our battery uses the NIH Toolbox for Assessment of Neurological and Behavioral Function (http://www.nihtoolbox.org) in order to generate an efficient and comprehensive battery of cognitive assessment. NIH Toolbox is a web-based computerized platform. Subjects will perform tasks as part of the 'Cognitive Domain' assessment including the 'Episodic Memory' (Picture Sequence) task and 'Working Memory' (List Sorting) task. In addition, we propose to use a standardized measure of Fluid Intelligence appropriate for use across the full age range to be tested. The NIH Toolbox for Cognitive Function is a web-based assessment; we have attached a description and some screenshots from the testing (NIH TOOLBOX BROCHURE).

After the cognitive assessment, the subject will be escorted to the MRI room in LCNI for the MRI assessment following LCNI SOPs. The operator will communicate with the subject via an intercom system between scans to assess level of comfort and willingness to continue, to prompt the subject to lie still, and to let the subject know when an upcoming scan will sound differently than the one before.

Scan sessions last approximately 60 minutes. We will collect anatomical images, one functional MRI task assessing memory performance, diffusion imaging, arterial spin label (ASL) and high-resolution T2 oblique coronal images of the hippocampus. We will also collect field maps to correct for field inhomogeneities. Total scan time is 52 minutes in length.

Session 2 will be a follow-up repeat of the same measures from session 1 (PSQI, cognitive assessment battery, and MRI scan). It will last no more than 2.5 hours.

Projected effect sizes and statistical power. To estimate effect size for the primary outcome variables of systolic and diastolic blood pressures, we bracketed over several scenarios with varying pre- to post-intervention differences (i.e., with the fall in pressure following heat therapy being 4.0, 4.5, or 5.0 mmHg greater than following exercise training), and using a SD of 4.5 mmHg from our pilot work and published studies using similar interventions and approaches, which resulted in minimum hypothesized effect sizes of 0.89, 1.00, and 1.11 for Aim 1. Effect sizes for blood pressures were the lowest of our outcome variables; thus, powering for blood pressure will allow adequate power for numerous measurements giving us a global picture cardiovascular and autonomic health. Our prospective power estimates based on a simpler model (two-sample RCT design, modeled as a two-sample t-test), to detect a minimum hypothesized effect size of 0.89 (which is smallest effect size of interest across outcome variables; power 90%; α=0.05), indicate the need to enroll 50 subjects (50:50 ratio of study groups; sample size assumes 20% dropout for the per-protocol analysis). Of those preliminarily screened who appear to be eligible, we will consent and enroll up to 250 individuals for further screening for eligibility anticipating only 50 individuals will be eligible for participation in the study. We need 50 study participants to account for rate of attrition once participants are enrolled in the formal study to ensure completion of 40 participant needed for statistical purposes.

D. Research Population, Recruitment Methods, and Compensation

Protocol 1:

The trial will be conducted in a cohort of 50 physically inactive to moderately active men and women with high blood pressure (Elevated, Stage 1, and Stage 2, based on current guidelines)[4] from the local Eugene and Springfield, OR communities. We will attempt to recruit subjects that represent the ethnicity of Eugene, Oregon and the University of Oregon.

Subjects will be recruited locally (newspaper advertisements, flyers, radio advertisements, in-person recruitment events, online posts, as well as Craigslist and other online advertisements including ad campaigns designed and hosted by marketing companies), as well as referred to us by local physicians and clinics.

If candidates encounter an online advertisement, they will be directed to the ONLINE RECRUITMENT QUESTIONNAIRE. If the candidate appears to qualify based on this questionnaire, they will have the opportunity to make a phone screening appointment with the Research Coordinator, during which candidates will be further screened using the IPAQ. Candidates who do not fill out the ONLINE RECRUITMENT QUESTIONNAIRE will be pre-screened by the Research Coordinator over the phone or in person using the PRE-SCREENING PHONE SCRIPT and IPAQ. Subsequently, subjects will be screened in-person following our SCREENING FORM, MEDICAL HISTORY FORM, and CORE TEMPERATURE PILL CONTRAINDICATIONS QUESTIONNAIRE. During this screening, we will assess medical history, BMI, fasting glucose, hemoglobin A1c, and lipid profile to verify eligibility. Further, seated blood pressure will be measured on two separate screening visits to determine resting blood pressure, and in each visit, blood pressure will be measured twice, performed according to clinical guidelines for assessing hypertension[4]. Subjects who undergo the screening and satisfy all inclusion criteria and no exclusion criteria will progress to Protocol 1.

Inclusion criteria: Systolic blood pressure120-180 mmHg or diastolic blood pressure 80-120 mmHg; ages 35-60.

Exclusion criteria: Hypertensive crisis or target-organ damage; chronic cardiorespiratory or metabolic disease other than hypertension; abnormal resting or exercise ECG (self-reported); currently on antihypertensive drugs; obesity Class 2 or greater (BMI ≥35); fasting glucose ≥126; hemoglobin A1c ≥7%; LDL ≥160 mg/dL; high level of physical activity based on the IPAQ; persons who are pregnant, nursing, or currently trying to conceive. People aged 34 years or younger, or 61 years and older, will not be eligible to participate in the study.

Participants will be compensated $450 for their inconvenience and time spent in Protocol 1. Participants will be compensated at a rate of $15 per hour for all Pre, Interim, and Post-test visits (9 hours x $15/hour = $135). Participants will also receive $5 per heat therapy or exercise training session (30 sessions x $5/session = $150), $45 for each 24-hour urine collection/24-hour BP (3 collections x $45 = $135), and an additional $30 bonus for completion of all the sessions within 10 weeks. Thus, $450 is the total amount listed in the consent form. If a participant starts an experiment but stops before the experiment is completed, the amount of money they receive will be prorated at a rate of $15 per research testing hour, $5 per heat therapy or exercise training session, and $45 per 24-hour urine collection/24-hour BP that they complete.

Protocol 2:

At the time when subjects consent to participate in Protocol 1, they will be told about the opportunity to particpate in Protocol 2. If subjects are interested in participating in Protocol 2, the consent process for Protocol 2 will be completed on Screening Day 2. Then subjects will be pre-screened using the MRI PRE-SCREENING FORM, LCNI MRI SAFETY SCREENING QUESTIONNAIRE and MINI-MENTAL STATE EXAMINATION (MMSE). If subjects consent to Protocol 2, they will be scheduled for their first cognitive function battery and MRI scan before starting therapy sessions, and they will be scheduled for the second cognitive function battery and MRI scan after 8-10 weeks of heat or exercise therapy. It is understood that for various reasons (lack of time, contraindications for MRI, etc.) not all subjects who participate in Protocol 1 will be able or willing to participate in Protocol 2. For this reason we will attempt to recruit up to 40 subjects for Protocol 2.

Inclusion criteria: Meets the inclusion criteria for Protocol 1 and has consented to participate in Protocol 1. Right handed.
Exclusion criteria: Does not meet the exclusion criteria for Protocol 1 or is cognitively impaired (≤24 score on MMSE). Additionally, anyone who may have non-removable metal items imbedded in their body or is determined to be ineligible based on the results of the LCNI MRI Safety Screening Questionnaire or the opionion of the expert LCNI researchers, will be excluded from Protocol 2. Left handed.

Left-handers have different functional brain patterns for language and other cognitive processes and therefore must be excluded for any functional brain imaging studies including Protocol 2 of this study.

Subjects who participate in Protocol 2 will be compensated $75 for each of the two testing session at the LCNI. This is the standard rate for 1-hour of scans and associated cognitive testing. Participants will receive payment after completion of MRI scanning and cognitive assessments.

E. Informed Consent Procedure

Pre-Screening: Pre-Screening will be conducted in one of two ways: 1.) via an online questionnaire and phone, or 2.) via phone only as described below. Candidates will be given ample time to consider whether or not to participate in the pre-screening and provided with the research team's contact information to ask questions at any time.

1.) **Via online questionnaire and phone:** Candidates who encounter the ONLINE RECRUITMENT QUESTIONNAIRE will read and sign a pre-screening consent electronically. Once signed, the questions on the ONLINE RECRUITMENT QUESTIONNAIRE will populate and the candidate will fill in their answers. If the candidate's answers match the inclusion criteria and do not match the exclusion

criteria, the candidate will be given the opportunity to make an appointment for a phone screening with the Research Coordinator. During the phone screening, the Research Coordinator will read the study description from the PRE-SCREENING PHONE SCRIPT and answer any of the candidate's questions before further screened for eligibility using the IPAQ. This phone screening will take about 20 minutes. If the candidate appears to qualify during the phone screening, they will be invited to come into the lab for two in-person screening sessions, during which the informed consent process will take place for Protocol 1 and 2. If the candidate's answers to the ONLINE RECRUITMENT QUESTIONNAIRE do not match the inclusion criteria or match the exclusion criteria, the questionnaire will end and the candidate will be informed that they are not eligible for the study. If the candidate does not sign the PRE-SCREENING CONSENT, the RECRUITMENT QUESTIONNAIRE will end.

2.) **Via phone only:** Candidates who do NOT encounter the ONLINE RECRUITMENT QUESTIONNAIRE will be pre-screened by the Research Coordinator over the phone using the PRE-SCREENING PHONE SCRIPT. Candidates will be read the PRE-SCREENING CONSENT and will consent to the pre-screening verbally. The Research Coordinator will document that the candidate has verbally consented before administering the RECRUITMENT QUESTIONNAIRE and IPAQ verbally. The phone screening (including the pre-screening consent process) will take about 30 minutes.

> Due to the inability to obtain written consent over the phone, a *waiver of consent documentation* is requested to obtain verbal but not written consent for the pre-screening if it occurs over the phone. This waiver is not required if candidates complete the ONLINE RECRUITMENT QUESTIONNAIRE which is capable of collecting electronic signatures. The pre-screening activities conducted over the phone represent only minimal risk (i.e., the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests). Additionally, the pre-screening procedures would not normally require signed consent outside of the research context.

Electronic signatures for the pre-screening consent and ONLINE RECRUITMENT QUESTIONNAIRE answers will be collected in REDCap, a highly secure data management system. If candidates do not consent to the pre-screening, no information will be retained about the candidate. If candidates consent to the pre-screening but fail the pre-screening, their information will be retained only if they indicated that they want to be notified about future studies they may qualify for. Otherwise, their information will be deleted.

Screening Day 1: Written informed consent for Protocol 1 will be obtained from each subject at the beginning of Screening Day 1. Informed consent is obtained by the following process: 1) Subject will review the PROTOCOL 1 INFORMED CONSENT FORM, 2) PIs, researchers, or staff will meet with the subject to review the PROTOCOL 1 INFORMED CONSENT FORM, confirm the subject's understanding, and answer any questions, 3) Once the investigator or staff member is convinced that the subject has read the PROTOCOL 1 INFORMED CONSENT FORM, verbally demonstrates understanding, and agrees to the process, the PROTOCOL 1 INFORMED CONSENT FORM will be signed in the presence of a witness, 4) The investigator or staff member will sign the PROTOCOL 1 INFORMED CONSENT FORM and a copy of the signed consent will be offered to the subject, 5) Consent forms will be kept in a secure file cabinet in the physiology lab and will be retained for a minimum of 5 years after the study is completed.

Screening Day 2: If the subject meets the inclusion criteria and does not meet the exclusion criteria for Protocol 1, Protocol 2 will be described to the subject. If they are interested, consent will be obtained as described above (see Screening Day 1) using the PROTOCOL 2 INFORMED CONSENT FORM. Then, subjects are screened for Protocol 2 using the MRI PRE-SCREENIG FORM, LCNI MRI SAFETY SCREENING QUESTIONNAIRE, AND MINI-MENTAL STATE EXAM. If subjects meet the inclusion criteria, do not meet the exclusion criteria, and consent to participate in Protocol 2, they will be invited to participate in the cognitive battery assessment and MRI scan at LCNI. Subjects will be made aware that there is no penalty for declining to participate in Protocol 2 and their ability to participate in Protocol 2 will not affect their relationship with the primary investigators, researchers, or the University of

Oregon. Subjects will be reminded that their participation in the study is voluntary, and they may completely withdraw from continued participation at any point without penalty or loss of benefits to which they are otherwise entitled. Research team members obtaining consent will be trained by Drs. Minson and Burggren in conducting the informed consent process for clinical cognitive neuroimaging research, and Dr. Burggren will be made available to potential participants that have questions about scanning procedures. Potential participants will be encouraged to contact the PIs or other members of the research team to answer any questions. The follow-up session, given the duration between visits, will be treated like the initial session, where participants will revisit the consent document. Consent forms for Protocol 2 will be kept in a secure file cabinet in the physiology lab and will be retained for a minimum of 5 years after the study is completed.

F. Participant Privacy, Data Disposition, and Data Confidentiality

Confidentiality of data will be assured by coding of subject identities, and that coding will be known only to the investigators. The data will be coded by giving each subject a unique subject number. This subject number will be assigned to the subject after they have given written informed consent. The code list will be stored in a password protected document on a password protected computer in a locked office in the physiology lab. Subjects will only be referred to by code number, and the code list will be destroyed after all the data is analyzed and the results of the study are published, or 24 months after the last subject participates, whichever is first.

Phone screenings will be scheduled using HIPAA compliant software.

Heart rate data will be collected with a Polar Team Pro system. The license for this system is shared with other groups (sports teams) at the University of Oregon. Therefore, individuals outside of the physiology lab may have access to heart rate data from subjects in this study. However, all heart rate data is coded such that individuals outside of the physiology lab will have no access to identifiable information.

Blood samples for biomarkers will be labeled by subject code only (no name) and either analyzed by us or by a commercial lab that will only receive coded samples with no other identifying information.

Samples will be kept for five years. After the five years, the samples will be destroyed by discarding them into a biohazard waste container to be collected and destroyed by the Environmental Health and Safety department at the University of Oregon. Any excess samples will be discarded and destroyed in the same manner.

No identified individual data will be presented or published, rather group mean data will serve as the basis for comparisons. Individual data may be shown without identifying the subject to illustrate scientific principles. Coded data will be kept either in a locked file cabinet in the physiology lab or on the secure server after the study is completed. Non-identifiable data will be kept in our files indefinitely.

In order to comply with federal and state tax laws, individuals receiving ≥ $600 in compensation will be required to provide appropriate tax identification information to university officials. Tracking of this information is a limitation to confidentiality but is clearly disclosed to participants via the consent process and form.

As this study is defined as a clinical trial and is funded by the National Institutes of Health, it has been registered with clinicaltrials.gov (NCT03557502). Regular reports will be filed with clinicaltrials.gov as required by the PIs of the study. There are no further requirements for data sharing with central NIH repositories.

Data for Protocol 2 will not be stored in REDCap and will be coded and handled as described above.

Data for Protocol 1 will be stored in the University of Oregon's (UO) installation of REDCap, a highly secure and robust web-based research data collection and management system. Features of REDCap that protect participants' privacy and data security include:

- Physical Security: UO's REDCap software is housed on servers located in UO's Information Services Computing Center providing locked physical security.
- Electronic Security: The UO REDCap servers are housed behind the UO Information Services Datacenter firewall. All web-based data transmissions are encrypted with industry-standard SSL methods.
- Controlled User Access: REDCap employs a robust multi-level security system that enables researchers to easily implement "minimum necessary" data access for their research staff, including specification of data fields that are identifiers. This feature includes "single click" ability to provide completely deidentified (removing all identified data fields and shifting dates) for analysis or other purposes. User activities are logged to enable auditing of all data access. UO access is integrated with UO's Shibboleth Sign-On such that users who are also UO employees are authenticated against their UO network credentials.
- Data Integrity: UO REDCap is hosted on UO Information Services managed datacenter, ensuring fidelity of database configuration and back-ups. User activities are logged to enable auditing of all data changes.

Only de-identified data will be shared with researchers outside of the University of Oregon physiology lab for the purpose of statistical analysis.

G. Potential Research Risks or Discomforts to Participants

Protocol 1:

Risks from participation in these studies are not considered minimal but are considered low.

Exercise testing and training. The risks associated with graded exercise testing include light-headedness, fatigue, shortness of breath, emesis, risk of myocardial infarction and death. According to the American College of Sports Medicine, these general statements can be made regarding the safety of maximal graded exercise testing and include the risks for a wide range of clinical conditions. The risk of death during or immediately after an exercise test is <0.01%. The risk of myocardial infarction during or immediately after an exercise test is <0.04%. The risk of complication requiring hospitalization (including myocardial infarction) is approximately 0.1%. The magnitude of severity for risks of light-headedness, fatigue, shortness of breath from the VO_2 Peak testing is very low, but the probability of these risks occurring is moderate. The magnitude of severity for risks of myocardial infarction and death from the VO_2 Peak testing is very, very high, but the probability of these risks occurring is very, very low. Like the graded exercise testing, there is potential risk associated with high intensity aerobic cycling exercise including light-headedness, fatigue, shortness of breath, risk of myocardial infarction and death. The magnitude of severity for risks of light-headedness, fatigue, shortness of breath from the aerobic exercise is very low, but the probability of these risks occurring is moderate. The magnitude of severity for risks of myocardial infarction and death from the aerobic exercise is very, very high, but the probability of these risks occurring is very, very low. Heart rate will be constantly monitored during testing. Procedures will be terminated immediately if any subject feels light-headed, nauseated, or experiences any other adverse signs or symptoms. See Emergency Procedures section below. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is low.

Heat therapy. Subjects assigned to the heat therapy group will be immersed in hot water on 30 occasions (up to 45 min immersion in a 40.5°C bath). There are some risks associated with heat exposure, including: fatigue, light-headedness, muscle cramps, dehydration, and neurological detriments (i.e., heat stroke). However, these symptoms do not typically occur until core temperature rises above 40°C. Core temperature will be continuously monitored and recorded during the first 5 trials for any individual subject to establish their sensitivity to heating. Subjects will be removed from the hot bath immediately if either core temperature reaches 39.5°C or the subjects experience any symptoms of heat-related illness. All symptoms subside upon lowering core temperature. Ice packs will be on hand if rapid cooling is necessary. Heart rate will also be continuously monitored and recorded throughout heating. If HR increases >60 beats/min above resting or increases >20 beats/min with a 5-

min time period, subjects will be moved to a seated position if they were previously fully submerged or removed from the hot tub if they were already sitting up. Additionally, heat exposure may have detrimental effects on a developing fetus. Thus, subjects who are pregnant or trying to conceive will be excluded from the study. Additionally, repetitive use of hot baths has been reported to lower sperm counts and sperm motility in some males; however, the decreased sperm counts are reversed within a few months after stopping regular sauna bathing. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is low.

Core temperature pills. Core temperature is measured by an ingestible pill, about the size of a multi-vitamin, which is designed and approved for human use and is accurate to 0.1ºC. This pill will harmlessly pass through the subject's intestinal tract. The pill is not recovered, is disposable, and every subject receives a new pill for each study day. The risks include mechanical injury to the mucus membranes if adequate care is not used. There is a small risk of electrical shock if there is a current leak. Risks of the temperature pill will be minimized by explaining the procedures to the volunteers. Volunteers with history of obstructive diseases of the gastrointestinal tract including diverticulosis, diverticulitis, inflammatory bowel disease, peptic ulcer disease, Crohn's disease, ulcerative colitis, or previous GI surgery should not use a telemetric pill. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is moderate.

Rectal thermistors. The use of rectal thermistors to measure core temperature carries minimal risk. The primary risk is damage to the lining of the rectum; however, this risk is very slight as we use a flexible thermistor that is designed for this purpose. Subjects with recent rectal, anal, vaginal or prostate surgery should not use a rectal thermistor. In addition, subjects who have a personal history of heart disease should not use a rectal thermistor, as the use of a rectal thermometer can cause a vagal reaction, increasing the potential for arrhythmias and fainting. There is also the risk of infection, either by the subject not washing their hands properly or due to a poorly cleaned thermistor. Each subject receives a sterilized thermistor which is labeled with their subject number. Thermistors are cleaned by the subjects and disinfected by the investigators between uses. The risk of infection is similar to that of having a bowel movement and is considered minimal. There is also the risk of embarrassment. This approach is typically well tolerated by subjects, and the investigative team is professional in regard to their treatment of the subjects. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is low.

Digital aural and oral thermometers. There are no risks associated with the use of digital aural and oral thermometers.

Cardiac output. There are no risks associated with breathing acetylene or helium, particularly in such low concentrations.

Blood sampling. There is the possibility of bruising, bleeding or infection at the site of venous access. In regard to probability of harm, in a published study with 4,050 subjects who had venipuncture performed, 12% experienced minor bruising. Serious complications were observed in less than 3% of patients, syncope only occurring in less than 1% of patients[15]. On each pre-, interim-, and post-intervention testing timepoint, we will take an intravenous sample using sterile venipuncture and withdrawal 40ml of blood immediately following the 24-hour urine collection. Subjects may choose to do the vascular studies and exercise test during the same visit after the blood draw. The vascular studies take about 2 hours and occur before the exercise test. This is sufficient time for the venipuncture site to seal by forming a platelet plug. Therefore, there are no additional risks to performing the exercise test on the same day as the blood draw. Approximately 130 ml of blood will be obtained over the duration of the protocol. The amont of blood obtained is less than the associated standard blood donation programs, where 450-500ml of blood is routinely withdrawn during a single hour long donation. As part of the screening procedures, subjects will be asked if they have donated blood recently or participated in studies in which blood was withdrawn. Subjects will be excluded if they have donated blood in the 8 weeks prior to experiment enrollment. Additionally, subjects will be asked not to donate blood or participate in other studies in which blood is drawn for 8 weeks following the experiments. This requirement is consistent with Association of American Blood Banks standards for blood donation. If the

subject is having blood drawn as part of a medical exam, they will be asked to inform their medical provider that they provided blood about equal to that of a standard donation. The magnitude of severity for risks of infection or syncope from the blood sampling is very low, and the probability of these risks occurring is very low. Bruising is temporary and does not pose any long-term risks to the subject, aside from mild discomfort. Pressure will be applied to the site after removal of the needle to assure that any bleeding ceases quickly. A band-aid and/or small gauze pressure wrapping will be applied to the subject's arm to keep the site protected. The risk of infection at the site is low. The subject will be instructed on how to keep the site clean during the days following the study. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is low.

Subject Rights and Withdrawal. Taking part in this research is a voluntary decision made by the research subject. The subjects can stop at any time and their decision whether or not to participate will not affect their relationship with the University of Oregon. By partaking in this research, subjects do not waive any liability rights for personal injury. The investigators may stop a subject from taking part in this study at any time if it is in their best interest, if the subjects do not follow the study rules (e.g., compliance of less than 70% for heat or exercise sessions, or failure to follow study guidelines despite several reminders), or if the study is stopped. For example, if the investigators determine the study is overly stressful for the subject (as indicated by body language and an extremely elevated heart rate and/or blood pressure) they may stop the subject from participating in the study. Or, if subjects express pain or discomfort beyond what is normally expected, the investigators will stop the subject from participating in the study.

Emergency Procedures. In the unlikely event of a medical emergency, we will follow the "Guidance for Human Physiology Investigators on Emergency and Non-Emergency Procedures for Human Subject Research," approved previously by the IRB. In addition, we would provide responding emergency healthcare providers with the MEDICAL HISTORY FORM provided by the subject.

As this human subject research meets the definition of Clinical Trial, we have attached our DATA AND SAFETY MONITORING PLAN.

Protocol 2:

MRI Scan. Given the physics of MRI, there are three potential pathways for risk associated with MRI scanning: heating, sound, and the static field itself. The static magnetic field of the scanner presents the most significant risk if ferromagnetic objects are brought into the room. There is a risk these objects may be strongly attracted to the MRI machine. They may also lead to heating and burns. This can occur if the participant or LCNI equipment are not positioned properly inside the scanner by creating conductive loops. To minimize this risk, LCNI-trained staff will conduct a thorough screening for any potential problems (research staff usually do this once before they arrive and LCNI does it again right before the scanning session), to make sure we have accounted for all MRI-related concerns. Participants who do not pass screening are not run. LCNI has a very conservative approach to screening, whereby any uncertainty by staff or participants about the veracity and completeness of the screening leads to cancelation of the scan. Participants are very carefully positioned in the magnet with special insulating pads to ensure no conductive loops are present. The MRI scanner makes loud thumping, pounding and whining sounds during scans, which reaches levels that could cause hearing damage. Thus, every person in the magnet room during scanning must wear hearing protection (earplugs and/or special headphones). Some people become anxious and/or tired and/or claustrophobic from lying in the MRI machine. LCNI offers 'practice' sessions in a mock scanner (a duplicate environment without the strong magnetic field) before the session to everyone and encourage their use, especially in people who do not know whether they are claustrophobic or not. The mock scanner looks and sounds just like the real scanner. This practice environment allows LCNI staff and/or investigators to pre-test and prepare patients for the real environment and to provide demonstration of details about the procedure. All participants are told that they may ask to stop the practice or real MRI scans at any time. They are given an emergency squeeze ball during the real MRI and instructed in its use. They may squeeze this ball at any time to stop the scan and be removed from the scanner. Although there are no known risks to having an MRI during pregnancy, it is a time when the heart and

brain are still developing and is listed as a reason for exclusion on our screening form. LCNI strongly encourages anyone who may be pregnant to wait until their pregnancy is finished to participate in research. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is moderate.

Psychological or Emotional. Participants may experience discomfort when completing questionnaires about their personal development and disclosing sensitive personal information. Participants may also feel fatigue, anxiety or frustration while undergoing tests or neuroimaging scans. To minimize these risks, subjects will be informed of the exact nature of the study procedures and given ample time to ask questions of study staff and investigators. Participants will be carefully screened and are acclimated to MRI scan procedures through a thorough screening and information process before the scan. Participants will be allowed to take breaks as needed and it will be made clear to all participants, both during the informed consent process that they are free to discontinue their participation at any time without penalty. Any participant who experiences significant discomfort during assessment will be able to terminate the procedure immediately. The ***probability*** of the adverse reactions discussed above is low, and the ***severity*** is low.

H. Potential Benefits of the Research

The potential benefits of the study in terms of knowledge to be gained outweigh the risks of the study.

Protocol 1:

Hypertensives are at an elevated cardiovascular risk. The outcomes of this project may greatly benefit the general population in terms of the information that may be gleaned from this project. It is possible the outcomes of this research may impact the future clinical treatment of patients at elevated cardiovascular risk. Thus, the potential benefits of the study greatly outweigh the risks subjects may experience in Protocol 1.

Protocol 2:

Heat therapy may be used to target elevated systolic blood pressure and Stage-1 hypertension, as well as to improve numerous biomarkers of cardiovascular disease risk including arterial stiffness. As vascular and cognitive function are closely related, this led us to hypothesize that heat therapy in the form of hot water immersion *may reduce the risk of ADRD*. Therefore, 30 sessions of heat therapy may improve cognitive function and biomarkers of dementia risk assessed using fMRI. As we are performing high resolution cardiovascular phenotyping of our subjects, we will be able to relate changes in biomarkers of cardiovascular health with cognitive function and fMRI-based biomarkers of brain aging and markers of ADRD pathology in mid-life adults. Collectively, these studies will examine relationships between SBP, large artery stiffness (outcome measures in Protocol 1), and tests of cognitive function/brain health (additional outcome measures from Protocol 2) with heat therapy. Thus, the potential benefits of the study greatly outweigh the risks subjects may experience in Protocol 2.

I. Investigator Qualifications, Roles, and Training

Dr. Minson has been performing research on biomarkers of cardiovascular health, autonomic-vascular regulation, and human adaptations to environmental extremes for the last 22 years. He has extensive experience performing translational studies in humans utilizing state-of-the-art research and clinical approaches. He has been a leading researcher on vascular and microvascular function in humans in numerous disease states and in understanding the basic mechanisms of blood pressure and blood flow regulation. He has recently published a clinical trial demonstrating that heat therapy can greatly improve biomarkers of cardiovascular health in young, sedentary but otherwise healthy adults.

Dr. Halliwill has extensive experience with exercise physiology, vascular regulation, and has been conducting human studies using many of the proposed methods for 25 years.

Dr. Hawn is a cardiologist and will provide assistance with the recruitment and screening of individuals with hypertension, as well as provide advice as needed regarding clinical issues which might arise in the execution of this research.

Aaron Harding, Emily Larson, Brendan Kaiser, Brandon Gibson, Emma Reed, and Jessica Atencio are all graduate students working under the direction of Dr. Minson and Dr. Halliwill. Dr. Christopher Chapman is a postdoctoral fellow working under Dr. Minson and Dr. Halliwill.

Karen Needham is a research technician working under the direction of Dr. Minson and Dr. Halliwill.

Lindy Comrada is the study coordinator. She will be the primary person performing subject recruitment and scheduling. She will also assist with the screening procedures (including taking blood) and will be trained in many of the research techniques. She will also assist as needed with the heat therapy or exercise sessions.

Alison Burggren, Ph.D., a Research Associate in the Robert and Beverly Lewis Center for Neuroimaging (LCNI) at the University of Oregon, will serve as a Coinvestigator of the proposed study. Dr. Burggren is an expert in fMRI imaging, and Alzheimer's related dementias with 15 years of experience conducting clinical and preclinical neuroimaging research in older adults. Dr. Burggren will oversee all of the proposed cognitive tests and MRI measurements in the administrative supplement, and will oversee the analyses of scans, including individual analytical methods for each imaging modality detailed in the proposal.

References

1. Egan BM, Li J, Hutchison FN, Ferdinand KC. Hypertension in the United States, 1999 to 2012: Progress Toward Healthy People 2020 Goals. *Circulation*. 2014;130:1692–1699.
2. Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB. Clustering of metabolic factors and coronary heart disease. *Arch Int Med*. 1999;159:1104–9.
3. Berry JD, Dyer A, Cai X, Garside DB, Ning H, Thomas A, Greenland P, Van Horn L, Tracy RP, Lloyd-Jones DM. Lifetime Risks of Cardiovascular Disease. *N Eng J Med*. 2012;366:321–329.
4. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, MacLaughlin EJ, Muntner P, Ovbiagele B, Smith SC, Spencer CC, Stafford RS, Taler SJ, Thomas RJ, Williams KA, Williamson JD, Wright JT. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *J Am Coll Cardiol*. 2017;doi: 10.1016/j.jacc.2017.11.006.
5. Zaccardi F, Laukkanen T, Willeit P, Kunutsor SK, Kauhanen J, Laukkanen JA. Sauna Bathing and Incident Hypertension: A Prospective Cohort Study. *Am J Hypertens*. 2017;30:1120–1125.
6. Laukkanen T, Khan H, Zaccardi F, Laukkanen JA. Association between sauna bathing and fatal cardiovascular and all-cause mortality events. *JAMA Intern Med*. 2015;175:542–548.
7. Brunt VE, Howard MJ, Francisco MA, Ely BR, Minson CTT. Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. *J Physiol*. 2016;594:5329–42.
8. Torgrimson BN, Meendering JR, Miller NP, Kaplan PF, Minson CT. Endothelial function, endothelin-1, and fibrinogen in young women using the vaginal contraceptive ring. *Fertil Steril*. 2009;92:441–447.
9. Lynn BM, Minson CTT, Halliwill JR. Fluid replacement and heat stress during exercise alter post-exercise cardiac haemodynamics in endurance exercise-trained men. *J Physiol*. 2009;587:3605–3617.
10. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, Hond E Den, McCormack P, Staessen JA, O'Brien E. Superiority of ambulatory over clinic blood pressure measurement in

predicting mortality: The Dublin outcome study. *Hypertension*. 2005;46:156–161.

11. Fagard RH, Celis H, Thijs L, Staessen JA, Clement DL, De Buyzere ML, De Bacquer DA. Daytime and nighttime blood pressure as predictors of death and cause-specific cardiovascular events in hypertension. *Hypertension*. 2008;51:55–61.
12. Lynn BM, McCord JL, Halliwill JR. Effects of the menstrual cycle and sex on postexercise hemodynamics. *Am J Physiol Regul Integr Comp Physiol*. 2007;292:R1260–R1270.
13. Romero SA, McCord JL, Ely MR, Sieck DC, Buck TM, Luttrell MJ, MacLean DA, Halliwill JR. Mast cell degranulation and de novo histamine formation contribute to sustained postexercise vasodilation in humans. *J Appl Physiol*. 2017;122:603–610.
14. Brunt VE, Jeckell AT, Ely BR, Howard MJ, Thijssen DHJ, Minson CT. Acute hot water immersion is protective against impaired vascular function following forearm ischemia-reperfusion in young healthy humans. *Am J Physiol - Regul Integr Comp Physiol*. 2016;311:R1060–R1067.
15. Galena HJ. Complications occurring from diagnostic venipuncture. *J Fam Pract*. 1992;34:582–584.