NCT03557502
Heat Therapy Versus Exercise Training in Hypertension
Statistical Analysis Plan Document
Last Edited May 22, 2024

TRIAL FULL TITLE	Heat Therapy versus Exercise Training in Hypertension
SAP VERSION	5
SAP VERSION DATE	05/22/2024
TRIAL STATISTICIAN	Robin Baudier
Protocol Version (SAP associated with)	IRB # 05042018.006, Last modified: 06.29.2022
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2 Abbreviations and Definitions

ADRD	Alzheimer's disease and its related dementias
ASL	Arterial spin label
AE	Adverse Event
BMI	Body mass index
BP	Blood pressure
CA1, CA2, CA3	Corpus Ammons fields 1, 2, and 3
CRF	Case Report Form
CVD	Cardiovascular disease
ERC	entorhinal cortex
eTIV	estimated total intracranial volume
fODF	Fibre orientation distribution function
GM	Gray matter
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
HTN	Hypertension
IMP	Investigational Medical Product
LDL	Low-density lipoprotein
LLD	Lower limit of detection
SAP	Statistical Analysis Plan
ULD	Upper limit of detection
WM	White matter
WMH	White matter hyperintensities

3 Introduction

3.1 Preface

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. In fact, in 2010, high blood pressure was the leading cause of death and disability-adjusted life years worldwide. 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, 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. 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. 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, 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. 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. 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.

3.2 Scope of the analyses

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.

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).

4 Study Objectives and Endpoints

4.1 Study Objectives

Protocol 1
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
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.*

4.2 Endpoints

In **Protocol 1,** the primary and secondary outcomes from the original grant were updated after interruption from the COVID-19 pandemic shut-downs led to not being able to collect originally planned Aim 2 outcomes: MSNA; FMD; β-stiffness index; Wall thickness, Dynamic arterial compliance; Reactive hyperemia; and Baroreflex sensitivity. In **Protocol 2,** The primary cognitive outcome is a memory domain composite score calculated as the average of age-corrected list-sorting and age-corrected picture sequence z zcores.The primary brain biomarker of ADRD risk outcomes are arterial transit time variables mean perfusion in gray matter and mean

perfusion in white matter. The primary and secondary outcomes are shown by protocol and aim in **Table 1** below.

Table 1. Outcomes by Protocol and Aim

Protocol 1			
Outcomes	**Aim 1**	**Aim 2**	**Aim 3**
Primary	Clinic systolic and diastolic pressures; Ambulatory systolic and diastolic pressures	Pulse Wave Velocity (measure of arterial stiffness)	Acute and chronic pressure reductions
Secondary	Heart rate; Cardiac output; Stroke volume; Total peripheral resistance; Secondary BP outcomes: waking, sleeping, dipper vs. non-dipper	Blood-borne biomarkers	
Protocol 2			
	Cognitive	**Brain biomarkers of ADRD risk**	
Primary Outcomes	Memory domain composite score*	Arterial transit time: mean perfusion in gray matter, mean perfusion in white matter	

*average of age-corrected list-sorting and age-corrected picture sequence z-scores

5 Study Methods

5.1 General Study Design and Plan

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, an aerobic-exercise intervention. We will specifically study individuals with Elevated or Stage 1 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. The table at the right depicts the workflow for this clinical trial protocol, which is as follows:

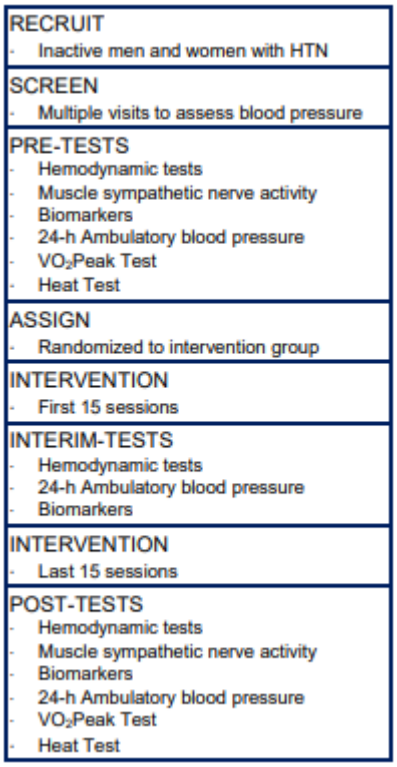
RECRUIT: The trial will be conducted in a cohort of physically inactive to moderately active men and women with high blood pressure (Elevated and Stage 1 or 2 Hypertension, based on current guidelines)[4] from the local Eugene and Springfield, OR communities (details under section D). Potential subjects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

Figure 1. Study Time Points



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 cordinjured 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 1_{st} 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.

5.2 Inclusion-Exclusion Criteria and General Study Population

INCLUSION CRITERIA: Systolic pressure ≥120 and <140 mmHg or diastolic pressure ≥80 and <90mmHg; ages ≥35 and <60. 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).
EXCLUSION CRITERIA: Secondary hypertension and/or target-organ damage; CVD other than hypertension; Abnormal resting or exercise ECG; Currently on antihypertensive drugs; Obesity Class 2 or greater (BMI ≥35); fasting glucose ≥126; hemoglobin A1c ≥7%; LDL ≥160 mg/dL; Participation in aerobic exercise ≥ 30 min/session and ≥ 1 sessions/week, based on self-reported exercise habits over the prior 12 months; Women who are pregnant, nursing, or desiring to become pregnant.

5.3 Randomization and Blinding

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.

5.4 Study Assessments

Timing and frequency of study assessments are shown in **Figure 1**. Descriptions of outcome variables collected are shown below in **Table 3**. See **Derived Variables** section below for additional calculated outcomes.

Table 3. Non-derived variables used in study assessments

Variable	Aim/Primary or Secondary	Description
Protocol 1		
syst_bp_mean	Aim 1/Primary	Continuous; average of 3 systolic BP measurements collected in clinic at pre-, interim-, and post-intervention timepoints
diast_bp_mean	Aim 1/Primary	Clinic diastolic BP; Continuous; average of 3 diastolic BP measurements at 2 screening

		visits collected in clinic at pre-, interim-, and post-intervention timepoints
mean_rest_hr	Aim 1/Secondary	Continuous; average of 3 resting heart rate measurements collected in clinic at pre-, interim-, and post-intervention timepoints
Mean_qc	Aim 1/Secondary	Continuous; average of 3 cardiac output measurements collected in clinic at pre-, interim-, and post-intervention timepoints
Mean_qc	Aim 1/Secondary	Continuous; average of 3 cardiac output measurements collected in clinic at pre-, interim-, and post-intervention timepoints
Protocol 2		
mean_pcalib_gm	Brain biomarkers of ADRD/primary	Continuous; Mean perfusion calibration in gray matter, measure of arterial transit time
mean_pcalib_wm	Brain biomarkers of ADRD/primary	Continuous; Mean perfusion calibration in white matter measured by MRI, measure of arterial transit time

Note: means calculated in REDCap are not considered derived variables for the purposes of this table.

5.5 Derived variables

For ambulatory blood pressure measures, subjects self-report waking and sleeping hours. The BP assessment interval for waking hours is every 20 minutes, compared to every 60 minutes during sleeping hours. For 24H ambulatory BP, day and night readings were weighted to account for the higher number of daytime readings.

Table 4. Derived variables used in study assessments

Derived Variable	Aim/Primary or Secondary	Description
Protocol 1		
abp_syst	Aim 1/Primary	Continuous; weighted average 24H ambulatory systolic BP
abp_dyas	Aim 1/Primary	Continuous; weighted average 24H ambulatory diastolic BP
abp_day_syst	Aim 1/Secondary	Continuous; average day ambulatory systolic BP
abp_night_syst	Aim 1/Secondary	Continuous; average night ambulatory systolic BP
abp_day_dyas	Aim 1/Secondary	Continuous; average day ambulatory diastolic BP
abp_night_dyas	Aim 1/Secondary	Continuous; average night ambulatory diastolic BP
sv	Aim 1/Secondary	Continuous; stroke volume is calculated as mean_qc*1000/ mean_qc_hr rounded to 3 decimal places
tpr	Aim 1/Secondary	Continuous; total peripheral resistance is calculated as mean_map/mean_qc rounded to 3 decimal places, where mean_map is mean arterial pressure
sbp_dipper	Aim 1/Secondary	Categorical; non-dipper <10%, dipper ≥10% to <20%, and extreme dipper ≥20% change in systolic BP at night calculated from ambulatory BP day and night averages

dbp_dipper	Aim 1/Secondary	Categorical; non-dipper <10%, dipper ≥10% to <20%, and extreme dipper ≥20% change in diastolic BP at night calculated from ambulatory BP day and night averages
pwv	Aim 2/Primary	Continuous; average of three pulse wave velocity measurements collected at pre-, interim-, and post-intervention timepoints
il6_lg2	Aim 2/Secondary	Continuous; Biomarker IL-6 with the lower limit of detection (LLD) of 0.031 imputed for <LLD, log2 transformed for normalcy
crp_lg2	Aim 2/Secondary	Continuous; Biomarker CRP with the lower limit of detection (LLD) of 0.031 and upper LD (ULD) of 50 imputed for <LLD and >ULD, log2 transformed for normalcy
et1_lg2	Aim 2/Secondary	Continuous; Biomarker ET-1 with the lower limit of detection (LLD) of 0.031 imputed for <LLD, log2 transformed for normalcy
alb_lg2	Aim 2/Secondary	Continuous; Biomarker urine albumin with the lower limit of detection (LLD) of 0.031 imputed for <LLD, log2 transformed for normalcy
Supine.SBP.low	Aim 3/Primary	Continuous; average of three lowest consecutive supine systolic blood pressure measurements taken every 5 minutes for an hour post-intervention during supine recovery (for Heat Therapy: syst_bp55_ht_s1 to syst_bp110_ht_s1; for Exercise Training: for Heat Therapy: syst_bp55_ex_s1 to syst_bp110_ex_s1)
Supine.DBP.low	Aim 3/Primary	Continuous; average of three lowest consecutive supine systolic blood pressure measurements taken every 5 minutes for an hour post-intervention during supine recovery (for Heat Therapy: syst_bp55_ht_s1 to syst_bp110_ht_s1; for Exercise Training: for Heat Therapy: diast_bp55_ex_s1 to diast_bp110_ex_s1)
Supine.SBP.1.ch	Aim 3/Primary	Change from average pre-session resting SBP to Supine.SBP.low
Supine.DBP.1.ch	Aim 3/Primary	Change from average pre-session resting DBP to Supine.SBP.low
eGFR	Aim 1/Exploratory	eGFR was calculated using the CKD-EPI Creatinine-Cystatin Equation (2021) using the CKDEpi_RF.creat.cys function in the nephron R package and rounded to two decimal places
uacr	Aim 1/Exploratory	Urine albumin creatinine ratio (mg/g) was calculated by dividing urine albumin by urine creatinine/1000 rounded to 2 decimal places
Protocol 2		
cognitive_score	cognitive/Primary	Averaged z-scores (centered & divided by standard deviation) of age-corrected picture sequence score (age_cor_score)

		and age-corrected list-sorting score (list_age_cor_scpre)

6 Sample Size

Projected effect sizes. 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. For MSNA, we used a pre-intervention mean value of 39±7 bursts/100beats based on our pilot data with 4 HTN subjects, and determined that a post-intervention mean value of 29±7 b/100bts would represent a clinically significant change (of 10 b/100bts). Thus, for MSNA, d = 1.43. Similarly, from our pilot work and our published studies using similar interventions and approaches[78,84,88], calculated effect sizes for β-stiffness d = 1.43, DAC d = 1.70, sympathetic baroreflex d =1.33, FMD d = 2.55, carotid wall thickness d = 3.33, for Aim 2. 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.

Statistical power. 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).

7 General Analysis Considerations

7.1 Timing of Analyses

The final data analysis will be performed by an externally contracted biostatistician after study visits have been completed for all enrolled subjects.

7.2 Analysis Populations

Intention-to-Treat will be the primary analysis approach. However, as one goal is to compare outcomes when subjects maintain compliance with heat therapy and exercise training, we will also employ per-protocol analysis as a secondary approach, including subjects with ≥90% compliance. As an exploratory step, we will conduct analyses of variance to compare characteristics of dropouts vs. completers, as this may inform future endeavors.

7.2.1 Intention to Treat or Modified Intention to Treat

- *All participants who received any intervention and who participated in at least one post-baseline assessment*

7.2.2 Per Protocol Population

- *All subjects who adhere to the major criteria in the protocol with a ≥90% compliance*
- *If ≥90% of participants have full compliance, this analysis will not be performed*

7.3 Covariates and Subgroups

Protocol 1

Protocol 1 covariates included in analyses in section 9.1 include age, sex, body mass index,

pre-intervention 24 hour ambulatory systolic and diastolic blood pressure, and average of three systolic and diastolic blood pressure readings prior to the first Heat Therapy or Exercise Training session.

Protocol 2

Protocol 2 covariates/potential confounders that will be characterized for their relationship with each other and Protocol 1 and 2 outcomes as described in section 9.1 include are listed in **Table 5**.

Table 5. Protocol 2 Covariates and Potential Confounders

	Cognitive	**Brain biomarkers of ADRD risk**
Covariates/ Potential Confounders	PSQI (sleep measure), MMSE (general cognitive assessment at baseline)	Hippocampal regions: Whole hippocampus, Left ERC, Right ERC, eTIV (for normalizing ERC measures) White matter hyperintensities (WMH): WMH volume, WMH Mean

7.4 Missing Data

Descriptive statistics will be computed to understand the nature of the data collected, and to identify outliers and missingness patterns. We will follow procedures to minimize biases and limit power loss as a result of attrition, such as the use of mixed effect models that allow for missing data, and employ multiple data-imputation methods if greater than 10% of a predictor or covariate of interest is missing. Proposed analyses are designed to limit biases even when assumptions of data missing completely or missing at random do not hold. Participants with any study visits post-intervention will be included in analyses.

7.5 Multiple Testing

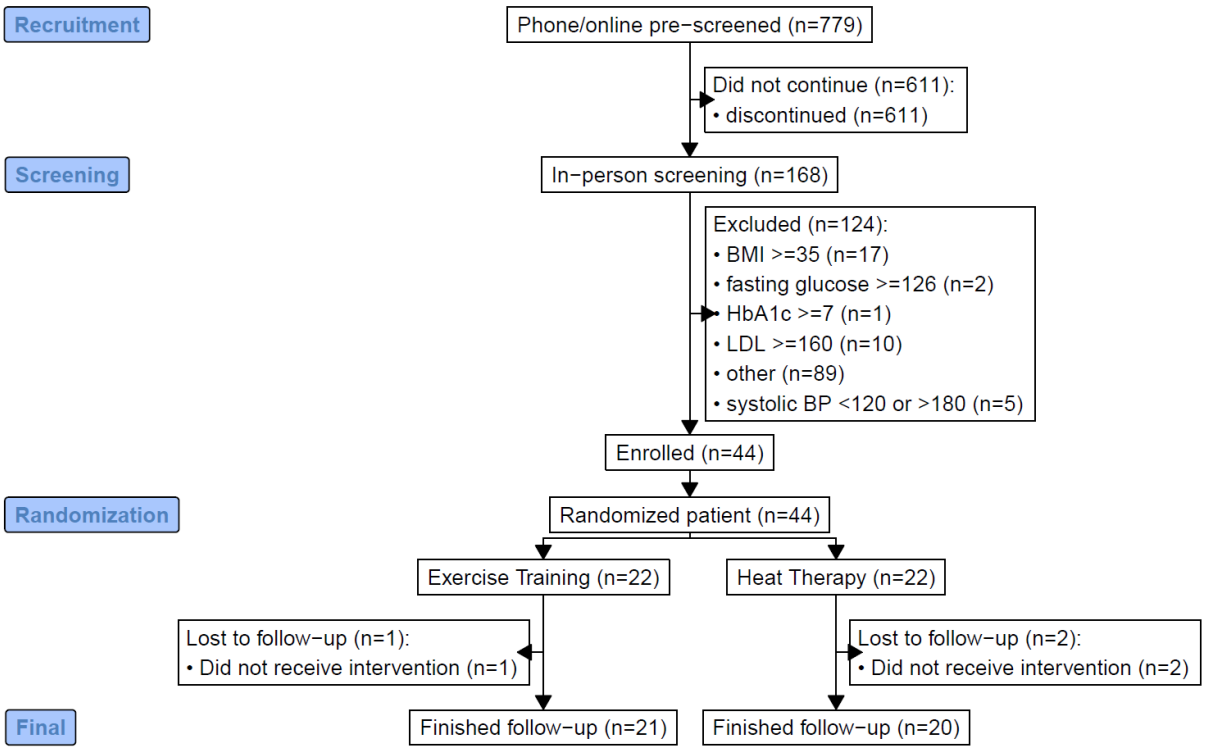
As this study is focused on learning and hypothesis generation and does not control for multiple comparisons.

8 Summary of Study Data

Continuous variables will be summarized as: mean (standard deviation), median (IQR), and frequency % as appropriate for parametric, nonparametric, and categorical variables, respectively. Summary tables will be structured with a column for study group (Heat Therapy, Exercise Training) and overall and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

8.1 Subject Disposition

A consort flow diagram will be created using study coordinator tracked numbers of pre-screenings and screenings not fully captured in REDCap. In-person screening numbers determined in REDCap by those with redcap_event_name values of "screening_visit_1_arm_1." Exclusion reasons determined for age, systolic BP, diastolic BP, pregnant/nursing/trying to conceive, BMI, HbA1c, glucose, total LDL, cardiovascular disease, medications, and IPAQ category of physical activity values for REDCap instrument screening_day_1_data_sheet variables age, syst_bp_mean, diast_bp_mean, preg_nurse_conc, bmi, hba1c, gluc, tot_ldl, cvd, drugs, and ipaq_cat respectively. Participants enrolled was determined based on ID list from clinical coordinators. Participants randomized determined by having a value for the intervention variable int in the REDCap randomization instrument.



8.2 Protocol Deviations

Protocol deviations uncovered during analysis included one Heat Therapy participant that should have been excluded for LDL values ≥160 who completed the study. Following published recommendations on handling of ineligible participants that are inadvertently randomized,[1] this participant is included in the intention to treat analysis dataset.

8.3 Demographic and Baseline Variables

The following variables for sex, age, race, ethnicity, body mass index (BMI), glycated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein (HDL) , low-density lipoprotein (LDL), LDL/HDL ratio, non-HDL, triglycerides, fasting glucose, total cholesterol, mean systolic BP, mean diastolic BP, and heart rate will be described at baseline: sex, age, race, ethnic_group, bmi, hba1c, tot_chol, hdl, , tot_ldl, ldl_hdl, non_hdl, triglyc, gluc, tc_hdl, syst_bp_mean, diast_bp_mean, and hr_mean. Summary statistics will be reported as described in section 9.

8.4 Treatment Compliance

Compliance will be defined as participation in Heat Therapy or Exercise Training intervention visits.

9 Efficacy Analyses

For primary analyses for Protocol 1 Aims 1, primary outcomes will be compared between groups (heath therapy vs exercise training) over time (within subject repeated measurements) using two-way repeated measures mixed model including an interaction between intervention group and timepoint with pre-defined contrasts. For Specific Aim 1, to test the hypothesis that heat therapy improves blood pressure (both in clinic and 24H ambulatory) better than dynamic aerobic exercise, the null hypothesis will be that the interaction between group and timepoint is equal to 0. For Aim 2, to determine whether heat therapy will improve biomarkers of arterial health (arterial stiffness & biomarkers of systemic

inflammation and oxidative stress) and compare responses to exercise training, the null hypothesis will be that the interaction between intervention group and timepoint is equal to 0. For Aims 1 and 2 exploratory analyses, ANCOVA will be performed for primary and secondary outcomes, with pretest values entered as a covariate and treatment condition specified as a fixed factor. Demographic and physiological variables (age, sex, BMI) and pre-intervention blood pressure will be added one at a time to the ANCOVAs to test for an interaction on treatment effects. For Aim 3, we will use ANCOVA models to determine the predictive potential of acute post-exercise hypotension response for chronic changes in blood pressure, with pretest values entered as a covariate and systolic and diastolic blood pressure specified as a fixed factor. Analyses will be performed on the Intention to Treat population. Additionally, change from pre- to post-intervention in ambulatory and in clinic systolic and diastolic pressure will be correlated with change from pre- to post first intervention session supine systolic and diastolic blood pressure by Pearson correlation.

For Protocol 2, where composite score cognitive function and brain biomarkers of ADRD risk outcomes were not collected at the interim timepoint, separate linear regressions will be performed for each outcome and predictor combination, where the post-intervention timepoint of the measure is the outcome, the pre-intervention measure of the outcome will be included as a covariate, and predictors will be intervention group in Aim 1 and 24H ambulatory systolic and diastolic blood pressure and arterial stiffness measures mean perfusion calibration in gray matter or white matter in Aim 2. Diagnostic plots and Cook's D of linear regressions will be assessed for influential outliers. If found and not improved through transformation, Robust regression using Huber weighting will be utilized instead.

Continuous outcomes will be transformed for normalcy prior to analysis. Methods used to check model assumptions will include checking for extreme outliers by visual inspection and Shapiro test and checking for homogeneity of variance by visual inspection and Levene's test. Full model results will be saved in an appendix or csv file, for possible use in planning future studies, meta-analysis and to enable replication of analysis.

9.1 Exploratory Efficacy Analyses

In addition to Protocol 1 primary analyses, additional exploratory analysis will include analyses of covariance (ANCOVA) for each dependent variable of interest, in which pretest values will be entered as a covariate and treatment condition will be specified as a fixed factor in ANCOVA models. Demographic and physiological variables age, sex, and body mass index will be added one at a time to the ANCOVAs to test for an interaction on treatment effects using the following ANCOVA models with pre-intervention systolic or diastolic blood pressure (Pre SBP, Pre DBP) included as a continuous covariate. ANCOVA models based on the formula Interim or Post BP ~ Pre BP + <Interaction variable> + Intervention + <Interaction variable>*Intervention and included the following terms in addition to Pre BP: Model 2, Pre BP*Intervention; Model 3, Age + Age*Intervention; Model 4, Sex + Sex*Intervention interaction; Model 5, BMI + BMI*Intervention interaction. SBP, DBP, age and body mass index (BMI) willl all included in models as continuous variables with means estimated at selected values of interaction terms to illustrate how blood pressure measures did or did not vary by subgroup.

For Protocol 1 Aim 3, which is an exploratory aim, analyses of covariance (ANCOVA) models will be used to determine the predictive potential of acute responses for chronic changes in blood pressure. To test the association between acute decreases in blood pressure following an initial heat therapy session or exercise peak test and sustained reduction in resting blood pressures as measured by post-intervention ambulatory blood pressure, we will perform ANCOVA models with pre-session systolic or diastolic blood pressure (Pre-session SBP, Pre-session DBP) included as a continuous covariate. Pre-session BP is an average of three pre-session measurements and Post-session BP is lowest

average of three consecutive readings taken every five minutes in the 55-110 minutes following the hour-long intervention. Group means for ambulatory blood pressure at the post-intervention timepoint will be estimated using the following formulas: Model 1, Post ambulatory BP ~ Pre-session BP + Intervention; Model 2, Post ambulatory BP ~ Pre-session BP + Intervention + Intervention* Pre-session BP; Model 3, Post ambulatory BP ~ Pre-session BP + Intervention + Post-session BP + Intervention* Post-session BP. BP measures will be included in models as continuous variables with means estimated at selected values of interaction terms to illustrate how blood pressure measures did or did not vary by subgroup. Additionally, exploratory correlations and PCA were performed among the following Project 1 variables: age, body mass index (BMI), HbA1c, total cholesterol (TC), Interleuken-6 (IL-6), C-reactive Protein (CRP), urine albumin to creatinine ratio (UACR), estimated glomerular filtration rate (eGFR), 24H ambulatory systolic and diastolic blood pressure (Amb. SBP, Amb. DBP), arterial stiffness measure pulse wave velocity (PWV), IPAQ long form continuous score MET-minutes/week (IPAQ), pre-first session resting SBP and DBP, maximum drop in supine SBP or DBP in the 55-110 minutes post-first heat or exercise session (Supine SBP or DBP drop), and total peripheral resistance (TPR), pulse wave velocity (PWV), renin, angiopoietin-2 (Ang2), endothelin-1 (Et1), and urine aldosterone.

In Protocol 2 exploratory analyses, the relationship between outcomes and the highly related covariates/potential confounders listed in **Table 5** at baseline and over time will be characterized through Pearson or Spearman correlations as appropriate based on distributions of data. Clustering of individuals and variables will be characterized through principal component analyses and unsupervised hierarchical clustering. Variables will be centered and scaled prior to principal component analysis. Additional exploratory correlations and PCA were then subsequently performed among the following Project 1 and 2 variables: age, body mass index (BMI), HbA1c, total cholesterol (TC), Interleuken-6 (IL-6), C-reactive Protein (CRP), 24H ambulatory systolic and diastolic blood pressure (Amb. SBP, Amb. DBP), IPAQ long form continuous score MET-minutes/week (IPAQ), pre-first session resting SBP and DBP, mean perfusion calibration of gray matter (Pcalib. GM) and white matter (Pcalib. WM), white matter hyperintesity (WMH) mean and volume, and mean arrival time in gray matter (Arr. Time GM) or white matter (Arr. Time WM), cognitive measure mini-mental state exam (MMSE, measured at pre-intervention only), list age-corrected sorting score and age-corrected picture sequence score, memory domain composite score (memory score, average z-scores of list age-corrected sorting score and age-corrected picture sequence score)and brain biomarkers of ADRD risk, whole hippocampus, right and left entorhinal cortex normalized to estimated total intracranial volume (LERC/eTIV, RERC/eTIV).

10 Safety Analyses

When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, severity, etc., each subject will only be counted once and any repetitions will be ignored; the denominator will be the total population size.

Per ClinicalTrial.gov reporting requirements, three tables summarizing adverse events are required and will be created:

- All-Cause Mortality (only required if primary outcome completion date is on or after 01/18/2017): A table of all anticipated and unanticipated deaths due to any cause, with number and frequency of such events in each arm/group of the clinical study.

- Serious Adverse Events: A table of all anticipated and unanticipated serious adverse events, grouped by organ system, with number and frequency of such events in each arm/group of the clinical study. (See Adverse Events definition below).

- Other (Not Including Serious) Adverse Events: A table of anticipated and unanticipated events (not included in the serious adverse event table) that exceed a frequency threshold (for example, 5 %) within any arm of the clinical study, grouped by organ system, with number and frequency of such events in each arm/group of the clinical study.

10.1 Adverse Events

When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, severity, etc., each subject will only be counted once and any repetitions of adverse events will be ignored; the denominator will be the total population size. The summary statistics will be produced in accordance with section 9.

10.2 Pregnancies

The study did not perform any pregnancy tests as trying to conceive was an exclusion factor.

11 Reporting Conventions

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as “<0.001”. The mean, standard deviation, quantiles, and any other statistics will be reported to two decimals. Estimated parameters will be reported to 2 significant figures.

12 Quality Assurance of Statistical Programming

Final statistical analysis will be performed using R on an Oregon Health and Sciences encrypted computer with Windows operating system that is stored in a locked office. R packages to be utilized will include lme4, lmerTest, emmeans, Rmisc, and rstatix for statistical analyses; tidyverse, GGally, ggpubr for data visualization; table1, crosstable, flextable, sjPlot, and stargazer for table creation.

To provide high quality code that is understandable, and allows reproduction of the analysis the following points will be followed: At the start of any code file there will be a set of comments that give: the author; the date and time of writing; references to inputs and outputs; reference to any parent code file that runs the child code file.

13 Summary of Changes to the Protocol and/or SAP

04/08/2024 updates to the SAP were made to include the analysis plan for Protocol 2 and the Exploratory ANCOVA analysis plan to include corrected definition of acute reductions of post-session supine blood pressure.
04/11/2024 updates to the SAP were made to 1) utilize the memory and list-sorting age-corrected scores rather than percentiles to calculate the Project 2 Aim1 primary outcome composite cognitive score due to their more normal distributions and 2) update the primary efficacy analysis model from mixed effect model to robust linear regression with Huber weighting. Due to there being no interim data collection timepoint for cognitive and MRI measures, pre-intervention values can be included as covariate rather than the more complex approach of including a time x group interaction. Assessment of residual plots indicated potential influential outliers, the impact of which can be mitigated through use of a

robust regression approach.
05/22/24 The SAP was updated to include correlations between pre- to post-intervention in ambulatory and in clinic systolic and diastolic pressure and change from pre- to post first intervention session supine systolic and diastolic blood pressure.

14 References

15 Listing of Tables, Listings and Figures

Below is a list of tables and figures to be produced.

Protocol 1

Table 1. Study group characteristics at screening

Figure 1. Flow diagram of the Heat Therapy vs Exercise Training in Hypertension clinical trial

Figure 2. Ambulatory systolic and diastolic blood pressure of participants in heat therapy or exercise training intervention groups averaged over 24 hours or daytime or nighttime measurements alone across study timepoints.

Figure 3. Clinic systolic and diastolic blood pressure of participants in heat therapy or exercise training intervention groups at each study timepoint.

Table 2. Estimated effects of heat therapy compared to exercise training on 24H ambulatory and in clinic blood pressure measures across timepoints by mixed effect model

Supplementary Table 1. Estimated effects of heat therapy compared to exercise training on daytime and nighttime ambulatory blood pressure measures across timepoints by mixed effect model

Supplementary Figure 1. Estimates of ambulatory systolic and diastolic blood pressure of participants in heat therapy or exercise training groups averaged over 24 hours or daytime or nighttime measurements alone across study timepoints.

Supplementary Figure 2. Estimates of systolic and diastolic blood pressure of participants in heat therapy or exercise training groups at pre-, interim-, and post-intervention timepoints.

Supplementary Figure 3. Percent change in average ambulatory systolic blood pressure from day to night of participants in heat therapy or exercise training groups across study timepoints.

Supplementary Figure 4. Percent change in average ambulatory diastolic blood pressure from day to night of participants in heat therapy or exercise training groups across study timepoints.

Table 3. Systolic and diastolic blood pressure dipper status by group and timepoint

Figure 4. Clinic secondary outcomes of participants in heat therapy or exercise training intervention groups at each study timepoint.

Supplementary Table 2. Estimated effects of heat therapy compared to exercise training on secondary in clinic outcome measures across timepoints by mixed effect model

Figure 5. Arterial stiffness of participants in heat therapy or exercise training intervention groups at each study timepoint.

Table 3. Estimated effects of heat therapy compared to exercise training on pulse wave

References

1. Yelland LN, Sullivan TR, Voysey M, Lee KJ, Cook JA, Forbes AB. Applying the intention-to-treat principle in practice: Guidance on handling randomisation errors. *Clin Trials*. Aug 2015;12(4):418-23. doi:10.1177/1740774515588097