

TITLE: Smartphone app-guided inspiratory muscle strength training for lowering systolic blood pressure

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GENERAL RESEARCH STAFF

General research staff will assist on this protocol. The Integrative Physiology of Aging Laboratory has a large number of graduate students and professional research assistants (usually 8-12) who are well trained on the clinical tests and sample processing procedures that will be used for this study, as these procedures are already well-established in the laboratory. General research staff who have gone through CITI and biohazard training will assist with collection and analysis of raw data from clinical tests and with sample processing and storage of blood samples. Only those graduate students and staff who have demonstrated proficiency, and been approved by the PI, will perform these procedures.

The roles the general research staff will play in data collection include:

- Administering survey instruments
- Running a computer while the PI performs assessments of vascular function
- Analyzing raw data files
- Entering data into a spreadsheet
- Centrifuging, pipetting, and freezing blood samples

I. OBJECTIVES

This study will pilot-test a smartphone app that independently guides users through an *at-home* inspiratory muscle strength training (IMST) intervention. The efficacy of at-home, app-delivered IMST will be directly compared to an established clinic-based, investigator-supervised IMST program in a randomized pilot clinical trial. The primary outcome will be the change in SBP. The change in vascular function, the change in PI_{MAX} , and adherence to IMST (% of prescribed training sessions completed) also will be assessed. In addition, we will employ a novel 12-month follow-up period in which app use is remotely monitored in all participants under free-living conditions without investigator interaction; this study design element will provide critical insight into app use under *real-world* conditions.

Hypothesis 1: In men and women with above-normal SBP, 6 weeks of at-home IMST will reduce SBP to a similar extent as IMST delivered in a supervised, clinical research setting. At-home and clinic-based IMST will both be safe and tolerable.

Aim 1: To determine changes in casual and home SBP after 6 weeks of at-home vs. clinic-based IMST (75% PI_{MAX} , 30 breaths/day, 6 days/week in both groups) in men and women age ≥ 18 years with initial SBP ≥ 120 mmHg. Safety and tolerability of at-home and clinic-based IMST also will be assessed.

Hypothesis 2: Six (6) weeks of at-home IMST will improve vascular function, increase inspiratory muscle strength, and promote adherence to a similar degree as clinic-based IMST.

Aim 2: To determine **a)** brachial artery flow-mediated dilation (measure of endothelial function), carotid-femoral pulse wave velocity and carotid compliance (measures of large elastic artery stiffness); and **b)** PI_{MAX} before and after 6 weeks of at-home or clinic-based IMST. Adherence also will be assessed in both groups.

Hypothesis 3: During a 12-month free-living period, adherence to IMST will remain high ($\geq 80\%$ of prescribed training sessions performed).

Aim 3: To assess adherence (% of days performing IMST) in all study participants after up to 12 months of app use free from investigator interaction.

II. BACKGROUND AND SIGNIFICANCE

Above-Normal Blood Pressure and Chronic Disease Risk

Having above-normal blood pressure (BP), i.e., ≥ 120 mmHg systolic BP (SBP) and/or ≥ 80 mmHg diastolic BP (DBP), increases risk of developing cardiovascular diseases (CVD), cognitive decline/dementia, chronic kidney disease, and other chronic health problems¹⁻⁴.

Approximately 60% of all US adults have above-normal BP, primarily driven by **above-normal SBP**⁵. Despite the availability of medications to treat above-normal SBP, *~75% of adults with hypertension fail to achieve BP control*⁶. This has led to a *45% increase in the number of deaths attributable to high BP* over the past decade⁷. Currently, above-normal SBP is the single largest *modifiable* risk factor for CV mortality in the United States⁸. Thus, developing **novel strategies** for lowering SBP is an **urgent public health and biomedical research priority**.

Aerobic Exercise for Lowering SBP

Based on growing evidence that above-normal SBP is linked to increased risk of CVD, stroke, cognitive decline, chronic kidney disease, and other chronic conditions, the American College of Cardiology (ACC) and the American Heart Association (AHA) co-released updated Guidelines for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults in 2017⁹. These guidelines emphasize regular aerobic exercise as a first-line intervention for *all stages of above-normal SBP*⁹. Aerobic exercise causes clinically significant reductions in SBP of 2-8 mmHg, with the largest effects apparent in those with the highest initial (baseline) SBP (i.e., stage 2 hypertension; SBP ≥ 140 mmHg)⁹.

Current guidelines call for ≥ 150 minutes of aerobic activity per week¹⁰. However, only ~50% of US adults meet these aerobic exercise guidelines¹¹. The **greatest reported barrier** to achieving aerobic exercise guidelines is **lack of time**¹²⁻¹⁴, while other common barriers include existing physical ailments, financial cost, facility access, and transportation issues¹⁴⁻¹⁷. Therefore, there is a need to develop novel lifestyle strategies that lower SBP and *also overcome critical barriers to adherence* to improve public health.

High-Resistance Inspiratory Muscle Strength Training



Figure 1

Inspiratory muscle strength training (IMST) is a novel **exercise-based** lifestyle intervention that utilizes the diaphragm and accessory respiratory muscles to inhale against resistance using a handheld device (**Figure 1**); exhaling is unimpeded¹⁸. Most biomedical research studies assessing IMST have used a model in which low/moderate resistance to inspiration (e.g., ~30% of maximal inspiratory pressure [PI_{MAX}]) is applied over longer individual training sessions (e.g., ≥ 30 min/session), with multiple sessions per week, resulting in an overall weekly time commitment similar to moderate-intensity aerobic exercise guidelines, i.e., ~150 min/week^{19–21}. However, I have completed a pilot study (described in detail below) in which subjects performed only 30 breaths per session against a *high* inspiratory

resistance (75% of PI_{MAX}) that required only **~5 min/day**, 6 sessions/week, or **~30 min/week of total training time**. Therefore, high-resistance IMST is a highly **time-efficient** mode of physical training. Moreover, by using small, portable, and affordable training devices, IMST can be performed at home or during travel, without requiring special facilities, further *reducing barriers to adherence*²².

III. PRELIMINARY STUDIES

My **AHA Postdoctoral Fellowship**-supported pilot trial²³ (6 weeks, double-blind, randomized, sham-controlled design) assessed **high-resistance IMST** in men and women with above-normal SBP (≥ 120 mmHg) at baseline. The **primary goals** were to establish: 1) the safety, tolerability, and adherence of IMST; and 2) the efficacy of IMST for lowering BP while also assessing potential improvements in vascular function.

Study Design and Results. Men and women aged 50-79 years with SBP ≥ 120 mmHg were randomized to high-resistance IMST (30 breaths/day, 75% PI_{MAX} , 6 days/week) or low-resistance Sham training (15% PI_{MAX}) for a 6-week period. Outcomes were assessed before and after the 6-week intervention. During the training period, participants were assessed weekly in the laboratory under the direct oversight of a research assistant who: 1) documented adherence to the previous week of training; 2) measured subject PI_{MAX} to record IMST-induced improvements in inspiratory muscle strength and adjust their absolute training intensities; and 3) supervised an in-person training session. The other weekly training sessions were unsupervised.

Thirty-six subjects (19M/17F, 68 ± 7 years) completed the study. The cohort included a mix of subjects prescribed antihypertensive medications and unmedicated individuals per 2017 ACC/AHA guidelines recommending combined lifestyle and pharmacological intervention for the management of high BP⁹. Of note, use of antihypertensive pharmacotherapy did not influence the response to the intervention. Subjects were otherwise free of chronic diseases but were enrolled if they had CVD risk factors (e.g., hypercholesterolemia).

Adherence, Safety, and Tolerability: Adherence to clinic-based IMST was excellent, with the IMST group completing 94% of all prescribed training sessions at the target frequency and intensity of inspiratory maneuvers; adherence was similar in the Sham subjects (90%), with no difference in adherence between groups ($p=0.17$). Data also suggest IMST is safe, with only two minor adverse events (neck muscle soreness, transient lightheadedness) in the IMST group that resolved without treatment, and tolerable (no dropouts due to adverse events). These highly promising preliminary results support IMST as a **safe** lifestyle intervention that promotes **excellent adherence** in adults with above-normal SBP.

Maximum Inspiratory Pressure: PI_{MAX} increased from 64 ± 5 mmHg at baseline to 74 ± 4 mmHg at post-testing in the IMST group ($p < 0.01$), whereas PI_{MAX} was unchanged in the Sham group (baseline: 69 ± 5 mmHg, post-testing: 72 ± 4 mmHg; $p = 0.33$). These data support this high-resistance IMST intervention (6 weeks, 6 days/week, 75% PI_{MAX}) as an **effective training stimulus**.

Blood Pressure: Casual (resting) SBP was 9 ± 2 mmHg lower after high resistance IMST but was unchanged with Sham training (**Figure 2A**; $*p < 0.001$ vs. baseline), such that *the reduction in SBP with IMST was significantly greater than the reduction with Sham training* ($p = 0.02$). This reduction in SBP is greater than or equal to reductions observed with aerobic exercise^{9,24}, while requiring only ~30 minutes per week. This improvement in SBP is clinically meaningful as this magnitude of reduction is associated with a 30-40% lower risk of death from CVD²⁵. Six weeks of high-resistance IMST also decreased casual DBP by 2 ± 1 mmHg, with no change in DBP occurring in the Sham training group (**Figure 2B**; $*p = 0.03$ vs. baseline), such that the difference between groups approached significance ($p = 0.08$). This reduction in DBP occurred despite our participants having clinically normal DBP at baseline, further supporting the BP-lowering effects of IMST.

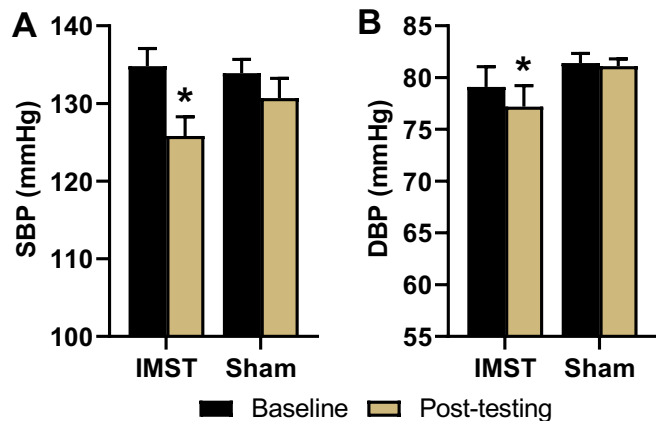


Figure 2

Vascular Endothelial Function: Vascular endothelial dysfunction is an independent CVD risk factor²⁶⁻³⁰. I observed an average improvement in vascular endothelial function (brachial artery flow-mediated dilation; FMD_{BA}) of ~45% following 6 weeks of IMST, with no change following Sham training (**Figure 3**; $*p < 0.001$ vs. baseline), such that *FMD_{BA} was significantly greater after IMST vs. Sham training* ($p = 0.01$). The average improvement in FMD_{BA} measurement units in response to IMST was $\sim 2.5 \Delta\%$, which represents a potentially clinically meaningful effect as each 1 $\Delta\%$ unit increase in FMD_{BA} is associated with an 8-13% lower risk for incident CVD²⁶⁻³⁰.

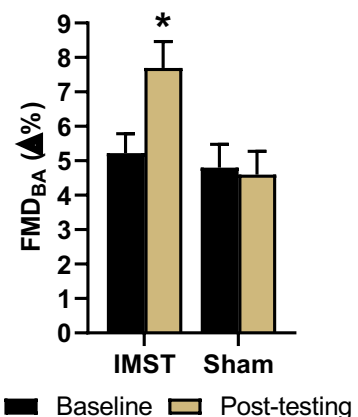


Figure 3

Multi-Trial Analysis of the Antihypertensive Effects of Clinic-Based IMST

I subsequently sought to confirm the above findings by performing a combined, retrospective analysis of the results from 5 pilot trials to date that have assessed clinic-based high-resistance IMST for improving SBP and DBP in various populations³¹. In a larger cohort (67 IMST, 61 Sham), I found that 6 weeks of high-resistance IMST lowers SBP by 9 ± 1 mmHg and DBP by 4 ± 1 mmHg, largely matching the above results from my pilot trial. This analysis also added **key information critical for the public health translation** of IMST. First, it established that age only *very modestly* impacted the effects of IMST on SBP and confirmed the effectiveness of IMST in adults as young as 18 years old (i.e., the lower age limit in this study). Second, it found that use of antihypertensive medication does not impact the SBP-lowering effects of IMST, indicating that IMST could be an effective adjunct treatment in adults taking BP-lowering medications.

IV. RESEARCH STUDY DESIGN

Experimental Design: A randomized, controlled, single-blind, parallel group design clinical trial will be conducted to assess the efficacy of 6-weeks of IMST (55%-75% maximal inspiratory pressure; PI_{MAX}) delivered in the research clinic by the study investigators (i.e., clinic-based IMST) vs. IMST delivered entirely via a smartphone app without investigator involvement (i.e., app-based IMST) for lowering casual (resting) and home SBP in adults aged 18 years and older with above-normal SBP (120-160 mmHg) at baseline. We also will assess the effect of IMST on endothelial function, large-elastic artery stiffness, and potential mechanisms of action.

Subjects will undergo pre-screening and then in-person screening to establish meeting inclusion/exclusion criteria. Subjects deemed eligible will begin baseline testing. Two baseline testing visits will occur in the CTRC, with subjects following the restrictions described below (see section XI. Procedures), in order to assess baseline blood pressure, vascular function, and to obtain blood samples for mechanistic insight. Investigative team members involved in the acquisition and analysis of outcome measurements will be blinded to group assignment.

Following baseline testing, subjects will be randomly assigned to clinic-based or app-based IMST (balanced randomization by age, sex, and SBP) by a research assistant who will remain unblinded for the trial duration. Investigative team members not involved in outcome measure data collection and analysis will oversee IMST, as this duty inherently necessitates unblinding. Participants in both groups will train 6 days/week for 6 weeks. Each training session will involve 30 inhalations against resistance (exhaling is unimpeded) using the PowerBreathe Plus – Medium Resistance inspiratory muscle strength training device. The 30 inhalations will be performed as 5 sets of 6 successive resisted inhalations with a 1-minute recovery period (normal breathing) between each set. Prior to starting IMST, maximal inspiratory pressure (PI_{MAX}) will be assessed by having each participant perform repeated maximal inspiratory maneuvers against a closed airway (the PowerBreathe Plus set to maximum resistance [186 cmH₂O]); the maneuver will be repeated until there is no further improvement in PI_{MAX} and there is less than a 10% difference between the two highest values. PI_{MAX} will be determined as the peak pressure produced during these maneuvers as measured by a pressure transducer placed in the PowerBreathe Plus headset. In the clinic-based group, a study investigator will walk the participant through the PI_{MAX} testing procedure. In the app-based group, the smartphone app will walk the participant through the same procedure.

During week 1, participants in the will train at 55% of their PI_{MAX} . Resistance will then be increased to 65% PI_{MAX} for week 2 and to 75% PI_{MAX} starting week 3; resistance will remain at 75% PI_{MAX} throughout the rest of the intervention. Resistance is set by turning a numbered dial at the base of the PowerBreathe device. Researchers will set the dial to the appropriate setting for the clinic-based IMST group. The smartphone app will instruct participants in the app-based IMST group on the correct numeric dial setting. Participants in the clinic-based group will return to the CTRC for 1 supervised training session per week during which PI_{MAX} will be reassessed, training intensity adjusted, and a training session will be supervised by an unblinded research assistant. Participants in the app-based group will perform all training sessions on their own (i.e., outside of the CTRC environment), with PI_{MAX} assessment and training intensity guided by the smartphone app. The smartphone app automatically instructs participants to reassess PI_{MAX} weekly (i.e., matching the same schedule as the clinic-based group) and instructs participants on the appropriate resistance setting based on the most recent PI_{MAX} assessment and training week (i.e., automatically instructs a resistance setting equivalent to 65% PI_{MAX} during week 2 and 75% PI_{MAX} during week 3).

After 6 weeks of IMST, both groups will continue with their prescribed training program until the completion of post-intervention testing. The frequency of training sessions will be adjusted to allow for a 24-48 hour period between the last IMST bout and the experimental test in order to isolate the chronic (vs. acute) effects of the intervention. To accommodate this adjustment in training schedule, study staff will instruct participants on the appropriate timeframe for their last IMST training session when sending visit reminder emails (i.e., to perform their last training session between Monday at 8am and Tuesday at 8am if the next post-testing session is scheduled for Wednesday at 8am). Post-testing will be done identically to baseline testing.

Expected duration: Baseline (visits 3-4) and end-intervention (visits 5-6) measurements will be scheduled over a 2-10 day period. The order of these visits can be switched as needed in order to facilitate scheduling). Subjects will continue to perform IMST until all post-testing has been completed. In the event of a conflict after the intervention begins (due to scheduling, illness, etc.), the intervention can be shortened to 5 weeks or lengthened to 7 weeks. We project the typical time lapse between completion of screening (visits 1 and 2) and the beginning of baseline testing (visits 3 and 4) will be 2 weeks. Based on the timing information above, the study will take 9 weeks on average to complete but may take between 7-11 weeks depending on visits scheduling and intervention length. The study will require ~11 hours of the subject's time.

Optional follow-up data collection: After completing post-testing, all participants (including those previously randomized to the clinic-based IMST group) will be given the IMST smartphone app and allowed to keep their PowerBreathe Plus – Medium Resistance device. Participants will be asked if the study team can remotely monitor their app use (i.e., training frequency and intensity) for up to 12 months. This is being done to assess habitual use of the app under “real world” conditions when participants are not actively participating in a randomized clinical trial, providing critical insight for eventual public health translation. Participants will be required to opt-in to this 12-month observation period (see Informed Consent document) and participation in this observation period is not required for enrollment into the 6-week clinical trial.

Lyft Transportation Option

Participants will be offered the option to utilize Lyft, a HIPAA-compliant transportation solution to enable access to care and services. Many potential participants, especially those living outside of the Boulder area, have expressed to us that transportation to our lab is a barrier to their participation. Our partnership with Lyft aims to minimize this barrier to ensure individuals without regular access to transportation can take part in our studies.

This Lyft option will be offered to participants living within a 40-mile radius of our facility. Exceptions to this radius will be considered on a case-by-case basis by the study PI/Co-I.

We will utilize Lyft services to (1) either request rides for participants to and from our facility or (2) send a ride link to participants to allow them to request a ride for themselves to and from our facility. We anticipate booking the ride for the participant in most cases as they have study visit times scheduled in advance; however, on rare occasions, participants may ask to book rides themselves for minor study activities (i.e., dropping off a monitor, coming in for a blood re-draw, etc.). The study coordinator will familiarize the participant with the details of the Lyft transportation service and provide them with the Lyft Information handout, emphasizing that the drivers only wait up to 5 minutes at the pickup spot. Participants will be given the option to utilize Lyft before consenting to participate in the study—they will be sent the Lyft consent document

over DocuSign or mail prior to the coordinator booking their first ride to the study site to undergo the consent process.

Study Team Requests Ride for Participant:

Our study coordinator will be provided individual sign-on information to access our Lyft Concierge Business account. If the participant has consented to use Lyft and would like a ride to the study site, the coordinator will input the participant's name*, phone number, location and date/time of pickup/drop-off into the ride request. This can either be done day-of or 30 days in advance of their visit. Once the driver is enroute, the participant will be sent an SMS text message or receive a call with the driver's name, vehicle information (model, license plate number), and estimated time of arrival. If the participant misses the original call, they should call Lyft back to receive ride details at 1-800-836-LYFT (5938) as Lyft will not leave a voicemail. Participants do not need an app or smartphone to use the service as it is all coordinated by the study team via their Lyft Concierge Business account. Participants only need a landline or basic cell phone, so they can receive updates on their ride. If they are uncomfortable providing their phone number to Lyft, the study team will input the study coordinator's phone number in the phone number field and communicate driver information (mentioned above) to the participant. The coordinator can monitor the Concierge dashboard to see where the participant is in real-time during the ride. If the driver or participant cancels the trip, the participant will be instructed to contact the study coordinator to request another ride or reschedule the study visit.

Participant Requests Ride Via Link:

If the participant would like to request the ride themselves, the study coordinator can schedule a "flexible ride". To schedule this, the study coordinator will enter the participant's name*, phone number, location and date of pickup/drop off location into the ride request. The participant will receive an SMS text with the link to request a ride. The participant could also call 1-800-836-LYFT (5938) to request the ride once this info has been submitted by the coordinator. The ride expires at the end of the day it was scheduled for. Study coordinators can schedule flexible rides up to 30 days in advance. If the driver or participant cancels the trip, the participant will be instructed to contact the study coordinator to request another ride or reschedule the study visit.

Text: When the participant receives the SMS text, they will confirm the ride details and click "Request Now" to be matched with a nearby driver. Once the driver accepts the ride, the participant will receive another text to track their ride on a map as the driver picks them up. The participant will receive updates about their ride via SMS text.

Call: When the participant is ready to be picked up, they should call 1-800-836-LYFT (5938). They will hear a script confirming their pickup and drop off locations and asking them to press "2" to request their ride. Once they press "2" and hang up, they'll be matched with a nearby driver. Once the driver accepts the ride, the participant will receive updates about their ride via SMS text.

Only participants who have access to a phone with SMS capabilities will be able to schedule flexible rides.

Participant Information and Safety:

Lyft receives the information the study team inputs during the ride request, including: the program name (ours is APP), the name and email address of the person requesting the ride

(study coordinator), participant first and last name*, phone number, pickup and drop-off address, and any pickup or internal notes the study coordinator chooses to include. Importantly, the program name or notes will not include the IRB protocol number or other wording indicating that the trip's purpose is for research. The Lyft driver only receives the participant's first name, pickup and drop-off address, and pickup notes. If the rider has an existing Lyft account (Lyft's systems detect this based on the phone number entered in the ride request), their profile photo and rating will also be shown to the driver. Once the trip is completed, pick up and drop off addresses are removed from the driver's view of their own ride history. All drivers undergo a background check and screening process before they can drive with Lyft; additionally, they are not told that participants are part of a study.

*Last name will be input as "CUB" (CU Boulder) to help maintain participant privacy.

Cost:

The Lyft trip is billed directly to our lab. The participants do not incur any costs for the Lyft ride, nor are they responsible for tipping the driver.

DETAILED DESCRIPTION OF STUDY MEASUREMENTS

SCREENING MEASURES:

- **Casual blood pressure:** Blood pressure will be measured in triplicate (Dinamap) under quiet, comfortable ambient laboratory conditions using a standard blood pressure cuff around the upper arm. The same procedure will be used when assessing blood pressure as an outcome measure (described below).
- **Body mass index:** Subject height and body weight will be measured using a height rod and digital scale (Tanita BwB-800), respectively. Body mass index (BMI) will be calculated as weight (kilograms) divided by height squared (meters²).
- **Medical history.** A researcher will ask subjects about their medical history (current prescription medications, pregnancy status, etc.) that was reported during phone, mail, or secure online screening. Subjects will be prompted to report any additional medical history of medication use that was not previously disclosed. Subjects who report a chronic over medical condition that makes it unsafe to participate in the study (as determined under the discretion of the Medical Director, Dr. Michel Chonchol) will be excluded from the study.

SUBJECT CHATACTERIZATION MEASURES: These measurements are made to characterize the study participants and determine if certain subject factors 1) impact the effectiveness of or adherence to IMST.

- **Family history questionnaire:** A family history questionnaire will be used to obtain information regarding family history of cardiovascular diseases, cancer and diabetes in order to determine if family history may account for any differences in the efficacy of IMST in improving cardiovascular function.
- **Modifiable Activity Questionnaire (MAQ):** Weekly energy expenditure and physical activity will be estimated using the Modifiable Activity Questionnaire, which takes ~15 minutes to complete.

- **Sleep questionnaire:** A custom designed questionnaire (<10 minutes to complete) will be used to estimate sleep quality and duration as these are factors known to influence physiological function. Both sleep quality and duration will be evaluated as possible confounders during data analysis.
- **Gynecological History Survey:** A custom design questionnaire (~5 minutes to complete) will be filled out by women enrolled in the study as gynecological history is known to influence cardiovascular adaptations to exercise training. Information from this survey will be used to identify potential confounders during data analysis.
- **Mini Mental State exam:** This questionnaire will be conducted with a member of the research team and is intended to estimate overall cognitive function.
- **Social Determinants of Health Questionnaire:** Questions adopted from the PhenX Social Determinants of Health Assessments Collection from the National Institute of Minority Health and Health Disparities will be used to assess social determinants of health. The questionnaire will ask questions about housing/living environment, employment and education status, and food and health care access. The questionnaire will be administered using the online data collection platform, REDCap, only at baseline.
- **Other paperwork:** Subjects will fill out the **CTRC Subject Information Form**, the **Medical Review Form**, and fill out and sign a **CU Boulder CTRC health release** document, authorizing the CTRC and research team to obtain and use medical information gathered as part of the study. The Medical Review Form also includes instructions for post-care of blood draw/IV catheter sites.

INTERVENTION ADMINISTRATION AND SUBJECT MONITORING

- **Maximal inspiratory pressure:** Maximal inspiratory pressure will be assessed weekly either at the start of each supervised training session (clinic-based group) or when prompted by the app (app-based group). Maximal inspiratory pressure will be measured near a residual volume after a maximal expiration. Participants will inhale with maximum effort for 2-5 seconds through a PowerBreathe Plus device set to maximum resistance (186 cmH₂O). The pressure waveform will be recorded by a pressure transducer in the PowerBreathe Plus headset and the highest 1-second average inspiratory pressure produced by the participant will represent maximum inspiratory pressure. The maneuver will be repeated until there is no further improvement and there is less than a 10% difference between the two highest values. This protocol is delivered identically via the study team (clinic-based group) or the smartphone app (app-based group), which walks participants through the procedure step-by-step.
- **Inspiratory muscle strength training:** Participants will perform 5 sets of 6 inspiratory efforts (30 breaths total) with the POWERBreathe Plus training device during each training session (6 training sessions/week). Each inspiratory effort will last approximately 2 seconds, with 2 additional seconds between each inspiratory effort for expiration. Approximately 1 minute will be allowed to elapse between each set of inspiratory efforts. Participants in the clinic-based group will perform 1 training session per week in the CTRC supervised by an unblinded study investigator; the other 5 training sessions will be performed unsupervised on their own. Participants in the app-based group will perform 6 training sessions/week guided by the smartphone app, all without study team supervision.

- **Adherence to the intervention:** Participants in both groups will perform 6 training sessions per week for 6 weeks. Adherence will be monitored either through completing a training diary (clinic-based group) or automatically via the smartphone app (app-based group). The training diary in the clinic-based group will be returned weekly during supervised training sessions. Adherence in the app-based group will also be assessed weekly by unblinded members of the study team, though no action will be taken by the study team to influence adherence in the app-based group.
- **Menstrual cycle phase (premenopausal women only):** Menstrual cycle phase has been shown to impact vascular endothelial function assessed as brachial artery flow-mediated dilation (secondary outcome of this study). Therefore, it is important to document menstrual cycle phase each time endothelial function is assessed so that menstrual cycle phase can be included as a potential co-variate when analyzing the results. Because we are documenting cycle phase, for this study, menstrual cycle phase will not influence the timing of vascular visits. Women identified as premenopausal based on the gynecological history survey will complete a short questionnaire that asks them about the date of their last menstrual period (question 1). Women identified as premenopausal and not using hormonal contraceptives will be asked to answer few additional questions about their current vaginal discharge characteristics (questions 2 to 4). These additional questions are independently validated to accurately assess menstrual cycle phase. This questionnaire takes <5 minutes to complete and will be filled out during each experimental visit. Participants will complete the survey on their own, using a computer provided by the laboratory. The questionnaire will state that questions 2 to 4 are optional and that participants do not need to answer all of the questions.

MEASURES TO ADDRESS PRIMARY AIMS:

- **Casual blood pressure:** Systolic and diastolic blood pressure will be measured in triplicate (Dinamap) under quiet, comfortable ambient laboratory conditions using a standard blood pressure cuff around the upper arm.
- **Home blood pressure:** Participants will be given a home blood pressure monitor (Omron 7 series) and instructed on how to operate it. Participants also will be given written instructions on how to operate the monitor (see Blood Pressure Monitor Instructions_23-0411). Systolic and diastolic blood pressure will be measured in triplicate, at home, under quiet, comfortable ambient conditions using a standard blood pressure cuff around the upper arm. Participants in each group will receive an email twice during baseline and twice during post-testing, and once per week (same day each week for an individual participant) during the intervention, instructing them to perform a home blood pressure assessment (see 23-0411 home BP email and 23-0411 home BP email for experimental testing documents). The email will include a link to a REDCap data collection form which participants will use to report their blood pressure (see 23-0411 REDCap home BP form).

MEASURES TO ADDRESS SECONDARY AIMS:

- **Brachial artery flow-mediated dilation (BA-FMD):** Endothelium-dependent dilation will be assessed with BA-FMD. The brachial artery will be imaged using Doppler ultrasonography. After an acceptable image is obtained, a blood pressure cuff placed

around the forearm just distal to the elbow will be inflated to 250 mmHg for 5 minutes. Brachial artery blood flow and vessel diameter will be recorded for 1 minute of baseline and for 2 minutes following rapid cuff deflation. Endothelium-dependent vasodilatory function will be determined by measuring the degree of dilation (peak change in vessel diameter) and forearm reactive hyperemia will be determined by measuring the blood flow response following cuff deflation.

- **Aortic Pulse Wave Velocity (PWV).** Aortic PWV, a measure of large elastic artery stiffness will be determined via transcutaneous tonometry (Noninvasive Hemodynamics Workstation, Cardiovascular Engineering Inc.) of the carotid, brachial, radial, and femoral arteries, recorded consecutively. The time delay (transit time) between the foot of pressure waves measured at each location will be determined by using the R-wave of an ECG recording taken during the tonometry procedure as a timing reference. PWV will be calculated as the distance between measurement sites divided by transit time of the arterial pulse wave.
- **Carotid Artery Compliance (CC).** Arterial compliance (a measure of large artery distensibility) will be measured non-invasively using high-resolution ultrasonography of one artery and applanation tonometry of the opposite artery. This procedure will be performed on the carotid artery. Compliance will be calculated as the change in artery diameter for a given change in pressure.

MEASURES TO ADDRESS TERTIARY AIMS:

- **Blood collection:** Blood will be collected at the CTRC during visits 4 and 6 (~6.4 tablespoons, 95 mL). CTRC nursing staff will place an IV and draw blood. IV placement is required due to the volume of blood being drawn.
 - **Circulating Markers (serum/plasma):** Circulating markers of oxidative stress and inflammation, as well as factors known to modulate physiological function will be measured in serum/plasma at the University of Colorado Anschutz Medical Campus. These measures are: endothelin-1, interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)- α , C-reactive protein (CRP), oxidized LDL (OxLDL), and total antioxidant status (TAS). Blood will also be drawn for a comprehensive metabolic panel and lipid profile, which will be analyzed at Boulder Community Hospital.
 - **Peripheral Blood Mononuclear Cells (PBMCs):** PBMCs will be isolated from blood samples collected in CPT mononuclear cell preparation tubes. To isolate the mononuclear cells, anticoagulated blood in CPT tubes will be centrifuged at 1600 x g in a swing out rotor for 20 minutes at 25 °C. Following centrifugation, the PBMC layer will be pipetted into a 15 mL conical tube. The PBMCs will be repeatedly mixed with sterile PBS and centrifuged at 300 x g. The resulting pellet of PBMCs will be repeatedly washed with Hank's balanced salt solution and reconstituted to a concentration of 5×10^6 cells/mL in Hank's balance salt solution. Isolated PBMCs will be used for the stress resistance analysis described below.
 - **Cell culture experiments:** Serum and plasma will be used to perform experiments in which purchased cultured endothelial cells will be exposed to 10% serum/plasma from human subjects. We will measure protein markers of improved nitric oxide (NO) bioavailability (e.g., endothelial NO synthase), reduced oxidative

stress (e.g., nitrotyrosine and MnSOD), and reduce inflammation (e.g., NFkB activation) in cells in order to determine the effects of circulating factors upregulated by IMST on endothelial function. To gain insight into which factors in the circulation may be involved, exosomes will be isolated from plasma and used in cell culture experiments. Recent evidence indicates exosomes (microparticles from all cell types that contain the same molecules as the parent cell cytoplasm) are important mediators of cell signaling. Importantly, we can also identify the parent cell types, providing powerful mechanistic insight and targets for future related research.

OPTIONAL DATA COLLECTION (FOLLOW-UP TESTING)

- **Long-Term Adherence:** During the optional 12-month follow-up testing period, adherence will be determined at the number of training sessions correctly performed by the participant divided by the number of training sessions recommended by the app (multiplied by 100) to calculate % adherence. Training sessions performed at the incorrect intensity will not be counted as adherent. Training sessions data will automatically be measured by the smartphone app. Average adherence will be calculated for each month and compared over time to assess potential changes in adherence over time.
- **Long-Term P_{IMAX}:** During optional follow-up testing, the smartphone app will continue to prompt participants to measure their P_{IMAX} on a weekly basis. P_{IMAX} data will automatically be measured by the smartphone app. Average P_{IMAX} will be calculated for each month and compared over time to assess potential changes in inspiratory muscle strength over time.

SAMPLE SIZE CALCULATIONS & STATISTICAL ANALYSES

Statistical Power. We intend to enroll a total of 48 participants (24/group). This sample size is based on feasibility and availability for this pilot clinical trial, not on pre-specified statistical power. Based on my experience with previous trials testing IMST, this will account for up to a 20% subject dropout rate and result in a final sample size of at least 40 participants (20/group) for final data analysis. While statistically comparing the pre-post change in SBP between groups is the primary objective, more emphasis will be placed on estimating the difference between the two conditions in the pre-post change in SBP (i.e., comparing the effectiveness of at-home and clinic-based IMST for lowering SBP), informing the design of a future larger study.

Based on previous research testing 6 weeks of high-resistance IMST²³, a standard deviation (SD) of the pre-post change in SBP could vary between 5.5 mmHg and 10 mmHg. With n=20 per group, the distance from sample mean to the limits of the 95% confidence interval will vary from 3.5 mmHg to 6.4 mmHg. Assuming the SD of the change to be 7 mmHg and 8 mmHg, then the distance will be 4.5 mmHg and 5.1 mmHg, respectively. Similarly, for FMD_{BA} (secondary outcome), the SD of pre-post change could vary from 1.7 Δ% to 2.9 Δ%, and the distance from the sample mean to the limits of the 95% confidence interval will vary from 1.1 Δ% to 1.8 Δ%. Assuming the SD of change to be 2.0 Δ% and 2.2 Δ%, then the distance will be 1.3 Δ% and 1.4 Δ%, respectively.

Randomization. To avoid severe imbalance in the number of participants assigned to either group, a blocked randomization scheme will be used to balance for sex (male/female), age (within 5 years), and baseline SBP (elevated BP: 120-129 mmHg; stage 1 hypertension: 130-140 mmHg; stage 2 hypertension: 140-160 mmHg). The block randomization scheme will be

stratified such that participants within each of the strata will be blocked randomized to either clinic-based or app-based IMST. Randomization will be performed by a research assistant, who will remain unblinded for the duration of the protocol. The investigators responsible for the collection of outcome variables will be blinded regarding group assignment.

Statistical Analyses. Descriptive statistics will be calculated for all baseline and outcome variables. Means and SDs will be calculated for continuous variables and frequency and proportion for categorical and ordinal variables. If a continuous variable has a skewed distribution, median and interquartile range (IQR) will be calculated, and data transformation will be performed before further analysis if appropriate. The 95% CI also will be calculated if appropriate. Tables and charts will be used to present the results. All analyses will be performed using SAS 9.4 or higher (SAS Institute, Cary, NC). The 2-sided significance level of 0.05 will be used in making a conclusion.

The 95% CI of pre-post change in outcome measures will be calculated for each group, respectively. Furthermore, the 95% CI of the difference between the two groups in the pre-post change in outcome measures will be calculated. To examine the intervention effect of app-delivered IMST on outcomes compared with clinic-based IMST, the measures at end-intervention (i.e., after 6 weeks of training) will be regressed on the baseline measures and randomization group. Furthermore, age, sex, and other covariates (e.g., blood lipids) can be added for adjustment. Finally, the percentage of prescribed training sessions performed and its 95% CI will be calculated. Observational data will be collected for up to 12 months and primarily analyzed descriptively to inform long-term performance of app-delivered IMST under real world conditions.

Missing Data and Sub-Group Analyses. Given the study design, conditions of the intervention, and outcome assessment protocols, we anticipate that missing data due to subject dropout will not be more than expected, i.e. 20%. There is no reason to expect that if a subject decides to withdraw from the study because of the intervention condition that the dropout will be associated with the study outcomes. Thus, we assume missing data will be missing at random (MAR) if not missing completely at random (MCAR). Given this, complete case analysis will be employed in the analysis of all outcomes. My sample size has taken potential dropouts into account, so we anticipate having appropriate power to detect differences in our primary outcome measure (casual SBP). The planned sub-group analyses will include race, sex, and age groups.

Name of procedure/instrument/tool	Purpose (i.e., what data is being collected?)
Family history questionnaire	Obtain information about family history of cardiovascular disease, cancer, and diabetes to determine if family history influences outcome measures.
Modifiable Activity Questionnaire, MAQ	Obtain information about normal physical activity levels to determine if chronic physical activity influences outcome measures.
Sleep questionnaire	Obtain information about sleep quality and duration to determine if sleep influences outcome measures.

Medical history	To ensure participants are healthy enough to participate in the study.
Mini mental state exam	Obtain information about participant cognitive function.
Gynecological history survey (women only)	To determine if gynecological history influences outcome measures
Social determinants of health questionnaire	Obtain information about social factors and determinants to elucidate if these factors influence the response to fisetin.
Height measurement	Establish subject characteristics (BMI).
Body weight measurement	Establish subject characteristics (BMI).
Casual blood pressure	Establish subject characteristics and to determine if IMST improves resting blood pressure
Home blood pressure	To determine if IMST improves blood pressure measured in the home setting.
Flow-mediated dilation	Measure of vascular endothelial function.
Blood draw	To evaluate general blood chemistry, and mechanistic markers.
Maximal inspiratory pressure	Used to determine IMST training intensity.
Aortic pulse wave velocity	Measure of arterial stiffness.
Carotid artery compliance	Measure of arterial elasticity.
Adherence	Used to monitor the percentage of prescribed training sessions completed.
Menstrual cycle phase (premenopausal women only)	Obtain information about participant menstrual cycle phase during experimental testing as phase can impact cardiovascular function.

V. FUNDING

This research is being funded by the National Institutes of Health (R03 HL171108).

VI. ABOUT THE SUBJECTS

After obtaining their written informed consent, men and women of all races and ethnic backgrounds with SBP 120-160 will serve as subjects. A total of 48 subjects (24/group) will be randomly assigned to the: app-based group or clinic-based IMST group. To be eligible to participate in this research study, volunteers must meet the following criteria:

Total planned enrollment: 96

This will account for subject disqualification based on inclusion/exclusion criteria (~50%) and dropout/failed testing (~20%) and ensure that 40 subjects (20/group) complete the study.

Inclusion criteria:

- Age 18+ years
- Ability to provide informed consent

- Willing to accept random assignment to condition
- Systolic blood pressure 120-160 mmHg, determined as the average from resting blood pressures taken during screening visits 1 and 2 by Integrative Physiology of Aging Laboratory staff
- Owns an Apple or Android smartphone
- Body mass index $<40 \text{ kg/m}^2$ as measured by study staff during Visit 1 (vascular function measurements can be inaccurate in people with a BMI $\geq 40 \text{ kg/m}^2$)
- Subject report of being weight stable in the prior 3 months ($<2 \text{ kg}$ weight change) and willing to remain weight stable during the 6-week intervention period
- Subjects taking antihypertensive medications will be included provided they meet the other inclusion criteria, including systolic blood pressure. Medication regimen (prescription and dosing) must be stable for at least 3 months prior to enrollment in the study and must remain stable during the 6-week intervention period. These medications will not be withheld prior to experimental protocols.
- If woman of childbearing age:
 - Not pregnant (defined as self-report of pregnancy)
 - Willing to be abstinent or use approved contraception (i.e., hormonal contraception, intrauterine devices, barrier methods such as condoms with spermicide, and surgical sterilization) throughout the duration of the study

Exclusion criteria:

- Age <18 years
- Subject report of a chronic overt medical condition (e.g., unstable cardiovascular disease, recent myocardial infarction or stroke, cancer) that may make it unsafe to participate in the study under the discretion of the Medical Director, Dr. Michel Chonchol.
- Inability to abstain from consumption of alcohol for 12 hours on experimental days (subject report)
- Subject report of blood donation within 8 weeks prior to enrolling in the study or unwillingness to abstain from donating blood for 8 weeks after completing the study
- Subject report of current ruptured eardrum or any other current condition of the ear
- Subject report of recent abdominal surgery (past 3 months) or current abdominal hernia
- Subject report of current asthma with very low symptom perception, frequent and severe exacerbations, or abnormally low perception of dyspnea
- Subject report of past or current costochondritis (inflammation of the cartilage that joins the ribs to the breastbone)

Dr. Chonchol will make final decisions on whether it is safe for all subjects to participate in the study. The PI will verify Dr. Chonchol's approval and verify that **all** inclusion/exclusion criteria are met before participants are enrolled in the study.

Subject Population(s)	Number to be enrolled in each group
Men and women of all races and ethnicities, age 18+ years with SBP 120-160 mmHg.	96 sign the informed consent and enrolled in the study; 48 randomized app-based or clinic-based IMST (24/group).

VII. VULNERABLE POPULATIONS

Vulnerable populations will not be considered for this study.

VIII. RECRUITMENT METHODS

Recruitment Materials: Participants will be recruited from Boulder County and the surrounding areas with the materials and methods outlined in IRB protocol #24-0146.

These recruitment methods represent those that we have successfully applied to other studies previously implemented by the Integrative Physiology of Aging Laboratory, which has successfully recruited men and women with above-normal blood pressure for studies over the past 25 years. The Denver/Boulder recruitment area exceeds 2.5 million in total population, over 50% being female. Thus, we anticipate no significant problems in recruiting the necessary numbers for this study.

Pre-consent: Participants will respond to study advertising by email or telephone. Those responding by email will be emailed the email screening script, which begins with pre-consent; those responding by phone will immediately undergo a telephone pre-consent and be read the phone screening script. Both forms contain language to inform subjects that they can choose to complete the pre-screening questions online (via REDCap, refer to protocol #24-0146 for details on the General REDCap Screening Form) or over the phone (read the *phone screening script*), depending on the participant's preference. A study information form will be included in the materials emailed to participants.

Participants who meet the inclusion criteria will then be contacted and scheduled for their screening visit (Visit 1) or scheduled at a time when they meet inclusion criteria (e.g., have been weight stable for 3 months). This screening may take place under the present protocol or under IRB protocol #24-0146. If individuals screened under protocol #24-0146 pre-qualify for the present study, the data collected under IRB protocol #24-0146 may be used as screening data for the present protocol (e.g., height, body weight, blood pressure) as long as it has been no more than one month since that data was collected. Participants who do not meet the inclusion criteria will also be notified and may choose to be considered for other ongoing studies in our laboratory.

Pre-screening form: The screening form will be administered by phone or securely online through Research Electronic Data Capture (REDCap). Refer to protocol #24-0146 for details on the pre-screening form and process.

List recruitment methods/materials and attach a copy of each in eRA

Refer to #24-0146

IX. COMPENSATION

Subjects will receive monetary reimbursements for completion of experimental sessions. Subjects will be paid approximately \$20 per hour for each experimental testing day and \$10 for each week of the intervention completed (up to \$60 total for the 6-week intervention) in the form of a check. If subjects complete the entire research study, they will receive **\$165** upon completion. If subjects choose to withdraw before the conclusion of this study, the prorated amounts subjects will receive for each visit are outlined below. There is no compensation for the screening sessions (Visits 1 and 2) because subjects will receive important blood pressure information from screening. Compensation does not include travel time – we will compensate

subjects for their travel for all visits (including Visits 1 and 2), if they wish, at the current CU approved rate.

Subjects will be asked to fill out a **Payment Form & W-9** to verify receipt of compensation. Subjects will be informed that any research compensation greater than \$600 within one calendar year will be subject to IRS reporting.

Visit	Approx. time per visit	Compensation
3	2 hours	\$40
4	45 minutes	\$15
5	1.5 hours	\$30
6	1 hour	\$20
1 week of IMST	30 min/week	\$10 (\$60 for all)
Total	8.25 hours	\$165

Subjects that complete the study will also be allowed to keep the POWERbreathe Plus IMST training device (monetary value: \$65) that they used in the study.

X. INFORMED CONSENT

Informed consent: Informed consent will be obtained in a quiet, private room at the Clinical Translational Research Center, located within the Ramaley Biology Building, or online via Zoom and DocuSign. The PI or other key study personnel who have completed the CCTSI CTSA course CTSA2: Informed Consent: Overview of a Process, and have been delegated the responsibility of obtaining informed consent by the PI will explain all study procedures to potential participants prior to their participation in any experimental procedure. Participants will then be given time to read through the informed consent document and invited to ask any questions. A member of the research team will also ask participants questions about the study procedures to ensure participant comprehension and autonomy (e.g., “how long will you be in this study?” or “what is the treatment/intervention?”). If the subject is unable to answer these questions within reason (e.g., believes the study to be 1 week or does not know that the intervention involves a type of breathing exercise), they will not be included.

It is possible that a person enrolled in the study could be an employee. We tell the person that participation in the study is voluntary and no aspect of their participation or non-participation has an effect on their employment or salary. The person conducting the screening and consenting is not the employee’s supervisor.

Process to document consent in writing: If an individual decides to participate in the study, he or she will be asked to sign the last page of the consent form to document their consent. The investigator obtaining consent will also sign the last page of the consent form. A copy of the signed consent form will be provided to the participant.

XI. PROCEDURES

OVERVIEW OF STUDY VISITS

Recruitment and Screening: the screening procedures described below will be performed over a 2-10 day period to obtain informed consent and determine if subjects qualify for the study.

Note: Visit 1 can either take place completely in person or it can be split into two visits (1a and 1b). Visit 1a will take place over Zoom and will include reviewing and signing the informed consent document and going over medical history. Visit 1b will take place, as described below, at the CTRC and will include the measures not performed during Visit 1a (i.e., resting blood pressure and height and body weight). The time commitment for Visits 1a and 1b will be about 1 hour total.

During the duration of the study (visits 1 through 6) Subjects will be asked to avoid major lifestyle changes including large changes in physical activity, body weight, and diet unless their physician determines changes are needed. Subjects will be asked to inform the research staff if there is a change in any of these (either because they are recommended by their physician or they choose to make changes) or if they get sick to reschedule visits. Subjects will be requested to maintain a stable medication regimen for the duration of the study, or to notify the research team should their physician recommend a change in medication during the course of the study.

- **Phone recruitment and REDCap screening survey** (after obtaining pre-consent)
- **Visit 1: Informed consent and screening, CTRC (~1 hour):** *Prior to this visit, subjects will be asked to abstain from non-prescribed over-the-counter medications and supplements for 48 hours, food and caffeine for 5 hours, alcohol and strenuous exercise for 24 hours.*
 - Informed consent
 - Resting blood pressure
 - Height and body weight (to calculate BMI)
 - Medical history
 - CU Boulder CTRC Health Release, Medical Review, and Subject Information forms

Note: If participants undergo the general screening visit described in IRB protocol #24-0146, Visits 1 and 2 can be combined, and participants will not need to repeat measurements performed in the general screening visit (e.g., blood pressure) so long as it has been no more than 1 month since the general screening visit.

- **Visit 2: Screening, CTRC (~45 minutes):** This visit will occur 1-9 days after completion of Visit 1. *Prior to this visit, subjects will be asked to abstain from non-prescribed over-the-counter medications and supplements for 48 hours, food and caffeine for 5 hours, alcohol and strenuous exercise for 24 hours.*
 - Resting blood pressure
 - Blood sampling: **~6.4 tablespoons** (~95 mL) will be drawn
 - Assays to be run at BCH: comprehensive metabolic panel, lipid profile, complete blood count (CBC)
 - Assays to be run at the University of Colorado Anschutz Medical Campus: interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)- α , C-reactive protein (CRP), oxidized LDL (OxLDL), and total antioxidant status (TAS)
 - Assays to be run by the research team: serum and plasma collection for use in cell culture experimental, exosome isolation, PBMC protein isolation

Experimental Baseline Testing: Subjects who qualify based on screening visits 1 and 2 will be notified and schedule baseline testing. Baseline testing will occur over a 2-10 day period; visits may be rearranged to facilitate scheduling.

- **Visit 3: Blood pressure, vascular function, CTRC (~2 hours):** *Prior to these visits, subjects will be asked to abstain from non-prescribed over-the-counter medications and supplements for 48 hours, food and caffeine for 5 hours, alcohol and strenuous exercise for 24 hours.*
 - Blood pressure at rest
 - Questionnaires/paperwork:
 - Family history questionnaire
 - Physical activity (Modifiable Activity Questionnaire, MAQ)
 - Cognitive function (Mini Mental State Exam, MMSE)
 - Sleep questionnaire
 - Gynecological History Survey (women only)
 - Menstrual cycle phase (premenopausal women only)
 - Social determinants of health questionnaire
 - Body weight measurement
 - Aortic pulse wave velocity
 - Carotid artery compliance
 - Brachial artery flow-mediated dilation
 - Home blood pressure monitor device pickup: participants will receive an email (see 23-0411 home BP email for experimental testing document) instructing them to perform 2 home blood pressure measurements prior to initiating the intervention.
- **Visit 4: Blood pressure, vascular function, CTRC (~45 minutes):** *Prior to these visits, subjects will be asked to abstain from non-prescribed over-the-counter medications and supplements for 48 hours, food and caffeine for 5 hours, alcohol and strenuous exercise for 24 hours.*
 - Blood pressure and heart rate at rest
 - Brachial artery flow-mediated dilation
 - Menstrual cycle phase questionnaire (premenopausal women only)

Intervention: *After completing Visits 3 and 4, participants will be randomized into either the clinic-based or app-based IMST group.*

Clinic-based IMST:

- Participants in the clinic-based group will be given a PowerBreathe Plus IMST device and shown how to use it by an unblinded research assistant.
- Participants will perform 30 breaths/day, 6 days/week, for 6 weeks at an intensity of 55% PI_{MAX} during week 1, 65% PI_{MAX} during week 2, and 75% PI_{MAX} during weeks 3-6.
- One training session per week will be performed in the CTRC, supervised by a research assistant. The other 5 training sessions/week will be performed unsupervised with training documented by subjects completing a training log (see *Training Log* document).
- PI_{MAX} will be reassessed at each weekly training session and training intensity will be adjusted by the research assistant at the training session.
- Home blood pressure will be assessed weekly when prompted via email from the study team.

App-based IMST:

- Participants in the app-based group will be given a PowerBreathe Plus IMST device and a study team member will help them install the IMST app on their phone.
- Participants will perform 30 breaths/day, 6 days/week, for 6 weeks at an intensity of 55% PI_{MAX} during week 1, 65% PI_{MAX} during week 2, and 75% PI_{MAX} during weeks 3-6.
- All training sessions will be performed unsupervised with training documented by the IMST app.
- PI_{MAX} will be reassessed weekly, when prompted by the smartphone app. The app will then notify participants to adjust the resistance on the PowerBreathe Plus to the appropriate intensity.
- Home blood pressure will be assessed weekly when prompted via email from the study team (see 23-0411 home BP email document).

Post-Intervention Testing: Participants will return to the laboratory for post-intervention testing after completing 6 weeks of clinic-based or app-based IMST. Post-intervention testing will occur over a 2-10 day period; visits may be rearranged to facilitate scheduling. Participants will continue to perform IMST while completing post-intervention testing, but with timing of training adjusted so that IMST sessions occur 24-48 hours prior to visit 5 and visit 6 (i.e., no IMST performed within 24 hours prior to a visit).

- **Visit 5: Blood pressure, vascular function, CTSC (~1.5 hours):** *Prior to these visits, subjects will be asked to abstain from non-prescribed over-the-counter medications and supplements for 48 hours, food and caffeine for 5 hours, alcohol and strenuous exercise for 24 hours.*
 - Blood pressure and heart rate at rest
 - Physical activity (Modifiable Activity Questionnaire, MAQ)
 - Body weight measurement
 - Aortic pulse wave velocity
 - Carotid artery compliance
 - Brachial artery flow-mediated dilation
 - Menstrual cycle phase questionnaire (premenopausal women only)
 - Home blood pressure monitor device return: participants will receive an email (see 23-0411 home BP email for experimental testing document instructing them to perform 2 final home blood pressure measurements prior to this visit.
- **Visit 6: Blood pressure, blood collection, vascular function, CTSC (~1 hour):** *Prior to these visits, subjects will be asked to abstain from non-prescribed over-the-counter medications and supplements for 48 hours, food and caffeine for 5 hours, alcohol and strenuous exercise for 24 hours.*
 - Blood pressure and heart rate at rest
 - Blood sampling: **~6.4 tablespoons** (~95 mL) will be drawn
 - Assays to be run at BCH: comprehensive metabolic panel, lipid profile
 - Assays to be run at the University of Colorado Anschutz Medical Campus: interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)- α , C-reactive protein (CRP), oxidized LDL (OxLDL), and total antioxidant status (TAS)

- Assays to be run by the research team: serum and plasma collection for use in cell culture experimental, exosome isolation, PBMC protein isolation
- Brachial artery flow-mediated dilation
- Menstrual cycle phase questionnaire (premenopausal women only)

Optional 12-Month Monitoring:

After completing post-intervention testing, those who opt into optional follow-up monitoring will continue to have their IMST data uploaded to the study database (described under Data Management).

Visit #	Procedures/Tools	Location	How much time the visit will take
Visit 1 (screening visit)	<ul style="list-style-type: none"> • Informed consent (40 min) • Resting blood pressure and heart rate (10 min) • Height (1 min) • Body weight (1 min) • Medical history (10 min) • CTRC forms (5 min) 	CTRC	~1 hours
Visit 2 (screening visit)	<ul style="list-style-type: none"> • Resting blood pressure and heart rate (10 min) • IV placement and blood draw (15 min) 	CTRC	~45 minutes
Visit 3 (experimental visits)	<ul style="list-style-type: none"> • Modifiable Activity Questionnaire (habitual physical activity levels - 15 min) • Mini mental state exam (5 min) • Sleep questionnaire (5 min) • Gynecological History Survey (women only – 5 min) • Menstrual cycle phase questionnaire (premenopausal women only – 5 min) • Social determinants of health (10 min) • Resting blood pressure and heart rate (10 min) • Body weight measurement (1 min) • Aortic pulse wave • Aortic pulse wave velocity (15 min) 	CTRC	~2.0 hours

	<ul style="list-style-type: none"> • Carotid artery compliance (15 min) • Brachial artery flow-mediated dilation (20 min) 		
Visits 4 (experimental visits)	<ul style="list-style-type: none"> • Resting blood pressure and heart rate (10 min) • Brachial artery flow-mediated dilation (20 min) • Menstrual cycle phase questionnaire (premenopausal women only – 5 min) 	CTRC	~45 minutes
Visit 5 (experimental visits)	<ul style="list-style-type: none"> • Resting blood pressure and heart rate (10 min) • Modifiable Activity Questionnaire (habitual physical activity levels - 15 min) • Body weight measurement (1 min) • Aortic pulse wave velocity (15 min) • Carotid artery compliance (15 min) • Brachial artery flow-mediated dilation (20 min) • Menstrual cycle phase questionnaire (premenopausal women only – 5 min) 	CTRC	~1.5 hours
Visits 6 (experimental visits)	<ul style="list-style-type: none"> • Resting blood pressure and heart rate (10 min) • IV placement and blood draw (15 min) • Brachial artery flow-mediated dilation (20 min) • Menstrual cycle phase questionnaire (premenopausal women only – 5 min) 	CTRC	~1 hour

Supervised training sessions (n=6; clinic-based IMST group only)	<ul style="list-style-type: none"> Maximum inspiratory pressure (10 min) Supervised training session (10 min) 	CTRC	~30 minutes
*please note, extra time is built into each study visit to account for subject check-in/check-out time			

XII. SPECIMEN MANAGEMENT

Whole blood, plasma and serum will be collected for this study. Except for a subset of blood samples (see next sentence), samples will be labeled with a unique subject identification number (coded) so that samples can only be linked back to individual subjects using a secure spreadsheet only available to the PI and research staff (see next paragraph for details). Blood/plasma samples that will be sent to Boulder Community Hospital for analysis will be labeled with the subject's name and not the identification number. These samples will be destroyed by Boulder Community Hospital after analysis. The names of subjects will not be identified in any publication arising from these studies. In most cases, only average data will be presented in publications. Samples will be de-identified upon completion of the study data analysis and closeout of the protocol, and de-identified biological samples will be kept indefinitely, with subject consent, for use in future (IRB reviewed and approved) studies related to physiological function. Subjects will still be allowed to participate in the rest of the study if they do not want to have their samples stored.

XIII. DATA MANAGEMENT

All subject identities and records will remain strictly confidential. Only the PI and research staff will have access to a majority of the data from this study. As PowerBreathe has developed and maintains the IMST smartphone app, they will have access to participant information such as phone numbers and email addresses. Sharing of this information is inherent with the use of most smartphone applications. Participants will be made aware during the informed consent process that this information will be transferred to PowerBreathe and instructed not to participate in the study if they are uncomfortable sharing this information. Importantly, **PowerBreathe is not sponsoring the study and the study team will not be sharing data or results with PowerBreathe.** PowerBreathe is making the app available to the study team for research purposes ahead of officially releasing the app to the public, which is expected to occur in early 2024.

The data we are collecting is considered Data Security Risk Level 1 (low). Data will be stored in the secure, HIPPA-compliant web platform REDCap. Only authorized individuals (PI and study team) will have access to the data on REDCap, and they will have their own account and password to access the data. REDCap servers are backed up on a daily basis, and the study team has access to every change made in the data via the REDCap "Logging Tool". All computers used to access REDCap are password-protected computers that have a fully patched operating system and applications and current antivirus software with current virus definitions.

App-based data will be transmitted in real-time to an online password-protected study database created by PowerBreathe for this study which will only be accessible to study team members. Data in the database will include the following non-sensitive information: subject code, all $\dot{V}I_{MAX}$ and training session data, dates for all $\dot{V}I_{MAX}$ and training sessions, and prescribed training protocol by date; no identifiable information will be included in the study database. This same process applies to data collected during the optional long-term observation period. The app will continue to collect and transmit data to the study database during the long-term observation period; PowerBreathe will retain access to participant email addresses and phone numbers, but not other data during this time. Data from the database will be exported in CSV format weekly and stored on REDCap. The names of the subjects will not be identified in any publication arising from these studies. With subject consent, biological samples (plasma, serum) will be kept for retrospective analyses related to aging and physiological function (this will not be conducted without IRB approval).

Refer to 24-0146 for details on data management for screening form information.

XIV. PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF PARTICIPANTS

Interested participants will be made aware of the study through recruitment efforts described in 24-0146. They will contact an investigator via phone or email information provided in the recruitment materials. Subjects are allowed to ask any questions they may have about the study prior to providing pre-consent. All email and/or phone communication with participants and potential participants will be kept confidential.

In-person screening and testing will take place in the CTRC, located on the CU Boulder campus. Only Integrative Physiology of Aging Laboratory investigators and CTRC clinical staff will be present during screening and experimental visits. Only information agreed upon in the informed consent will be collected.

Approved Integrative Physiology of Aging Laboratory investigators will have access to all subject data. While a subject is enrolled in the study, CTRC personnel will have access to the CTRC subject information form, CTRC health release document, and Medical Review form. These forms will be given to Integrative Physiology of Aging Laboratory investigators after the subject completes or withdraws from the study; CTRC personnel will not keep a copy. As described above in Section XIII, participants will be informed that they will have to provide their phone number and email address to PowerBreathe in order to use the IMST smartphone app.

Presented subject data (i.e., in manuscript or presentation form) will be presented as group mean data in most cases. Individual subject data will rarely be presented; when individual subject data are presented, the data will not be associated with information that could be used to identify participants in the study.

XV. WITHDRAWAL OF PARTICIPANTS

Primary exit/stopping criteria:

- Completion of the study
- Withdrawal of informed consent (participant's decision to withdraw for any reason)

- Any clinical adverse event (AE), laboratory abnormality or intercurrent illness, which, in the opinion of the medical director, indicates that continued participation in the study is not in the best interest of the participant.
- Significant non-compliance with the protocol (i.e., procedures, assessments, etc).

Study stopping criteria:

The investigators or medical director may decide to stop the study if:

- The data show a significant increased risk of serious adverse effects in one of the treatment groups.
- It becomes clear that successful completion of the study is not feasible (e.g., there is an excess of patient dropout, missing data, lack of recruitment, etc.).

The number of participants exiting the study and the reasons for exit will be carefully documented. We have accounted for approximately 20% of participants to withdraw from the study (plan to enroll 48 participants to ensure completion of 40 participants), so participants will not be replaced.

XVI. RISKS TO PARTICIPANTS

We see no psychological, social, or legal risks beyond those of participation in health-related research in general. The potential physical risks of participating in the proposed experiments are reasonably small. Importantly, the procedures have been used previously in the Integrative Physiology of Aging Laboratory without complications.

The risks associated with the experimental protocols include:

Inspiratory muscle strength training - While performing spirometry and breathing maximally, it is possible that participants may begin to feel light-headed, dizzy, or experience momentary shortness of breath. To minimize the likelihood of these events participants will be allowed recovery time between each task. There is also a chance that participants will experience ear discomfort during training; participants will be instructed to inform the study team if they experience ear discomfort. While unlikely, participants could experience pain while training. Participants will be instructed to stop training immediately if training feels painful and inform the study team. Inspiratory muscle strength training has been performed in the Integrative Physiology of Aging Laboratory since 2017 without any serious adverse events, or reports of ear discomfort or pain during training.

Blood draws - When the needle goes into a vein, it hurts for a short time and there may be redness or swelling around where the needle goes into the skin. There is a small chance they may feel lightheaded or faint. In about 1 in 10 cases, a small amount of bleeding under the skin will cause a bruise. A risk of a blood clot forming in the vein is about 1 in 100. The risk of infection or significant blood loss is less than 1 in 1,000. The maximum total amount of blood we are drawing over the duration of the study is about 13 tablespoons (190 mL). This amount is less than what is taken during a standard blood donation; however, participants will be instructed to not donate blood while participating in the 6-week intervention arm of this study or for 8 weeks before baseline testing or after post-testing.

Brachial artery flow-mediated dilation (vascular endothelial function) - Inflating the blood pressure cuff during this procedure may cause a mild to moderate “pins and needles or numbing” sensation. This feeling may persist for up to 5 minutes after the cuff is deflated.

Breach of confidentiality - As in all research involving human participants, there is a risk for breach of confidentiality. The IMST app is made by PowerBreathe International Ltd. As with most smartphone applications, using the IMST app involves transmission of user phone number and email address to the group that manages the app. Participants will be informed only to agree to participate if they are comfortable with PowerBreathe having this information about them.

XVII. MANAGEMENT OF RISKS

The potential risks of the proposed study will be minimized by:

- Allowing only men and women without self-reported overt clinical cardio-metabolic diseases or clinical manifestation of cardiovascular disease (CVD) to participate.
- Using only safe, well-established procedures with only qualified and experienced personnel performing the procedures, along with implementing planned procedures for managing expected adverse events
- Ensuring constant personal monitoring of each experimental session by the investigators
- Measuring electrocardiogram (ECG) continuously during tests of vascular function
- Employing record keeping processes with confidentiality. Individual participant data will not be associated with participant name.
- Providing appropriate supervision and emergency equipment in the CTRC
- Regular monitoring of the protocol by the PI and research coordinator
- Keeping all records in REDCap or in safe, locked facilities on the CU Boulder campus
- Not associating individual subject data with subject name (all stored data will be coded)
- Instating appropriate clinical supervision, availability of emergency equipment and medications (with well-planned emergency procedures), and the overall safety provided by the CTRC environment.

Specific Risks Associated with **inspiratory muscle strength training** will be minimized by:

- Excluding any individuals who report having a condition that would be contraindicated for inspiratory muscle strength training (i.e., individuals with unstable cardiovascular disease or pulmonary disorders, ruptured eardrum, costochondritis)
- Careful screening of participants
- Careful monitoring of participants during the intervention
- Monitoring the status of all participants throughout the protocol by the physician of record, Dr. Chonchol: Dr. Chonchol will review all subject information prior to enrollment (to determine eligibility), will be informed on the status of subjects regularly throughout the protocol by the research staff/PI, and will be informed immediately of any adverse events
- Gradual, progressive increase in inspiratory muscle strength training intensity

XVIII. POTENTIAL BENEFITS

Because the risks of participating in this study are small, the risk-to-benefit ratio is also relatively small. Participants will receive benefits associated with overall knowledge of their health from

the extensive testing performed to established participant characteristics (i.e., blood glucose status, plasma lipids and lipoprotein profile, blood pressure).

The findings of the proposed research should provide innovative and important new information regarding the effects of inspiratory muscle strength training on physiological function. This information will improve our understanding of a novel lifestyle intervention that could be used to lower blood pressure and prevent and/or treat hypertension-associated physiological dysfunction and cardiovascular disease.

XIX. PROVISIONS TO MONITOR THE DATA FOR THE SAFETY OF PARTICIPANTS

The PI, co-investigators and/or other research staff will continually monitor and report annually on adherence to the protocol with regard to the recruitment plan and outcome data. The PI and Research Coordinator with the assistance of the CU Boulder CTRC staff will maintain adherence to inclusion/exclusion criteria (see Subject Description) and collection of safety data. Abnormal laboratory values obtain from blood testing (e.g., blood chemistry tests) will be reviewed by the Medical Director, Dr. Michel Chonchol. Dr. Chonchol will determine if incidental findings from blood tests need to be reported to the study participant and advise on appropriate next steps. Adherence will be assessed by contrasting current procedures to documented approved procedures to ensure there are no discrepancies. Research records will be reviewed on a daily basis and any protocol violations, should they arise, will be reported within one week.

Medical oversight for subjects enrolled in CTRC studies at the Boulder CTRC occurs through the joint actions of the study investigators and Boulder CTRC medical staff. The latter are under the supervision of the Medical Director of the Boulder CTRC, who assumes full responsibility for ensuring that their activities conform to the policies of medical oversight. Furthermore, Dr. Michel Chonchol, board-certified nephrologist and Professor Adjunct in the CU Boulder Department of Integrative Physiology, will serve in the capacity of physician of oversight for the research protocol.

The initial protocol (following IRB approval) will be reviewed by the Boulder CTRC Safety Monitoring Committee (SMC) for feasibility. The SMC consists of the CTRC Research Subject Advocate, the CTRC Medical Director, the CTRC Administrative Manager, a CTRC staff physician, the CTRC Pharmacist, the CTRC Biostatistician, a representative of the CTRC nursing staff, two principal investigators, and such other members as the SMC may direct.

The investigators and CU Boulder CTRC staff will examine safety data on a continuous basis (as the tests are being performed) and will document any inconsistencies. They will assess the data based on the inclusion/exclusion criteria. Abnormal data from screening failures resulting in exclusion of participants from participation will be reviewed by the CTRC staff physician and Dr. Chonchol. Based on the staff physician's or Dr. Chonchol's recommendations the participant will be notified regarding the data, the implications, and whether a follow-up with their primary care physician is warranted.

1) Performance of Procedures

- a) Informed consent for all study subjects will be performed by the study investigator or a protocol specific member of the investigative team. As part of the informed consent process, study investigators will ask subjects participating in a procedure if they understand the risk and if they have any questions. To ensure the subject is able to give

informed consent, the individual obtaining informed consent will ask the participant questions about information discussed in the consent such as “how long will you be in this study?” or “what is the treatment/intervention?” If the subject is unable to answer these questions within reason (e.g., believes the study to be 1 week or does not know that the intervention involves breathing exercise), they will not be included.

- b) Boulder CTRC medical staff will perform medical procedures as outlined in study protocols that have been approved by the IRB in accordance with standard medical practice guidelines, evaluate and monitor subjects with ongoing adverse events, and respond to emergency situations that arise. All CTRC staff are familiar with the scientific purpose of the studies, the risks and benefits of the studies, and the procedures involved in the studies.
- c) Boulder CTRC medical staff are obligated to halt a procedure if they believe there is a potential risk to the subject that has not been disclosed or that the subject may be at greater risk than the usual study subject because of an underlying medical condition, regardless of whether that condition is part of the inclusion/exclusion criteria.
- d) Boulder CTRC medical and clinical staff are required to adhere to the inclusion/exclusion criteria and the experimental procedures specified in the Boulder IRB approved protocols. Any protocol deviations will follow the Medical Oversight for Studies Performed on the Boulder CTRC document and IRB’s requirements using their Reportable New Information Form (HRP-214-FORM).
- e) Boulder CTRC medical staff will report all adverse events or protocol deviations to the study PI.

2) Disclosure/Interpretation of Clinical Information

- a) Boulder CTRC medical staff will disclose and provide interpretation of medical information relating to CTRC studies to both the investigators and study subjects.
- b) Boulder CTRC medical staff will not withhold medical information from study subjects unless the withholding of that information is explicitly sanctioned in the study protocol and has been approved by the IRB. Any protocol in which medical information is to be temporarily withheld must specify when and by whom the medical information is to be transmitted to the study subject.
- c) All clinically significant medical information obtained through Boulder CTRC studies must be shared with study subjects and/or their physicians. There must be clear documentation in the Boulder CTRC medical records as to when and by whom this information was transmitted.

3) Management of Adverse Events

- An adverse event reporting form will be completed when an adverse/unanticipated event is considered to be related to the use of a medical treatment or investigational procedure during the protocol at the CU Boulder CTRC. An adverse event is defined as any unfavorable symptom, sign, or disease (including abnormal laboratory finding) temporally associated with the use of a medical treatment or investigational procedure that may be considered related to the medical treatment or investigational procedure (modified from the National Cancer Institute’s Common Toxicity Criteria). The Boulder CTRC follows the guidelines established by the National Cancer Institute’s Common Toxicity Criteria (<http://ctep.cancer.gov/reporting/ctc.html>) in defining and reporting adverse events. Adverse/unanticipated events may be identified and reported by the principal investigators as well as the CTRC personnel involved in the implementation of the protocol. Harm experienced by a subject or other individual, which in the opinion of

the investigator or CTRC staff is unexpected and probably related to the research procedures, will be reported to the Boulder IRB within 5 days. According to the Boulder IRB, a harm is “unexpected” when its specificity or severity are inconsistent with risk information previously reviewed and approved by the IRB in terms of nature, severity, frequency, and characteristics of the study population. According to the Boulder IRB, a harm is “probably related” to the research procedures if in the opinion of the investigator, the research procedures more likely than not caused the harm. Such events are considered reportable new information by the Boulder IRB and will be reported to the Boulder IRB within 5 business days. Reports to the Boulder IRB will use their Reportable New Information Form (HRP-214-FORM: Reportable New Information).

- All adverse events will be logged in a study adverse event log and the adverse event report and associated correspondence will be saved in individual subject files. All adverse events, including those events deemed *not* to be unexpected and probably related to the research, will be made available for review upon request by appropriate bodies (e.g., IRB, FDA, NIH, etc.).
- Any subject who is experiencing a **medical emergency** as a result of participating in this study will be instructed to call 911. If subjects experience any serious unexpected side effects related to the intervention, they will be instructed to discontinue the inspiratory muscle strength training intervention immediately.
- **Management of Non-Emergent Adverse Events During CTRC Business Hours (Monday, Wednesday and Friday from 7:00 AM to 3:30 PM and from 7:00 AM to 4:00 PM Tuesday and Thursday):**
 - All reports of adverse events that occur during regular CTRC hours during procedures performed by CTRC staff (e.g., blood draws) will be evaluated and managed by a CTRC nurse or physician; Integrative Physiology of Aging Laboratory personnel (present during all procedures) will document the actions taken and report/record the adverse event as described above. Dr. Chonchol will be informed of the adverse event by Integrative Physiology of Aging Laboratory staff, and be responsible for making a medical determination to the PI regarding the safety of future participation of the subject in the procedure or study.
 - Procedure-related adverse events that occur during procedures performed by Integrative Physiology of Aging Laboratory personnel in the CTRC (e.g., blood vessel function testing) will be reported by Integrative Physiology of Aging Laboratory study personnel directly to Dr. Chonchol, who will assist with the management and evaluation of adverse events and make a medical determination to the PI regarding the safety of future participation of the subject in the procedure or the study; Integrative Physiology of Aging Laboratory study personnel will document and report the adverse event as described above. Such events also will be reported to the CTRC, including the CTRC Research Subject Advocate, using the UCB CTRC Adverse Event Reporting Form.
 - Symptoms/side effects potentially related to the intervention will be reported to Integrative Physiology of Aging Lab personnel or CTRC staff:
 - If a participant reports experiencing unexpected symptoms/side effects to Integrative Physiology of Aging Laboratory personnel, the staff member will contact Dr. Chonchol for acute management

and evaluation. Dr. Chonchol will make a medical determination to the PI regarding the safety of future participation of the subject in the procedure or the study

- If a participant reports experiencing unexpected symptoms/side effects to CTRC medical staff, CTRC staff will inform Integrative Physiology of Aging Lab personnel so the event can be documented, and may contact Dr. Chonchol for acute management should this be deemed medically necessary by CTRC medical staff. In either situation, Dr. Chonchol will make a medical determination to the PI regarding the safety of future participation of the subject in the procedure or the study

- **Reporting Non-Emergent Adverse Events Outside of CTRC Business Hours:**

- All reports of adverse events that occur outside of regular CTRC business hours, or that related specifically to inspiratory muscle strength training, will be reported directly to the PI, Sophie Lalande, by calling his CU Boulder phone number (303-735-4172) that has been linked directly to the PI's personal cell phone. This phone number is provided in the consent form. The PI will immediately notify the physician of record, Dr. Chonchol, and Dr. Chonchol will be responsible for management of the adverse event. Dr. Chonchol may contact the subject if necessary or advise the PI on how to manage the adverse event (e.g., recommend the subject seek medical attention by contacting their physician or going to the ER or Urgent Care, stop the intervention, etc.).

- **Method for relaying medical information between the CTRC medical staff, PI, Physician and participant:**

- Should a participant have a non-emergent medical complaint, CTRC medical staff (business hours) or the PI (non-business hours) will have access to the study subject's un-blinded information, including their assigned treatment condition, and will be able to provide Dr. Chonchol with all of the information necessary to make an informed medical decision. If needed, after being briefed on the situation, Dr. Chonchol will follow up with the participant directly to discuss the ongoing adverse event and to provide advice for how to proceed. Dr. Chonchol may recommend that the participant seek medical attention (e.g., go to the ER or Urgent Care, or contact their personal physician).

- **Backup Investigator to the PI:**

- In the event that the PI will be unavailable by phone (e.g., due to travel or illness), phone calls will be routed to a backup co-investigator's (Matthew Rossman) personal cell phone number instead who will remain "on call" until the PI returns.

- Should a study patient contact a study investigator about an **ongoing** adverse event, the study investigator will contact Dr. Chonchol for guidance on how to manage the adverse event. Dr. Chonchol will be notified immediately by the investigator in the event of an ongoing serious event or an ongoing mild/moderate adverse event needing medical attention; Boulder CTRC medical staff will be notified no later than the next working day and may be notified sooner, if deemed appropriate by Dr. Chonchol. Dr. Chonchol will develop a plan to monitor the subject while the adverse event is ongoing, or recommend they seek medical attention (e.g., ER or Urgent Care, their personal

physician). Dr. Chonchol may refer the study patient to the Boulder CTRC medical staff for evaluation during business hours, as outlined above. Dr. Chonchol will work with CTRC clinical staff, study investigators and Integrative Physiology of Aging Laboratory personnel to communicate with the participant and manage the ongoing adverse event. A timeline including the conversations and actions taken shall be logged and available for review by the CTRC physician, the CTRC research subject advocate, and the SMC. The monitoring plan for all ongoing serious adverse events will be communicated to, approved and regularly reviewed by the Medical Director.

- The Boulder CTRC nurses and physicians will acutely manage an adverse event and will facilitate the provision of appropriate medical treatment for adverse events that occur during CTRC procedures, but are not responsible for the ongoing treatment of adverse events. The Boulder CTRC physician and Dr. Chonchol are responsible for determining when management of an adverse event should be transferred to an appropriate medical care provider.
- Boulder CTRC medical staff, including nurses and physicians, and Dr. Chonchol are responsible for evaluating and monitoring all ongoing adverse events until one of the following conditions is met: a) the event resolves; b) the subject refuses further evaluation or monitoring by the staff; or c) the adverse event has stabilized and, in the judgment of the medical staff, further monitoring would not be useful. If notification of the participant's physician was not deemed medically necessary during the management of the adverse event, Dr. Chonchol will determine whether or not it is medically necessary to notify the participant's physician about the adverse event. The participant's physician will be made aware of all serious adverse events either by the participant or Dr. Chonchol; Dr. Chonchol will be available to discuss the adverse event with the participant's physician.
- Once an ongoing adverse event has resolved, Dr. Chonchol will be responsible for making a recommendation to the PI regarding whether or not it is safe for the participant to continue in the study. If Dr. Chonchol deems it is medically safe for the participant to continue, the PI will decide whether to continue the subject in the study or withdraw the subject if the continued participation of the subject in the study would compromise the study findings.
- Under no circumstances will study investigators who are not trained medical professionals provide medical interpretation or medical advice to study subjects.

The Boulder CTRC will not pay for medical attention for study subjects outside of care provided on the CTRC premises.

All subject identities and records will remain strictly confidential. Individual subject data will be coded and will not be associated with the subject's name. The code key will be maintained on REDCap in a password protected format. The code key will be destroyed after completion of data analysis and submission of papers for publication. Electronic data will be collected and managed using REDCap electronic data capture tools. Any physical data will be stored in a locked file cabinet in the PI's office. The names of the subjects will not be identified in any publication arising from these studies. All of the ultrasound measurements will be recorded onto hard drive on the ultrasound; however, all of the ultrasound images will be coded with an identification number and not the name of the participant. Only the PI and research staff will

have access to these data. With subject consent, biological samples (plasma, serum) will be kept indefinitely for retrospective analyses related to aging and physiological function (this will not be conducted without IRB approval).

XX. MEDICAL CARE AND COMPENSATION FOR INJURY

If study participants experience a medical emergency, they are instructed to call 911. If they have a medical complaint or are experiencing a non-emergency adverse event, they are instructed to contact the Principal Investigator, Sophie Lalande (303-735-4172), who will always contact Dr. Michel Chonchol for medical instructions (see section XIX). The investigators will not provide any medical instructions unless directly instructed by Dr. Chonchol. The University of Colorado Boulder has no program to pay for medical care for research-related injury. Subjects will be informed (written in the informed consent) that we are not able to provide any compensation for injury and that they will be responsible for the cost of any treatment.

XXI. COST TO PARTICIPANTS

There are no costs to participants other than potential costs associated with traveling to and from the CU Boulder campus (e.g., gas). If subjects request, they may be partially compensated for travel costs for all visits.

XXII. DRUG ADMINISTRATION

N/A

XXIII. INVESTIGATIONAL DEVICES

The IMST smartphone app has been designed by PowerBreathe International Ltd. The app is being supplied to the study team for research purposes ahead of official public release expected in early 2024. The app remotely delivers the clinic-based IMST protocol previously tested by the PI and other investigators at the University of Colorado Boulder. The app will be downloaded onto participants personal Apple or Android smartphones. The app meets FDA guidelines for a Nonsignificant Risk Device because it serves as an accessory to the PowerBreathe Plus (described below), providing guidance and facilitating training with the Powerbreathe device. The app is not: a) intended as an implant; b) purported to support or sustain human life; c) for a use of substantial importance in diagnosing, curing, mitigating, or treating disease; or d) otherwise present a potential for serious risk to health, safety, or welfare of a subject.

The PowerBreathe Plus – Medium Resistance inspiratory muscle strength training device is a class 1 FDA device (FDA Device Listing No: [Class 1]: D3224044) and meets FDA guidelines for a Nonsignificant Risk Device because it is not: a) intended as an implant; b) purported to support or sustain human life; c) for a use of substantial importance in diagnosing, curing, mitigating, or treating disease; or d) otherwise present a potential for serious risk to health, safety, or welfare of a subject.

The PowerBreathe Plus training device is a handheld mechanical device that provides resistance to inspiration (breathing in), exhalation is unimpeded. In this study, volunteers will

perform 30 inspirations per day (6 days/week) with the PowerBreathe Plus. An inspiratory maneuver begins after a subject has fully exhaled, they then breathe in as powerfully and deeply as possible. All volunteers in the clinic-based group are provided in-person training for device operation. Instruction for all volunteers in the app-based group are provided by the IMST smartphone app.

The PowerBreathe device consists of a handset, a valve head, and a mouthpiece. The handset serves as a control unit to regulate inspiratory resistance via turning dial that adjusts spring-loaded resistance. The valve head is a mechanical component that generates resistance to inspiration and is controlled by the handset. The mouthpiece is a plastic attachment to the valve head that subjects breathe through to facilitate inspiratory muscle strength training. An intelligent adapter (a small, Bluetooth-enabled pressure/flow sensor) is placed between the mouthpiece and valve head for participants in the app-based group. The intelligent adapter communicates training data (i.e., inspiratory number and intensity) to the IMST app. No changes to the device are anticipated during the course of this clinical study.

Device disinfection/sanitation. Volunteers will be given verbal instructions on how to clean the training device between uses. Participants will be instructed to soak the mouthpiece and valve in warm water for 10 minutes, then run both pieces under warm running water, and finally leave both pieces out to dry, after each use. About once a week, the mouthpiece and valve should be soaked in soapy water or in water with disinfectant intended for use on equipment that comes into contact with the mouth (e.g., babies' bottles), run under warm water, and then left out to dry. The handset should be wiped down with a damp cloth or disinfecting wipe.

Participants who complete the 6-week intervention will be allowed to keep their PowerBreathe Plus device. Participants who do not complete the 6-week intervention will be asked to return the device. When a participant returns the training device, the study team will sterilize the device by soaking the mouthpiece and valve in Alconox for a minimum of 5 minutes, and then bleach (1:10 dilution with tap water) for a minimum of 15 minutes, before thoroughly rinsing both parts under warm running water and leaving them out to dry. The handset is cleaned with a disinfecting wipe.

XXIV. WORKING WITH OTHER INSTITUTIONS

Daniel Craighead from the University of Minnesota will provide general guidance on the protocol. No research activities will take place at the University of Minnesota, and identifiable data will not be analyzed at the University of Minnesota. Each institution will rely on the CU Boulder IRB for oversight of the research.

PowerBreathe maintains the IMST smartphone app but is not sponsoring the study. The study team will not be sharing data or results with PowerBreathe.

XXV. SHARING OF RESULTS WITH PARTICIPANTS

Subjects will receive a copy of all health-related information collected during the study in a packet that will be sent to them 3-4 weeks after completing post-testing or withdrawing from the study.

XXVI. REFERENCES

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