Sex Effects on Blood Pressure With Handgrip Training

Research protocol

This study will be designed as a randomized, controlled, cross-over, repeated measures experiment. Fifty young healthy adult participants (25 males and 25 females) will visit the laboratory three times to perform a fatiguing bout of isometric handgrip exercise before and after both 4 weeks of isometric handgrip training and a no-exercise training condition. Participants will be randomly allocated to the isometric handgrip and the non-exercise training conditions using an online program (http://www.randomizer.org/). Participants that start with isometric handgrip training will return to the laboratory after a 4-wk washout, as this duration has been shown to decondition cardiovascular outcomes following training (1–3). To eliminate diurnal variation, each participant will visit the laboratory at the same time of day. Main outcomes in each visit will be measured at rest, during the fatiguing bout of isometric handgrip exercise, and 5,10, 30, and 60-min post-exercise (see figure and Table 1). These measurement timings are aimed to characterize BP, and the cardiac autonomic and vascular function biphasic responses (4–6). Visits 2 and 3 will take place within 7 days of the last training session.

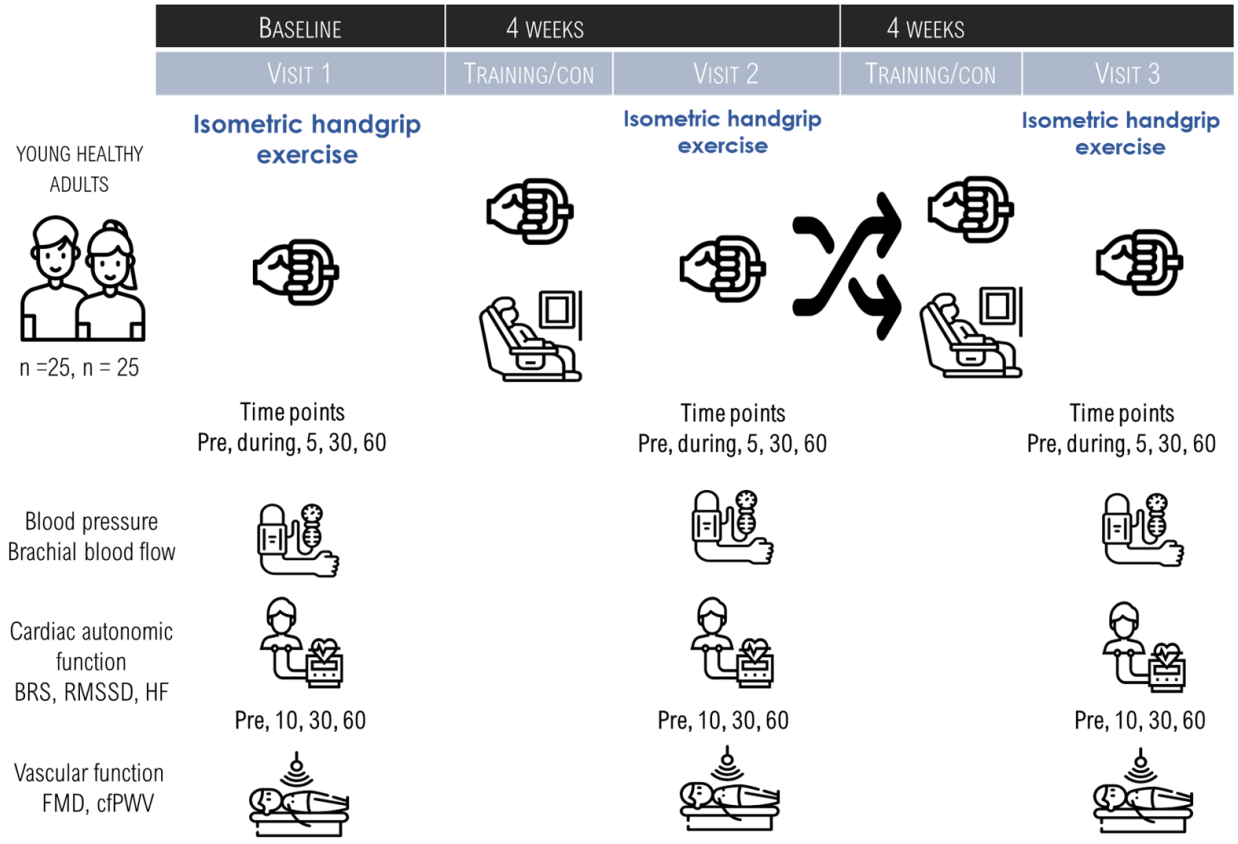

Figure 1: Experimental design, a randomized, controlled, cross-over, repeated measurements study. Abbreviations: CON; control, BRS baroreflex sensitivity, RMSSD, root mean square of

Table 1 Procedures to be conducted in each visit to the laboratory

Procedures	Time (min)
Rest	15
Measurements	
1) BP, arterial stiffness, cardiac autonomic function	5
2) Brachial endothelial function (FMD)	15
Fatiguing isometric handgrip exercise	~ 3-5
Last isometric contraction	
Measurements	
1) BP, brachial blood flow	
2) Cardiac autonomic function (BRS, RMSSD, HF)	
Post 5 min	
Measurements	
1) BP, arterial stiffness (cfPWV), cardiac autonomic function (BRS, RMSSD, HF)	5
Post 10 min	
Measurements	
1) Brachial endothelial function (FMD)	15
Post 30 min	
Measurements	
1) BP, arterial stiffness (cfPWV), cardiac autonomic function (BRS, RMSSD, HF)	5
2) Brachial endothelial function (FMD)	15
Post 60 min	
Measurements	
1) BP, arterial stiffness (cfPWV), cardiac autonomic function	5
2) Brachial endothelial function (FMD)	15
Total	~ 100

successive differences; HF, high-frequency power band; FMD; brachial artery flow-mediated dilation, cfPWV; central pulse wave velocity.

Study intervention

Baseline visit – fatiguing bout of Isometric handgrip exercise

All participants will rest for 15-min, and then the MVC will be determined based on three attempts, each separated by 30-s, with participants in a supine position. The highest MVC will be used to define the exercise workload (30% MVC). Then, participants will perform a fatiguing bout of unilateral isometric handgrip exercise in the supine position using a digital handgrip transducer intertwined with a data acquisition system (Powerlab 16/35; ADInstruments, BellaVista, NSW, Australia). Participants will use their dominant hand and will hold the isometric contraction until task failure. The failure criteria will be defined as the inability to sustain the contraction force within 5% of the target MCV percentage (i.e., 30%). We chose to set the intensity at 30% MVC as this is the standard target intensity of isometric handgrip exercise protocols (7–9) and no differential BP responses have been reported when different intensities (i.e., 20 vs 30 and 45% MVC) are

used (10–12). Based on previous research, we expect that participants will fatigue within 3 to 5 min (i.e., time to task failure) (12). Participants will receive both visual and auditory feedback to maintain the target exercise intensity throughout the session. The same fatiguing bout protocol will be used before and after the training intervention.

Isometric handgrip training intervention and control

Participants will train three days per week at home for 1 month using a reliable and validated digital handgrip dynamometer (DynX, MD System, Inc., Westerville, OH, USA)) in a seated position. The isometric handgrip training protocol will consist of four sets (2 for each hand) of two-min sustained contractions starting at 30% MVC interspersed by 1-min resting periods (a total of 12-min). All training sessions will be remotely supervised via Zoom or phone call by a research team member to ensure compliance with the exercise prescription. Before each training session, MVCs will be re-assessed over three repetitions, each separated by 30-s, to ensure an accurate exercise prescription (13). The highest value will be used to determine the target intensity of the session. In the control/washout conditions, participants will be instructed to maintain their normal daily routines.

Post-intervention visits – isometric handgrip exercise acute bout

All participants will return to laboratory to complete the same acute bout of isometric handgrip exercise detailed in the baseline visit after the isometric handgrip exercise training and respective control.

Study endpoints (clinical trials):

NA

Study main outcomes

The main outcomes of this study are brachial BP (i.e., SBP and DBP), cardiac autonomic function (i.e., RMSSD, HF, and cardiac baroreflex sensitivity), and vascular function (i.e., flow-mediated dilation (FMD), and central pulse wave velocity (cfPWV).

Study Population

Eligibility criteria

Inclusion criteria

Eligible participants for this study are 1) persons with normal BP as defined by the American Heart Association (<130/90 mmHg) and ages between 18-40 years; 2) those with BMI < 30 kg/m^2; 3) persons with no cardiovascular risk factors and no clinically diagnosed cardiovascular disease; 4) persons recreationally active ($\leq$ 2 days of structured physical activity); and 4) able to speak and comprehend English.

Exclusion criteria

Prospective participants will be excluded if they report 1) diagnosed cardiovascular (e.g., coronary artery disease, heart failure), musculoskeletal (i.e., osteoporosis and sarcopenia), and kidney disease; 2) taking antihypertensive or other vasoactive medications and cardioactive medication; 3) obesity (i.e., BMI > 30 kg/m^2); 4) hypertension (i.e., $\geq$ 130/90 mmHg; 5) diabetes mellitus (i.e., fasting HbA1 > 6.5%); 6) depression and anxiety disorders; 6) long COVID; 7) being a smoker; 8) pregnancy or menstrual irregularities among females.

Excluded vulnerable populations

Pregnant females will be excluded due to pregnancy-induced changes in hormonal concentrations and cardiovascular regulatory mechanisms.

Study Duration

We foresee that this study will be completed in one year. The estimated duration of individual participation in this study is 2 months and each visit to the laboratory will take approximately 2h.

Number of Participants

We aim to sample 50 young healthy adults, 25 males, and 25 females.

Study Setting

All study measurements will be conducted in Integrative Physiology Human Laboratory (IHPL) at the University of Massachusetts Boston located at 100 Morrissey Blvd in the Quinn Administration Building. The isometric handgrip training intervention will be conducted at the home of each participant enrolled in this study.

Prior approvals:

NA

External IRB/ethics review:

NA.

Local permissions/local context:

NA

Recruitment methods

Recruitment will be conducted at the University of Massachusetts Boston and in the greater metropolitan Boston area. We will ask to place recruitment flyers at the University of Massachusetts Boston and to use the email services. Word of mouth will also be used as a recruitment strategy. All communication, including electronic and social media, will only be used to spread information about the study. Persons interested in this study will initiate contact via our study phone call line or the Integrative Human Physiology Laboratory email for further study details and subsequent screening for eligibility. We will only contact potential subjects as a follow-up to those who initiate contact with study personnel first. Prospective participants will be screened over the phone using the Physical Activity Readiness Questionnaire for Everyone (PAR-Q Plus), the International Physical Activity Questionnaire (IPAQ), and a health history questionnaire along with age, sex, and menstrual cycle regularity. To minimize coercion and undue influence on participants verbal and non-verbal/behavioral expressions will be used to assess possible objections or resistance to taking part in the study. At any time during the study, participants can withdraw without any negative consequences.

Procedures involved

Study assessment procedures

Cardiovascular function

During the study visits, all non-invasive cardiovascular function measurements detailed below will be done in a quiet climate-controlled room (22-24º C) after a 15-min resting period, during, and after the fatiguing handgrip exercise with participants in a supine position. Participants will be tested in a fasted state (4h) and have refrained from exercise, caffeine/alcohol intake, and vitamins/supplements in the last 24h. Cardiovascular function testing in females will occur without controlling for the menstrual cycle (33,34), given that a growing body of literature supports little to no variation in BP, cardiac autonomic (i.e., BRS), and vascular function outcomes (i.e., brachial artery FMD and cfPWV) at rest and during acute exercise across the menstrual cycle or oral contraceptive pill cycle (25,27–32).

Blood pressure

Participants will be asked to wear a non-invasive inflating and deflating finger cuff during all study visits. This will allow beat-to-beat BP to be recorded using finger plethysmography (Finometer; Finapres Medical System, Amsterdam, The Netherlands) before, during, and after isometric handgrip exercise in the supine position. Beat-to-beat BP will be averaged offline over 2 to 5-min using Lab Chart 8 (ADInstruments, BellaVista, NSW, Australia). Beat-to-beat dynamic fluctuations in BP induced by acute isometric handgrip exercise will be quantified through BP variability indices including spectral methods using the adapted HRV module of Lab Chart 8. This will give insight into sympathetic outflow to blood vessels (14,15).

Brachial BP of the right arm will also be measured three times at 1-minute intervals before and after isometric handgrip exercise using an automated blood pressure cuff (HEM-7311-ZSA; Omron Healthcare Co, Ltd, Kyoto, Japan) (16). Measurements will be accepted if they do not differ > 4 mmHg, and the average of the last two will be used for data analysis (16). Mean arterial pressure will be estimated as 2/3 DBP + 1/3 SBP.

Central BP will be assessed via carotid artery tonometry (NIHem, Cardiovascular Engineering Inc., Norwood, MA). Brachial systolic and diastolic cuff BPs will be used to calibrate the peak and trough of the signal-averaged pressure waveforms. Carotid pressure waveforms will be calibrated with diastolic and integrated mean brachial cuff pressure. Central BP is then estimated from the calibrated carotid pressure. Central pulse pressure will be calculated as the difference between the peak and trough of the calibrated carotid pressure waveform.

24-hour ambulatory BP will be monitored with the AMBPro devices (SonmoMedics, Randersacker, Germany), which uses pulse transit time technology. The cuff will inflate every hour at night and every 30 min during the day, for measurement of standard brachial BP during the inflation/deflation cycle. BP between cuff inflation is determined continuously from pulse transit time measurements derived from concurrent electrocardiogram and photoplethysmography recordings. This device validated by the European Society of Hypertension can also be programmed to obtain pressures using conventional oscillometry during cuff inflation. Participants will be instrumented with four limb electrodes on their chest for the electrocardiogram recording. A cuff will be placed on their non-dominant arm that contains the photoplethysmography sensor. Data will be exported from the device for off-line analyses of the 24-hr BP and heart rate signal. The physical activity and movement sensors embedded within the device will allow for clear identification of daytime and nighttime pressures.

Cardiac autonomic function

We will place electrodes directly on the skin of each participant to obtain the ECG trace required to estimate both heart-rate variability and baroreflex sensitivity outcomes.

Heart rate variability and baroreflex sensitivity: R-R intervals will be sampled at 1000 Hz frequency to obtain digital R waves digital sequences using the single ECG lead module of PowerLab data acquisition system (ADInstruments, BellaVista, NSW, Australia). All data acquisition and offline analyses will be conducted following the standards of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (17). Offline heart rate variability (HRV) analyses will be performed over a 2-min time series for each time-point (i.e., baseline, during and 5, 30 and 60 min after exercise) using the HRV analysis module of Lab Chart 8 (ADInstruments, BellaVista, NSW, Australia) (18). Main time-domain indices of interest include the standard deviation of NN intervals (SDNN) – a measure of overall variability, and the root mean square of the sum of the squares of the differences between NN intervals (RMSSD) – a measure of cardiovagal modulation. In addition, non-linear time-domain parameters will be derived from the Poincaré plot, including the vertical deviation that reflects mainly cardiovagal modulation (SD1), the longitudinal deviation (SD2), and the non-linear ratio SD1/SD2 an index of autonomic balance analogous to LF/HF ratio (19). The time-frequency domain analysis will be conducted using the Lomb-Scargle Periodogram, which permits accurate estimation of low (0.04 to 0.15 Hz) and high-power frequency (HF) power bands (0.15 to 0.40 Hz) in absolute and normalized power units during exercise conditions. Cardiac baroreflex sensitivity (BRS) will be estimated via the spontaneous sequence method over 2 min through the Lab Chart 8 baroreflex macro. Briefly, this method identifies ramps where increases in SBP (> 1 mmHg) are coupled with increases of the RR interval (> 4 ms) (linearly correlated) that occur over at least 3 beats (minimum sequence). The minimum acceptable correlation coefficient between SBP and RR interval ramps will be 0.80. Then, baroreflex sensitivity is estimated as the average of all regression slopes between the SBP and RR interval.

Vascular function

During the vascular function measurements, we will ask participants to lie quietly in the supine position. They will also experience a forearm occlusion to permit the assessment of endothelial function.

Endothelial function: will be non-invasively assessed via brachial artery FMD, which represents nitric oxide-dependent vasodilation and closely correlates with the endothelial function of the coronary arteries (20,21). FMD will be assessed in the right brachial artery with an ultrasound (Arietta V750, Fujifilm, Tokyo, Japan) equipped with a 7.5-MHz linear array probe incorporating a 5-MHz Doppler transducer, placed ~4 cm above the antecubital fossa, and held by a stereotactic clamp following standard guidelines (22,23). Reactive hyperemia will be induced by rapid cuff deflation following a forearm occlusion (Hokanson SC10, Bellevue, WA 98005, USA) maintained for 5-min at 250 mmHg. Changes in intraluminal brachial artery diameter will be tracked with automated edge-detection software (FMD studio, Quipu srl, Pisa, Italy) allowing precise measurement of the artery diameter (23). Doppler measurements of peak hyperemic blood velocity with an insonation angle of ≤ 60º will allow calculation of FMD main stimuli - shear rate as (4 × peak blood velocity / $D_{baseline}$) (23). All FMD analyses will be done offline using the FMD studio. FMD will be presented both as an absolute change (FMD (mm) =D_{peak} – $D_{baseline}$) and as a relative change (% FMD = (D_{peak} -D_{bas}/$D_{baseline}$) ×100) in diameter. If a non-linear ratio between D_{peak} and D_{bas}

is observed (B coefficients < 1), %FMD will be allometrically scaled for $D_{baseline}$ (24,25). In addition, blood flow will be estimated as (Mean blood velocity) x (Brachial Cross-Sectional Area) x (60). Brachial artery blood flow will be measured in both exercising and non-exercising arms. All FMD scans will be conducted by the same researcher with inter-day measurements CV <15% (22,23).

Arterial stiffness: cfPWV the non-invasive gold standard measurement of central arterial stiffness will be assessed using an applanation tonometry automatic device (NIHem, Cardiovascular Engineering Inc) with participants lying supine. Briefly, common carotid artery and femoral artery pressure waveforms will be recorded using a high-fidelity strain-gauge transducer placed separately on both arteries permitting pulse transit time estimation through the ECG R-wave to the foot of the applanation waves (intersecting tangent foot-to-foot method). PWV is then calculated by dividing carotid-femoral distance (subtraction method) by pulse transit time (26). Pulse waveforms velocity from carotid-brachial and radial artery tracts will also be measured and interpreted as regional indices of upper limb stiffness. All measurements will be conducted by reliable operators (i.e., CVs < 5%) on the right side of the body and ensuring that repeated measurements differ by less than < 0.5 $m.s^{-1}$ (27). Brachial waveforms and BP will be used to calibrate central pressure waveforms.

Local carotid stiffness and blood flow will also be measured from the common carotid artery using a high-resolution ultrasound (Arietta 750, Fujifilm, Tokyo, Japan) and high frequency (7.5 MHz) linear probe. Carotid artery diameter and blood velocity will be measured using the wave intensity mode with the probe placed on the skin over the carotid artery approximately 1-2 cm to the proximal carotid bifurcation with a 60-degree insonation angle. Carotid stiffness indices such as β stiffness (eq.1) and PWV (eq.2) will be estimated according to the manufacturer's formulas:

$$(eq.1)\ \beta = \ln\left[\frac{SBP/DBP}{(D_{syst} - D_{diast})/D_{diast}}\right]$$

Where, β is beta stiffness; SBP, brachial systolic blood pressure, DBP, diastolic blood pressure; D_{syst}, brachial artery diameter during systole; D_{diast}, brachial artery diameter during diastole.

$$(eq,2)\ PWV = \sqrt{((\beta \times DBP)/(2\rho)}$$

Where, PWV is brachial artery pulse wave velocity; β, beta stiffness, DBP, brachial diastolic blood pressure; and r, is blood density - assumed constant (1050 kg/m^3).

Body composition

Participants will seat for 5-min inside the Bod Pod, (Body Composition System; Life Measurement, Incorporated, Concord, CA), a large egg-shaped chamber to have their body composition measured via air plethysmography. Participants will enter the Bod Pod only wearing minimal clothing (briefs for males and swimsuit for females), a swim cap and will be asked to breathe normally for 20-s and then into an internal breathing tube to measure thoracic gas volume.

This will permit the estimation of body density needed to calculate % of body fat via the Siri equation (28). The Bod Pod will be calibrated before each test following the manufacturer's instructions with the chamber empty while using a cylinder of known volume (50 L).

Pulsatile hemodynamics and wave separation analysis

Pulsatile hemodynamics and wave separation analysis

Aortic impedance will be measured following the method outlined by Mitchell et al (29,30). Aortic impedance describes pulsatile flow and pressure relations at the proximal aorta in early systole before the return of reflected waves. Firstly, 2D parasternal long-axis images of the left-ventricular (LV) outflow tract will be acquired using a 2-4-Mhz ultrasound transducer (Arietta V750, Fujifilm, Tokyo, Japan). This will permit the determination of aortic diameter defined as the largest diameter in early systole close to the aortic leaflets. Then, a 2-MHz continuous echo Doppler flowmeter (pedof) probe (Arietta V750, Fujifilm, Tokyo, Japan) will be placed at the suprasternal notch to estimate aortic flow as the product of blood velocity and LV outflow tract area. Lastly, carotid pressure waveforms will be acquired via applanation tonometry (NIHem, Cardiovascular Engineering Inc). Both aortic flow and carotid pressure signals will be integrated into the NIHem software to estimate aortic impedance as the ratio of carotid pressure by aortic flow. In addition, waveforms will be separated into forward (Pf) and backward waves (Pb) with their ratio considered an index of general wave reflection, using the NIHem software as previously described (30).

Follow up

This study will not have a follow-up.

Data Analysis Plan

Data integrity

All study procedures described in this protocol will be performed by trained personnel and all raw data will be checked within the same day of the visit by the research team who collected it. Biweekly examinations of the analyzed data will be performed by the PI to ensure high-quality analysis is being performed.

Sample size

Based upon an effect size of 0.160 and a drop-out rate of 15%, a priori power analysis (G-Power Version 3.1.9.3) for mixed ANOVA suggested a total of 50 participants are required to detect SBP mean differences of at least 5 mmHg over time and groups (α= 0.05, $1-\beta$=0.80). Despite this mean difference obtained from a study that characterized BP responses after acute isometric handgrip exercise (7), we also aim to examine the effects of training on acute blood pressure responses to isometric handgrip exercise. Thus, given these differences in study design, we additionally set the effect size considering the small, standardized effect sizes (≤ 0.20) for exercise training sciences (31).

Statistical analysis

Linear mixed models will be used to examine the effects of acute isometric handgrip exercise and training on BP, cardiac autonomic, and vascular function outcomes over time (i.e., aim 1, 2, and 3). These will be fitted with restricted maximum likelihood and applying Satterthwaite's method for approximating degrees of freedom for the F test using R lmerTest package (32). Fixed effects will be set as time, sex, training condition, and the random intercept as each participant. All models' residuals will be checked for normality and homogeneity of

variances using both Shapiro-Wilk and Levene tests, respectively, and through QQ plot inspection using the R performance package (33). Natural logarithm (Ln) transformations will be applied to HRV outcomes, as these are generally not normally distributed, but both transformed and untransformed data will be presented. Linear mixed models will be run with and without possible outliers identified through box plots as observations lying outside the 1.5 interquartile range. Unbiased effect sizes - partial omega squares (ω^2) will be estimated for each fixed effect and interactions terms and interpreted accordingly to the rough benchmarks set by Cohen (34) [small ($\omega^2 < 0.05$), medium ($\omega^2 < 0.25$), and large $\omega^2 > 0.25$) effects sizes]. Post-hoc comparisons will be performed using the Bonferroni correction, in the presence of significant fixed effects and interactions. MVC, %fat and free fat mass, and physical activity outcomes will be added to all linear mixed models as covariates, whereas resting HR and SR_{auc} will be added to FMD and HRV main outcomes, respectively. In addition, the repeated measures correlation coefficient will be used to test associations between isometric handgrip exercise-induced BP changes before and after the training intervention and of BP with cardiac autonomic and vascular function outcomes (35). Multiple imputation procedures using full information maximum likelihood will only be used in case of a significant amount of missing data on outcomes (>15%) and if missing completely at random, as linear mixed modeling provides robust estimates for small to medium amounts of missingness (36). All statistical analyses will be conducted using R software, version 4.2.3 (37) with a significant level (α) of 0.05.

Individual blood pressure responsiveness to isometric handgrip training

We aim to conduct an exploratory analysis of the individual BP responsiveness to isometric handgrip training. Participants will be classified as showing relevant (responder) or negligible (non-responder) training-related hypotensive effects using the ROPE + HDI decision rule to reject or non-reject the null – a Bayesian method (38,39). Briefly, this method estimates the percentage of the highest density interval (HDI, like CI in frequentist statistics) within the range of values around the null – region of practical equivalence (ROPE). This estimated percentage corresponds to different levels of significance that will guide the training-related hypotension individual response classification with non-responders defined as > 99 %, likely responders < 5%, and responders < 1% of HDI within the ROPE (38,39).

The HDI calculated as an 89% credible interval will be derived from each participant's posterior normal distribution obtained from 1000 simulations based on the individual pre-to-post change in SBP and DBP (delta) and respective standard deviations calculated as TE/2, where TE is the technical error calculated as CV/2 *100) (38–40). Individual normal distributions will be derived using the R's rnorm function. We will estimate the ROPE as 20% of the baseline SBP and DBP standard deviations in each group (sex) and this represents the smallest worthwhile difference (41–43). Both the HDI and the ROPE will be computed with R package bayestestR (44). Participants will then be classified according to the ROPE + HDI decision rule. This method of classifying responders mitigates some pitfalls of the traditional response threshold binary classification, such as not accounting for within-participant measurement error variability associated with blood pressure or the magnitude of group differences in mean response (40,41,45).

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