Study protocol and statistical analysis plan
for
24-hour blood pressure measurements and ischemic conditioning

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Study Protocol

Twenty-two apparently healthy normotensive middle-aged participants came to the laboratory following an overnight fast, participant demographics are presented in Table 1. Prior to testing, participants gave their written informed consent and completed a health history questionnaire. Participants were excluded from the study if they were pregnant, had a recent illness, history of diabetes, heart disease, or other cardiovascular problems, recent surgery, or any other medical intervention. Using the medical history questionnaire our participants reported an average of 2 (1) risk factors; including age, family history, smoking, blood pressure, total cholesterol, physical inactivity, psychological stress, high-fat diet, and overweight/obesity. All subjects gave their written informed consent prior to study participation, and the ethics committee of the University of Texas at Austin approved all procedures. This study was approved by the local IRB and registered with ClinicalTrials.gov (NCT03303404).

After 20 minutes of supine rest, endothelial function was assessed using flow-mediated dilation (FMD; index of endothelium-dependent vasodilation) (1). After another 20 minutes of rest, a second measure of endothelial function was performed. These two measurements were used to establish repeatability of endothelial function within the laboratory. For the data analyses, only the second measurement of FMD was used as the baseline (pre) measure of endothelial function. Following these procedures, the participants were given an ambulatory blood pressure monitoring device. After 24-hours, subjects

returned to the laboratory, and their endothelial function was reevaluated following 20 minutes of rest.

Flow-mediated dilatation was measured with an semi-automated diagnostic ultrasound system (UNEXEF38G, UNEX Corporation, Nagoya, Japan) while participants rested in a supine position (2). A blood pressure cuff was placed on the forearm with the proximal edge of the cuff below the subject's antecubital fossa. The cuff was inflated to 50 mmHg above resting systolic blood pressure for 5 minutes to occlude blood flow. The position of the probe was marked on each participant to ensure subsequent measurements were taken on the same region of the brachial artery. Shear rate (s^{-1}) was calculated by the following equation: 8 x blood flow velocity (m s^{-1}) / internal artery diameter (3).

Ambulatory blood pressure monitoring provides an insight into blood pressure changes in everyday life and an estimate of the overall blood pressure load exerted on the cardiovascular system over 24 hours (4). Blood pressure recordings were made using a noninvasive ambulatory monitor (Spacelabs 90227, Spacelabs Healthcare Inc., Redlands, Washington, USA) (5). Participants' arm circumferences were measured to determine appropriate blood pressure cuff sizes; 24-32 cm for adult, 32-42 cm for large adult, and 38-50 cm for extra-large adult cuffs. The cuff was programmed to inflate automatically to 170 mmHg every 15 minutes from 6 AM to 11 PM and every 20 minutes between 11 PM and 6 AM. Physical activity was monitored using a pedometer, which was attached to a belt around the participants waist. (OMRON HJ-320, OMRON Healthcare Inc., Hoffman Estates, Illinois, USA). The cumulative ischemic stimuli from the ambulatory blood pressure monitoring was

calculated by recording the average time the cuff was inflated above the participants systolic blood pressure and then multiplied by the number of measurements taken.

Statistical Analyses

Statistical analyses were performed with GraphPad Prism (version 7.05, San Diego, California, USA). Data were first tested for the normal distribution. Statistical significance between before and after ambulatory blood pressure monitoring was then evaluated by the Student's *t*-test for paired data. A post-hoc power analysis was conducted using the program G*Power (version 3.1.9.2). Considering the difference between the mean and standard deviation (Pre to Post) with an alpha level of 0.05 for FMD in response to ABPM, the overall sample size of 22 participants in this study achieved an adequate power (>80%). A p value <0.05 was used to determine statistical significance.

References

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