Study Protocol and Statistical Analysis Plan

Heat Waves in the Elderly: Reducing Thermal and Cardiovascular Consequences

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Experimental Protocol: All experimental trials began between 8:00 and 10:00 to avoid diurnal variations. Before experimental trials, participants were instructed to eat a light breakfast (without caffeinated beverages) on the morning of testing. Alcoholic beverages and aerobic/resistance exercise were prohibited 24 hours before testing. Upon arrival, participants provided a urine sample and weighed themselves nude. A euhydrated state before each trial was confirmed by a urine specific gravity value ≤1.020 (Atago Inc., Bellevue, WA). If urine specific gravity was between 1.020 and 1.024, participants ingested 500 mL of water before testing. If their urine specific gravity was ≥1.025, the trial was rescheduled to a later date. Participants rested in a temperate environment (~22°C and 40% humidity) for at least one hour while baseline measurements (e.g., heart rate, blood pressure, forearm blood flow, etc.) were performed. Following baseline measurements, participants entered the environmental chamber set to one of the following two extreme heat simulations: 1) DRY (47°C and 15% humidity; reflective of the 2018 Los Angeles heatwave) and 2) HUMID (41°C and 40% humidity; reflective of the 1995 Chicago heatwave). The duration of each exposure was 3 hours, which represents the approximate duration of peak environmental temperatures during a heatwave (4, 31). Before instrumentation, participants' body mass was measured using a precision balance scale with ±10 g accuracy (Mettler Toledo, OH). After a brief period (~5-minutes) of instrumentation, participants were seated in a semi-recumbent position on a chair with a breathable fabric thereby reducing impediments for thermal exchange from the regions in contact with the chair. To reduce the influence of clothing on thermoregulatory responses, participants wore athletic shorts (males) or athletic shorts and a sports bra (females). To decrease the effect of dehydration, participants consumed 3 mL/kg body mass of tap temperature water (14-16°C) every hour during the extreme heat simulation. This level of fluid ingestion was based on pilot data and was selected to maintain hydration to a level that would not compromise thermoregulatory, perceptual, or cardiovascular responses during heat exposure (16, 54, 55). Tap water temperature was used to mimic the water available to individuals during an actual heatwave. Following the extreme heat simulation,

participants were de-instrumented and provided a towel to wipe off excess sweat before they weighed themselves nude.

Insert Figure 1 here

Metabolic Heat Production: Participants performed seven 5-minute bouts of light physical activity (either cycling: n=39 or walking: n=1) dispersed throughout the 3-hour extreme heat exposure (Figure 1). The intensity of the activity was maintained at a working metabolic rate of ~3 METs to mimic activities of daily living such as cooking, cleaning, carrying groceries, moving around the home, etc. (1). Metabolic heat generation was verified using indirect calorimetry (PARVO Medics True-One Metabolic Measurement System, Parvo Medics, Salt Lake City, UT), with metabolic gases being collected during the first two 5-minute periods of exercise. That identified workload was used for all subsequent exercise bouts. The average rate of oxygen consumption, respiratory exchange ratio, and workload were used to determine metabolic heat production, which was calculated using standard formulae (19).

Instrumentation: Core temperature was measured via rectal temperature using a general-purpose thermocouple probe inserted 10 cm beyond the anal sphincter (Mon-a-therm, Mallinckrodt Medical, St. Louis, MO) (Young: n=17, Older: n=8) or a telemetric pill (e-Celsius performance pill ®, BodyCap©, Caen, France) used as a rectal suppository (Young: n=1, Older: n=1). If participants did not consent to rectal temperature measurement, or if there were technical issues with the rectal thermocouple probe, core temperature was measured using an orally ingested telemetric pill (Young: n=2, Older: n=11). In these participants, the ingestible telemetric pill was taken no less than 1 hour before beginning the extreme heat exposure. Importantly the time following ingestion of the telemetric pill (e.g., between 1 and 12 hours) does not influence the validity of pill temperature as an index of core temperature (49). In addition, there is good agreeability amongst these methods to measure core temperature (10, 29), and for participants who had simultaneous measures of pill and rectal temperature in this study (n=25) the intraclass correlation coefficient was 0.87. Mean skin temperature was obtained as the weighted average

of local temperatures measured via the chest (22%), upper back (21%), lower back (19%), abdomen (14%), anterior thigh (13%), and calf (11%) (58).

Forearm blood flow, as a surrogate of changes in skin blood flow, was measured from brachial artery blood velocity and diameter values obtained by duplex ultrasonography (iE33/EPIQ 7, Philips Medical Systems, Andover, MA). A linear transducer (L12-3, Philips Medical Systems, Andover, MA), operating at an insonation angle of 60°, was placed proximal to the brachial artery bifurcation where the best spatial resolution of the artery could be obtained. Imaging was performed by the same sonographer throughout each heat exposure, and to aid in consistency between measurements sonographers used anatomical landmarks (e.g., ~9 cm proximal to the medical epicondyle) to ensure similar probe placement. Blood velocity was measured using time-averaged mean velocity from pulsed-wave Doppler ultrasound. Offline 2D images of the brachial artery were used to measure vessel diameter at end-diastole. Brachial artery diameter and blood flow velocity were measured from recordings of 5 consecutive cardiac cycles at baseline and in the final 15 minutes of extreme heat exposure. Blood flow velocity and brachial artery diameter measurements were used to calculate forearm blood flow using a standard formula:

$$Flow\ (mL/minute) = v \cdot \pi \cdot \left(\frac{d^2}{4}\right) \cdot 60 \qquad \text{Eq 1}$$

Where *v* is velocity in m/s and *d* is diameter in cm.

Heart rate was obtained from a 6-lead electrocardiogram (GE Medical Systems, Madison, WI). Blood pressure was measured by automated auscultation of the brachial artery (Tango+, SunTech Medical, Morrisville, NC, USA). Local sweat rate was measured on the upper back using a ventilated capsule technique where anhydrous compressed nitrogen was passed through the capsule at a flow rate of ~300 mL/min. The water content of the nitrogen gas was then measured using capacitance hygrometers (Vaisala, Woburn, WA, USA) and normalized for the area under the capsule.

Thermal perception was measured using an 8-point scale, with 0.5 increments ranging from 0.0 (‘unbearably ‘cold’’), 4 (‘comfortable’), to 8.0 (‘‘unbearably hot’’). At the start and end of each extreme heat exposure, participants were given a modified environmental symptom questionnaire (53) to assess symptoms commonly associated with environmental stress such as “I feel lightheaded”, “I have a headache”, “I feel dizzy”, “I feel thirsty”, “I feel weak”, “I feel grumpy”, “It is hard to breathe”, “I have a muscle cramp”, “I feel tired”, “I feel nauseous”, “I feel hot”, “I have trouble concentrating”, “I feel ‘goose bumps’ or chills”, and “I can play at my best”, on a scale of 1 (“not at all”) to 6 (“extreme”).

Blood Sampling and Analysis: Blood was taken from an arm vein after 30 minutes of supine rest at baseline and the end of the extreme heat simulations. We measured hematocrit (microcapillary technique) and hemoglobin (ABL90 Flex, Radiometer, Brønshøj, Denmark) to calculate changes in plasma volume using the Dill and Costill (22) equation. Blood samples were centrifuged to isolate plasma and aliquots of plasma were sent to a nearby laboratory (Texas Health Presbyterian, Dallas TX) to determine plasma osmolality (Abbott Alinity, IL, USA).

Calculations: We calculated mean body temperature as:

$$\text{Mean body temperature } (^{\circ}\text{C}) = 0.8T_{core} \times 0.2T_{skin} \quad \text{Eq 2}$$

Where T_{core} and T_{skin} are core temperature and mean skin temperature, respectively.

We calculated mean arterial pressure (MAP) as:

$$\text{MAP (mmHg)} = \frac{SBP}{3} + \left(DBP \times \frac{2}{3}\right) \quad \text{Eq 3}$$

Where SBP and DBP are systolic and diastolic blood pressure, respectively.

We calculated forearm vascular conductance by dividing forearm blood flow by MAP. We calculated body mass loss as the difference in nude body weight (Mettler Toledo, OH) between pre- and post-extreme heat exposure, and whole body sweat loss (WBSL) after correcting for fluid ingestion and urine output, which was collected and measured for volume. We calculated whole body sweat rate by dividing the whole body sweat loss by total heat exposure time.

Data Acquisition: Rectal temperature, skin temperature, local sweat rate, and heart rate were sampled at 250 Hz (Biopac MP150, Santa Barbara, CA) and converted into 1-minute averages for data analysis. Core temperature measured via the telemetric pill (both ingested and as a suppository) was sampled every 30 seconds, and blood pressure was measured every 15 minutes during heat exposures.

Statistical Analyses: A power calculation was performed to detect a 0.5°C difference in the increase in core temperature between groups, with a standard deviation of that increase being 0.5°C. Based upon these estimates, and with an alpha of 0.05, 20 participants (10 participants per age group) would provide power of >0.80 to detect differences between age groups in the change in core temperature (power analyses from: NCSS PASS 2019). However, we enrolled 40 participants (20 participants per age group, with each age group comprising 10 males and 10 females) to account for possible attrition/missing data and to increase the capability to detect differences in other outcome variables that may have a greater degree of variability than core temperature. Due to technical difficulties, we were unable to obtain some measurements (e.g., skin temperature, local sweat rate, blood pressure, and limb blood flow) at either baseline or at the end of the extreme heat exposures in some participants. Similarly, we were unable to obtain metabolic heat production data in one young and one older participant. These missing data were excluded from final analyses, and the number of participants included for each variable is indicated in text, tables, and figures where appropriate. Before analyses, data were assessed to confirm that they met model assumptions (i.e., normality, equality of variance). Within each extreme heat simulation, we analyzed data using linear mixed effect models with main effects of time (within factor) and group (between factor; older vs young) or unpaired t-tests (two-tailed), as appropriate. Statistical analyses were performed in R studio (Version 2022.07.1), and graphs were generated using GraphPad Prism 9.4 (GraphPad Software Inc., La Jolla, CA, USA). Data in text, tables, and figures are presented as means ± standard deviations. Statistical significance was set *a priori* to $p < 0.05$.