STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Project: Establishing Evidence-based Indoor Temperature Thresholds to Protect Health (2018-026H)

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Relevance of the research project

Multiple epidemiological reports have detailed the relations between outdoor temperature and mortality and morbidity. These generally demonstrate that older adults are among the most at risk of adverse health events during heatwaves. Concurrently, laboratory-based physiology studies have demonstrated that older adults exhibit impaired thermoregulatory and cardiovascular responses to heat exposure compared to their younger counterparts. The majority of these studies have exposed participants to extreme ambient heat stress. However, most people, especially older adults, spend ≥70% of their time in the home, where temperatures can range anywhere from ≤22°C, if the home is actively cooled, to ≥35°C in the case of insulated and poorly ventilated domiciles, even for those living in temperate continental climates.

To protect individuals against heat stress in the home, a recent report published by Toronto Public Health recommended that the maximum indoor temperature limit for multi-unit residential buildings should be set at 26°C. The World Health Organization also provides recommendations for maximal indoor temperatures, though their threshold temperature is set considerably higher at 32°C. Importantly, much of the evidence used to support these guidelines is indirect, based primarily on thermal comfort and relations between mortality and outdoor temperatures. Moreover, both guidelines offer a 'one-size-fits-all' solution, such that neither explicitly considers the thermoregulatory and cardiovascular insufficiencies that may reduce the range of tolerable indoor conditions for vulnerable populations (e.g., older adults). Despite these limitations, the thermal and cardiovascular strain experienced in conditions representative of indoor environments, where older adults spend the majority of their time, has not been directly evaluated.

Research Project Objectives

The objective of this project is to evaluate the effect of a range of environments experienced during heatwaves on body temperature and autonomic cardiovascular function in older adults. This will be accomplished by comparing thermal and cardiovascular responses in a group of older adults resting for 8-hours in a range of conditions representative of those experienced indoors during heatwaves – from insulated and poorly ventilated domiciles (36°C), through currently recommended indoor

temperature thresholds set by the World Health Organization (32°C) and Toronto Public Health (26°C), to an actively-cooled environment (22°C). To assess how the strain experienced in these conditions affects haemodynamic regulation in turn, this study will also include clinically validated tests of cardiovascular function selected to mimic autonomic challenges experienced during activities of daily living (e.g., quickly standing from a lying position). Combining resting measurements of physiological strain with validated autonomic functional tests will allow for the assessment of i) the level of hyperthermia experienced by older adults resting in relatively cooler ambient conditions (e.g., indoor environments), and ii) how the associated hyperthermia may in turn impact the regulation of cardiovascular function and haemodynamic stability. The latter objective is of particular importance given that most acute injury during heatwaves are cardiovascular in origin.

Hypotheses

Indices of thermal and cardiovascular strain will not be appreciably modified in older adults resting in simulated indoor environments below the currently recommended thresholds set by Toronto Public Health (22°C and 26°C). However, at higher indoor temperatures (31°C and 36°C) thermal (rectal temperature) and cardiovascular strain (heart rate, rate pressure product) will be elevated compared to below-threshold conditions. The increased physiological strain in these conditions will be reflected in attenuated responses to validated cardiovascular autonomic functional tests.

Methods

Participants

A total of 16 older (age: 65-80 years) adults will be recruited for the proposed project with an approximately even distribution of men and women. Each participant will complete each of the 4 experimental arms of the study. Recruited participants will be homogenous for anthropomorphic characteristics as well as habitual physical activity levels as verified via standardized questionnaires. All participants will be non-smoking and will be screened for electrophysiological abnormalities. Written and informed consent will be obtained from all volunteers prior to participation.

Experimental Design

Pre-trial instructions

All participants will be asked to avoid strenuous activity and alcohol for 24 hours prior to all preliminary and experimental sessions and to eat a light meal 2 hours before the start of each session. Participants will also be asked to consume a minimum of 500 ml of water the night before and morning of each session to ensure adequate hydration. For the experimental sessions, hydration state will be verified upon arrival to the laboratory via urine specific gravity (euhydration operationally defined as a urine specific gravity <1.025). In the event that participants exceed this threshold, ~500 mL of tap water will be provided and urine specific gravity will be tested again after ~30 min. Participants will wear an athletic shirt and shorts. For the preliminary session, participants also will wear an athletic t-shirt.

Preliminary screening

All participants will complete one preliminary evaluation a minimum of 48 hours before the first experimental session. During this session they will be familiarized with all experimental procedures and measurement techniques and will complete the Get Active Questionnaire (GAQ) and the American Heart Association Pre-participation screening Questionnaire to assess their eligibility to participate. The GAQ will also be used to assess habitual activity levels along with the Kohl Physical Activity Questionnaire. Participants will also provide verbal and written informed consent at this time, Thereafter, participant

physical characteristics will be evaluated. Body height and mass will be determined via a physician stadiometer and a high-performance weighing terminal, respectively, and from these measurements body surface area will be calculated. Body density will be estimated via hydrostatic weighing and used to calculate body fat percentage.

Experimental Protocol

Experimental design

Each session will commence at approximately 08:00. Upon arrival to the laboratory the participant will provide a urine sample for measurement of urine specific gravity and insert a rectal temperature probe. Thereafter, participants will be instrumented for the measurement of skin temperature. A measurement of body mass (nude) will also be taken at this time. Participants will then be transferred to a thermal chamber regulated to 22°C and 45%. Following 10-min of supine rest, participants will perform a specialized cardiovascular test battery (~75 min; details provided below). Following this, they will rest in the seated position in a room immediately adjacent to the thermal (~22°C) chamber for ~30 min while baseline body temperature (rectal temperature, skin temperature) and cardiovascular measures (heart rate, arterial blood pressures) are recorded. A venous blood sample will be obtained at this time.

While baseline measurements are being taken, the thermal chamber will be heated to the conditions for that day's session. Each participant will complete four separate (separated by ≥7 days) sessions consisting of resting exposure to: i) 36°C, based on maximal measured indoor temperatures (i.e., 35°C) and similar to outdoor conditions experienced during recent heatwaves in North America (HOT, heat index: 41°C); ii) 31°C, similar average daytime temperature during the 2010 heatwave in Ontario and Quebec as well as the World Health Organization recommended indoor temperature limit during heatwaves (WARM, heat index: 32°C); iii) 26°C corresponding to recommended upper limits for indoor environments as set by Toronto Public Health based before protective cooling measures should be implicated (TEMPERATE, heat index: 26°C) and iv) 22°C, to simulate an air-conditioned environment (COOL, heat index: 21°C). In all sessions, relative humidity will be regulated to 45%.

Following baseline measurements, the participant will enter the thermal chamber where they will remain for 8 hours. They will spend the majority of this time resting in the seated (slightly reclined) position, except for at the 6th hour where they will again perform the cardiovascular test battery. Rectal and skin temperatures and heart rate will be measured continuously throughout exposure. Water (tap) will be provided ad libitum from a ~500 mL cup placed on a side-table positioned slightly behind the participant (so as not to be visible in the peripheral vision). Participants will also be allowed to eat a self-provided lunch (~300 g, low water content) between hours 1.5 and 3.5 of exposure (corresponding to ~12:00-14:00 hh:mm). Every 60 min, arterial blood pressure and heart rate will be measured in triplicate after which participants will stand to provide a measurement of body mass in order to determine sweat losses (corrected for consumed food and water). Participants will be allowed to remain standing and briefly perform light stretching, if desired (max ~5 min).

Autonomic control of heart rate and blood pressure will be assessed prior to and at the end of each session using a battery of cardiovascular tests, which, in Study B consists of: i) analysis of heart rate variability and spontaneous baroreflex sensitivity during supine rest, ii) Ewing's battery (modified) for assessment of cardiovascular autonomic function, and iii) analysis of integrated baroreflex sensitivity during cyclical perturbations in arterial pressure evoked by stand-squat maneuvers. Prior to the battery, participants will be instrumented with an integrated 3-lead ECG non-invasive blood pressure monitor for the integrated measurement of beat-to-beat heart rate and arterial blood pressure, estimated from the arterial pressure waveform measured at the left middle finger using the volume-clamp technique. Throughout the battery, the arm will be affixed to the chest using a medical sling with the finger held at the approximate level of the left ventricle. Participants will be instructed to breathe normally and, without speaking, carefully follow the instructions given by the test administrator. The same researcher will administer the battery for all sessions. Finally, note that the first two sections of the battery described below (i.e., resting baroreflex sensitivity and Ewing's test) will be performed twice in succession prior to the stand-squat procedures.

i. <u>Resting HRV and BRS</u>. Heart rate variability (HRV) and baroreflex sensitivity (BRS) will be evaluated during 8-min of supine rest. Participants will be instructed to breathe

(diaphragmatic) in time with a metronome set to 30 beats/min (15 breaths/min), to remove the influence of respiratory sinus rhythm on cyclical fluctuations in heart rate and blood pressure. Thereafter, the metronome will be stopped and 1-min of recovery (spontaneous breathing) will be given prior to the start of the Ewing's battery.

ii. Ewing's battery. The modified Ewing's battery to be employed consists of three tests (deep breathing, Valsalva's maneuver and lying-to-standing test) designed to assess autonomic control of heart rate (primarily parasympathetic) and blood pressure (primarily sympathetic).

- First, participants perform 1 min of deep diaphragmatic breathing at a rate of 6 breaths·min^{-1} (metronome set to 12 beats/min). Breathing will be performed to vital capacity; that is, through a maximal inspiration and expiration. 5-min of recovery will be given before the next test.
- The next test is the Valsalva maneuver. While still in the supine position, participants will exhale against a closed glottis into a narrow vinyl tube connected to an electronic pressure transducer that will be used to provide constant feedback to the participant on the generated expiratory pressure. The participant will be instructed to maintain a constant pressure of 40 mmHg for 15 s. Throughout expiratory straining, air will be vented via a small bleed to prevent generation pressure using the mouth. Upon release, heart rate and blood pressure will be monitored for 1 min.
- The final test in Ewing's battery is the lying-to-standing test. The test begins with 1-min of rest in the supine position. At this point, the participant is instructed to quickly (but safely) assume a standing position directly beside the bed (max ~1 min). The participant then stands quietly for 3 min.

iii. Squat-stand. Following the second Ewing's battery, the participant will rest in the seated position on a rigid chair for 10 mins. The participant will then be asked to stand for 90-seconds while the positioning of the sling and finger-pressure cuff is adjusted (if needed) to ensure the left hand is maintained at heart-level. After this, 5-min of squat-stands will be performed at a rate of 6 cycles/min (0.1 Hz). Each cycle will consist of a squat, held for 5 seconds, followed by standing for 5 sec. For these maneuvers, participants will be instructed to maintain a consistent depth for each squat (based on comfort), breath normally to avoid expiratory straining (Valsalva) and limit excessive

forward flexion at the hip. Participants will be verbally aided in timing for the first ~2-3 cycles and self-timed thereafter by a stopwatch located directly within their line of sight.

After the cardiovascular battery, participants will remain seated in the chamber for the remainder of the 8-hour exposure (~45 min). Following measurements of arterial blood pressure, a final venous blood sample will be obtained followed by a measurement of body mass. The participant will be provided with water and/or a commercially available sports drink before leaving the laboratory.

Data analysis

Continuous variables related to thermal strain (rectal and mean skin temperatures) and cardiovascular strain (heart rate, rate pressure product) will be converted to 15-min averages for baseline and at each hour of exposure. Likewise, seated arterial pressures (3 measurement average) will be presented at these time points. Rate pressure product (an index of myocardial work) will be calculated as heart rate x systolic blood pressure x 10^{-3}. The change in body mass from baseline values will also be determined at each hour of exposure to quantify the rate of fluid loss, corrected for fluid consumed and urination. The change in plasma volume and serum osmolality from baseline to end exposure will also be calculated from the venous blood samples obtained at the start and end of each session. HRV and resting spontaneous cardiac BRS will be determined from data collected during the 8-min controlled breathing period. Similarly, integrated BRS will be derived from collected blood pressure and ECG waveforms collected during the stand-squat manoeuvres.

For the clinically validated tests in the Ewing battery, analysis will be performed by a trained researcher blinded to the participant and session code (i.e., environmental conditions). Deep breathing will be analysed as the difference in heart rate between the average of the three lowest and three highest values recorded during inspiration and expiration, respectively (E/I HR_{Diff}). For the Valsalva maneuverer, the so-called Valsalva ratio (VALratio) is calculated as the longest R-R interval during recovery divided by the shortest R-R interval during straining. Finally, the lying to standing test is analysed by determination of the 30:15 ratio, which represents the longest RR interval measured between the 25-35th heart beat upon standing divided by the shortest RR interval between

the 10-20th beat after standing. Additionally, the systolic response to standing (SR_{stand}) is also taken as the 1-min average of systolic blood pressure after the second min of standing divided by systolic blood pressure during the pre-stand supine resting period (i.e., the reduction in systolic pressure upon assuming a standing position). The participant identifier and experimental arm will be blinded from the investigator responsible for analyzing outcomes in the cardiovascular battery. Values for each test will be taken as the average of each of the two recorded values.

Statistical analysis and sample size calculation

Statistical analysis will compare end-exposure data relating to thermal and cardiovascular strain as well as autonomic function (while correcting for between-session baseline variability). Body temperature (i.e., rectal temperature and mean skin temperature) and cardiovascular responses (i.e., systolic, diastolic and mean arterial pressure, heart rate, rate pressure product) at the end of exposure (i.e., hour 8) as well as the autonomic functional tests of the cardiovascular battery (i.e., E/I HR_{Diff}, VAL ratio, 30:15 ratio and SR_{stand}) will be evaluated using an analysis of covariance (ANCOVA) with the repeated factor of environmental condition (four levels: HOT, WARM, TEMPERATE and COOL) and baseline values modelled as a continuous covariate. Variables related to fluid loss (e.g., change in body mass, plasma volume and serum osmolality) will be analysed via a one-way ANOVA with the factor of condition (4 levels). For all statistical models, homogeneity of variances will be assessed using Levene's test and visual assessment of residual plots. Normal distribution of residuals will be assessed with Sharpiro-Wilks test and visual inspection of histograms and Q-Q plots and data will be log-transformed in the event that distribution of residuals deviates from normality. Pre-planned *post hoc* comparisons will be employed to identify differences between adjacent conditions (e.g., COOL vs TEMP, TEMP vs. WARM, WARM vs. HOT) in order to limit a large family of comparisons. For all analyses, alpha will be set at $P < 0.050$. Descriptive statistics will be presented as mean (standard deviation). Comparisons between groups or time-points (where appropriate) will be presented as means ± 95% confidence interval.

An *a priori* power analysis determined that a total sample size of 16 older adults is required to detect a difference in rectal temperature between groups with 80% statistical

power, after adjusting for multiple comparisons. The effect size (Cohen's *d*=1.0) was determined based on practically meaningful difference of 0.3°C between conditions, assuming a standard deviation of 0.3. The standard deviation was based on previously published studies from our laboratory, which demonstrated a 0.2°C (SD 0.3) difference in core temperature between young and older adults (Kenny GP et al. *Temperature (Austin)*. 2016 31;4(1):79-88) and a 0.0°C (SD 0.3) difference in core temperature between older adults with and without type 2 diabetes (Poirier et al. *Temperature (Austin)*. 2020 [in press]) following 3 hours of rest in a hot environment (40°C, 30% relative humidity).

Measurements

Primary Outcome measure

Body temperatures

Rectal temperature will be continuously monitored using a general-purpose thermocouple temperature probe (Mon-a-therm General Purpose Temperature Probe, Mallinckrodt Medical Inc., St-Louis, MO, USA) inserted ~12 cm past the anal sphincter. Skin temperature will also be assessed using surface temperature monitors (DS1922L Thermochron, OnSolution Pty Ltd, Australia) placed in 8 locations as described in ISO 9886:2004. Mean skin temperature will be subsequently calculated using the provided weightings: forehead (7%), right scapula (17.5%), upper left chest (17.5%), upper right arm (7%), right forearm (7%), left hand (5%), right anterior thigh (19%) and left calf (20%).

Secondary Measures

Cardiovascular measurements

Heart rate will be recorded continuously throughout each experimental session and stored every second using a Polar coded WearLink and transmitter, Polar RS400 interface and Polar Trainer 5 software (Polar Electro, Kempele, Finland). Arterial systolic and diastolic blood pressures will be intermittently taken as an average of three values measured at the brachial artery (~30 s between measures) via manual auscultation. Mean arterial pressure will be calculated as 2/3 diastolic pressure + 1/3 systolic pressure. Rate pressure product, an index of myocardial work, will also be evaluated as heart rate × systolic pressure x 10^{-3}. Brachial systolic and diastolic arterial blood pressures will also

be estimated from beat-to-beat recordings of arterial pressure waveform measured at the left middle-finger using the volume-clamp technique (NIBP, ADInstruments, Colorado Springs, CO, USA). In the specialized cardiovascular test battery, beat-to-beat arterial blood pressure measures will be coupled with beat-to-beat heart rates derived from R-R interval measured via an integrated 3-lead ECG (FE231 Bio Amp, ADInstruments, Colorado Springs, CO, USA).

Hydration-related variables

Baseline (start of session) urine specific gravity will be assessed with a hand-held total-solids refractometer. (Reichert TS 400 total solids refractometer, Reichert, Depew, NY, USA). Participants presenting to the laboratory with urine specific gravity <1.025 will be considered adequately hydrated to begin the experimental session. Throughout each intervention, fluid status will be monitored via changes in body mass. Pre and post heat exposure measurements will be obtained using a high-performance digital weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, Canada). This unit will also be used to monitor changes in body mass throughout the intervention.

Venous blood samples will be collected for determination of plasma volume and serum osmolality responses. Blood samples will be transferred directly into plasma (5.4 mg K2EDTA) and serum (no additive) Vacutainer tubes (BD, Franklin Lakes, NJ, USA). The K2EDTA blood is mixed by inversion and used to measure haematological parameters in duplicate (Ac·T diff, Beckman Coulter, Miami, FL, USA). Measured blood haemoglobin and haematocrit will be used to estimate changes in plasma volume. Non-additive blood is left to sit for 20 min to clot before centrifugation (~1380 g for 10 min). Separated serum is then transferred into polypropylene Eppendorf tubes, frozen at -20ºC, and stored at -80ºC until serum osmolality can be later analyzed via freeze-point depression (model 3320, Advanced Instruments, Norwood, MA, USA).

Delimitations and limitations

All participants recruited for this research will be sedentary or habitually active but non-endurance trained adults aged 65-80 years. The results may not be directly applicable to different population groups (e.g., children, adolescents or the extremely old

[80+ years]). Although an approximately equal number of men and women will be recruited for each intervention to ensure a sample representative of the general population, the experiments performed will not permit the assessment of the interaction of age and sex on thermoregulatory and cardiovascular responses to long-duration heat stress. Finally, behavioral factors in large part determine one's risk of mortality and morbidity during heatwaves. While the project will provide novel information on the physiological-basis of heat tolerance, it is important to consider that defining risk is a multi-faceted endeavour and thereby larger in scope than any one series of studies in a given domain.