STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Project: Physiological Responses in Young and Older Adults During a Prolonged Simulated Heatwave (H 05-16-07)

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Relevance of the research project

Older adults are among the most at risk of adverse health events during heatwaves due, in large part, to impaired homeostatic responses to heat stress. However, much of our understanding of the effects of extreme heat on physiological responses in older adults has come from studies that have employed short-term (<3 hours) exposure to conditions hotter than those typically experienced during heatwaves. Additionally, we are lacking information regarding the physiological consequences of currently recommended heat-mitigation strategies. For instance, The World Health Organisation suggests that when home-cooling is unavailable, individuals should visit a cooled location (e.g., cooling centre) for 1-2 hours in order to cool the body. Whether this intervention can provide lasting reductions in physiological strain is not clear, but it is likely that reductions in body temperature are only transient as heat will be rapidly gained upon re-exposure to elevated ambient temperatures. The proposed research will therefore provide important and novel information regarding 1) the effect of prolonged exposure to simulated heatwave conditions on whole-body heat loss and the resultant thermoregulatory and cardiovascular responses in young and older adults, and 2) the efficacy of currently recommended heat-mitigation strategies for protecting at-risk sectors of the population.

Research Project Objectives

The objectives of this project are: 1) evaluate the effect of age on whole-body heat exchange and the development of thermal and cardiovascular strain during day-long (9-hour) exposure to simulated heatwave conditions (Intervention 1); and 2) determine whether short-duration (2 hour) exposure to an air-conditioned environment following extreme heat exposure results in lasting reductions in thermal and cardiovascular strain upon return to the heat (Intervention 2).

The first objective (via Intervention 1) will be achieved by comparing whole-body heat loss and storage (measured using combined direct and indirect calorimetry) and the accompanying changes in body temperature (i.e., rectal, mean skin temperature) and haemodynamic responses (e.g., cardiac output, heart rate, rate pressure product, etc.) between young and older adults during a 9-hour resting exposure to 40°C and 15% relative humidity conditions. These conditions (heat index: 38°C) were chosen to simulate
peak temperatures experienced during heatwaves and are similar to peak conditions in heatwaves in Ottawa, Canada (2018; 34°C and 58%, heat index: 41°C) and Paris France (2003; 38°C and 25%, heat index: 38°C). This intervention will thereby allow for the determination of i) whether ecologically valid heatwave conditions exceed the physiological capacity for heat dissipation in older adults, and ii) how thermal and cardiovascular strain develops during long-duration exposure during heatwaves.

The second objective (via Intervention 2) will be accomplished by comparing physiological responses in the older adults from Intervention 1 to a separate age-matched group, removed from the heat to spend 2 hours in a cooler environment (22-23°C) approximately mid-way through the simulated heatwave exposure (hours 5-6). Comparing the rate of heat gain and changes in body temperature and cardiovascular responses before and after the ambient cooling intervention to the non-cooled group from Intervention 1 will allow for determination of whether short-term cooling leads to prolonged reductions in thermal and cardiovascular strain or if the cooling-induced physiological alterations occur only transiently.

Hypotheses

**Intervention 1**: Older individuals will exhibit reduced whole-body heat loss compared to young adults during a 9-hour exposure to simulated heatwave conditions (40°C, 15% relative humidity) such that heat balance (i.e., rate of heat loss balancing the rate of heat gain; thermal equilibrium) will not be attained in the older adults. As a result, greater thermal (i.e., rectal temperature) and cardiovascular (i.e., heart rate, rate pressure product, etc.) strain will be observed in the older adults and these between-group differences will be exacerbated as exposure progresses.

**Intervention 2**: Upon return to high ambient temperatures, body heat storage will be exacerbated in the older adults exposed to cooling (hours 5-6) compared to the older adults from Intervention 1 not removed from the heat. Consequently, comparable thermal and cardiovascular responses will be observed between groups by the end of exposure (i.e., 9 hours).
**Methods**

**Participants**

A total of 20 young (age: 18-30 years) and 40 older (age: 65-80 years) adults will be recruited for the proposed project. Young ($n = 20$) and older ($n = 20$) adults will complete **Intervention 1** and a separate cohort of older adults ($n = 20$) will complete **Intervention 2**. Each experimental arm will have an approximately even distribution of men and women. Details on the determination of the sample sizes for each intervention are provided below. Participants will be homogenous for anthropomorphic characteristics and as well as habitual physical activity levels as verified via standardized questionnaires. All participants will be non-smoking. Written and informed consent will be obtained from all volunteers prior to participation and older participants will be screened for cardiac electrophysiological abnormalities prior to the heatwave simulations.

**Experimental Design**

*Pre-trial instructions*

All participants will be asked to avoid strenuous physical 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. For all sessions, participants will wear athletic shorts and footwear (and a sport top for women). For the preliminary session, participants also will wear an athletic t-shirt.

*Preliminary screening*

All participants will complete one preliminary evaluation a minimum of 7 days before the first experimental session. During this session they will be familiarized with all 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 (Detecto, model 2391, Webb City, MO, USA) and a high-performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON, Canada), 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 (Intervention 1)**

**Experimental design**

Each session will commence at approximately 07:00. Upon arrival to the laboratory, the participant will provide a urine sample for the assessment of urine specific gravity, after which a measurement of nude body mass will be obtained. Participants will then insert a rectal temperature probe for the continuous measurement of rectal temperature. Thereafter, participants will be instrumented for the measurement of skin temperature and heart rate.

Baseline cardiovascular parameters will be evaluated via a brief (~45 min) cardiovascular test battery, performed as follows. First, cardiac output will be measured via inert gas rebreathing. Brachial arterial systolic and diastolic pressures reconstructed from arterial pressure waveforms measured at the right middle finger will then be collected for 10-min and subsequently used for evaluation of resting spontaneous cardiac baroreflex sensitivity (an index of cardiovascular autonomic function). Immediately thereafter, arterial systolic and diastolic pressures will be measured via manual auscultation. A second measurement of cardiac output will then be taken (allowing for ≥10 min between the first and second measurements to ensure full gas washout), after which calf and limb blood flows on the right side of the body will be measured via venous occlusion plethysmography. Throughout the short cardiovascular test battery, participants will remain seated in a semi-recumbent position with both feet placed on the floor and their hands resting comfortably in their lap, except for during the measurements of limb
blood flow, where the instrumented limbs will be elevated to facilitate venous drainage. Finally, a venous blood sample and body mass measurement will be obtained.

Participants will then be transferred to the whole-body direct calorimeter chamber, regulated to 40°C and ~15% humidity. These conditions were chosen to simulate peak temperatures experienced during heatwaves and are similar to peak conditions in recent heatwaves in North American in 2018 (Ottawa, Ontario; 34°C and 58%, heat index: 41°C) and Europe in 2003 (Paris, France; 38°C and 25%, heat index: 38°C). The participant will rest quietly for 3-hours (hours 1-3) within the calorimeter chamber while whole-body heat production and exchange are measured continuously. At the 3-hour mark, the participant will exit the calorimeter and the brief cardiovascular test battery will be performed followed by a measurement of body mass. Hours 4-6 will be spent resting in the heat in the thermal chamber adjacent to the calorimeter. During this time, participants will be allowed to consume a light (~300 g), self-provided lunch with low water content (e.g., peanut butter sandwich). Tap water will be provided *ad libitum* via a self-service insulated water cooler located in the thermal chamber (out of direct sight of the participant). At the half-way point of exposure (hour 4.5), a venous blood sample will be obtained. Another cardiovascular battery will then be performed followed by a measurement of body mass at the end of this period. The participant will then re-enter the calorimeter where the final 3 hours will be spent (hours 6-9). At the end of this period, the participants will undergo a fourth and final cardiovascular test battery and a venous blood sample and measurement of body mass will be procured. The participant will be provided with water and/or a commercially available sports drink before leaving laboratory.

**Data analysis**

Continuous variables related to whole-body heat exchange (i.e., the rates of evaporative heat loss, dry heat gain, net heat exchange, and body heat storage) will be converted to 30-min averages corresponding to each hour of the two calorimetry sessions. Cumulative heat storage will be calculated as the temporal summation of whole-body heat production and exchange over each 3-hour calorimetry period (i.e., hours 1-3 and 7-9). Similarly, continuous measurements of body temperatures (i.e., rectal and mean skin temperatures) and heart rate will be converted to 30-min averages for each hour of
the 9-hour exposure. Data collected during cardiovascular batteries (i.e., heart rate, arterial blood pressures, cardiac output, stroke volume, total peripheral resistance, rate pressure product, baroreflex sensitivity and calf and forearm blood flow and vascular conductance) and reductions in body mass (to estimate fluid loss) will be presented for each of the cardiovascular batteries (every ~3 hours). Changes from baseline will also be computed for all thermal and cardiovascular data. Similarly, changes in plasma volume and serum osmolality will be determined from the venous blood samples taken throughout the protocol.

Statistical analysis and sample size calculations

Statistical analysis for Intervention 1 will be performed to compare whole-body heat exchange and storage and the resultant progression of thermal and cardiovascular responses between the young and older adults throughout the 9-hour simulated heatwave. Calorimetrically-derived variables and variables related to thermal, cardiovascular and fluid regulatory responses will be evaluated using a linear mixed-effects model. Time will be modelled as a repeated within-subject fixed effect, and age-group will be modelled as a between-subject fixed effect, whereas participant identification is included as a random factor. Model parsimony will be used to determine variance/covariance structure of model errors and random and fixed effect structure in accordance with Akaike’s information criterion. When main effects or interactions are identified as statistically significant, post hoc multiple comparisons will be made on model estimated marginal means. For all statistical models, homoscedasticity will be assessed using Levene’s test and visual assessment of residual plots. Normal distribution of residuals will be assessed via visual inspection of histograms and Q-Q plots and data will be log-transformed in the event that distribution of residuals strongly deviates from normality. 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 19 young and 19 older adults is required to detect a difference in the rate of whole-body heat storage between groups at the end of each calorimeter session (i.e., hours 3 and 9), after
adjusting for multiple comparisons, with 80% statistical power. In lieu of clinically meaningful data (i.e., what would be considered a clinically meaningful change in whole-body heat storage), the standardized effect size (Cohen’s $d = 1.06$) was calculated from the difference in the rate of body heat storage between young and older adults over the final 30-min of a 3 hours heat exposure (young: -2 [26] kJ/hour, older: 43 [54] kJ/hour) in our previous work (Kenny GP et al. Temperature (Austin). 2016 31;4(1):79-88).

**Experimental Protocol (Intervention 2)**

**Experimental design**

Twenty older adults will complete the protocol for Intervention 2, which is identical to that of Intervention 1 except that after the first calorimeter session and the subsequent cardiovascular battery, participants will exit the thermal chamber and spend ~2 hours (hours 5-6) resting in an air-conditioned room (~23°C, ~50% relative humidity). Similar to protocol 1, participants will be allowed to eat a small self-provided lunch during this time and consume water (tap) *ad libitum*. In contrast to Intervention 1, a venous blood sample will not be procured at the 4.5-hour mark in Intervention 2. The third cardiovascular battery will be performed in the cooled environment. The level of significance will be set at $P < 0.050$. As in Intervention 1, participants will then re-enter the calorimeter for the final 3 hours, where they will rest in the heat for the remainder of the experimental session.

**Data analysis**

Data analysis for Intervention 2 will be identical to that for Intervention 1.

**Statistical analysis and sample size calculations**

Statistical analysis for Intervention 2 will be performed to assess whether mid-day exposure to a cooled room results in greater body heat storage following return to a hot environment such that physiological responses are similar to those of a non-cooled group by the end of the 9-hour heatwave simulation. Cumulative whole-body heat storage over the second calorimetry period (hours 7-9) will be compared between the group receiving the cooling intervention to the non-cooled older adults from Intervention 1 using a two-way independent samples t-test. Two one-sided tests (TOST; see Lakens. *Soc Psychol*
Personal Sci. 2017:8 (4), 355-362) will be employed to assess the equivalence of variables related to whole-body heat exchange and thermal and cardiovascular as well as fluid loss between groups at the end of each exposure period. This method of analysis is the most appropriate to assess our primary hypothesis of this Intervention – that the groups do not differ by the end of the second calorimetry session (i.e., 9 hours).

Briefly, the TOST procedure involves two one-sided tests to evaluate whether a between-group mean difference is within pre-specified bounds: ±1 Cohen’s d for our purposes (equivalent to an 0.3°C difference in rectal temperature). The null hypothesis is taken as a meaningful difference between groups and its rejection therefore corresponds to an equivalence of means. The level of significance will be set at $P < 0.050$. Descriptive statistics will be presented as mean (standard deviation) and comparisons between groups will be presented as mean ± 95% confidence interval.

An a priori power analysis determined that a total sample size of 18 older adults in each group (36 participants total) is required to confirm whether between-group differences in rectal temperature are within upper and lower bounds of +0.3°C and -0.3°C, respectively, with 80% power. This corresponds to an effect size (Cohen’s d) of 1.0, based on the pooled-standard deviation of 0.3°C, which was based on previously published data from our laboratory demonstrating 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 (44°C, 30% relative humidity)

Measurements
Primary Outcome measures

Whole-body heat exchange and body temperatures

The rates of whole-body evaporative and dry heat loss will be measured via the Snellen direct air calorimeter, which provides the only standard measure of these metrics. Calorimeter inflow and outflow values of absolute humidity and air temperature are measured at 8 second intervals using high precision dew point hygrometry (RH Systems
model 373H, Albuquerque, NM, USA) and resistance temperature detectors (Black Stack model 1560, Hart Electronics, UT, USA), respectively. Air mass flow is determined via differential thermometry over a known heat source in the effluent air stream. All data are displayed and recorded on a personal computer with LabVIEW software (Version 7.0, National Instruments, Austin, TX, USA). Heat loss via sweat evaporation is then determined using the outflow–inflow difference in absolute humidity, multiplied by air mass flow and the latent heat of vaporization of sweat (2426 J·g⁻¹). Dry heat loss is similarly derived from the outflow–inflow air temperature difference and specific heat capacity of air (1005 J·kg⁻¹·°C⁻¹). Throughout exposure, ambient temperature (40°C) will be greater than that of the skin (~35-36°C). Thus, measured dry heat loss will be negative, indicating heat gain from the environment. Net heat exchange at the skin surface will be calculated as evaporative heat loss minus dry heat gain.

Metabolic energy expenditure will also be measured using indirect calorimetry. Expired oxygen (O₂) and carbon dioxide (CO₂) content will be measured from using electrochemical gas analysers (AMETEK model S-3A/1 and CD 3A, Applied Electrochemistry, Bastrop, TX, USA) air drawn from a 6 L fluted mixing box located within the calorimeter. Expelled air is then recycled back into the chamber to account for respiratory heat exchange. The gas analyzers and turbine ventilometer will be calibrated ~30 min prior to each calorimetry measurement period (2 per experimental session). Endogenous metabolic heat production will be assumed to be equivalent to metabolic energy expenditure as no external work will be performed in either intervention. Body heat storage will be evaluated as the summation of heat production and net heat exchange (i.e., evaporative heat loss – dry heat gain) and reported as a rate (i.e., kJ·min⁻¹) as well as a cumulative value over each measurement period (i.e., kJ).

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 $\frac{2}{3}$ diastolic pressure + $\frac{1}{3}$ systolic pressure. Rate pressure product, an index of myocardial work, will also be evaluated as heart rate × systolic pressure x $10^{-3}$.

Cardiac output will be taken as average of duplicate values measured non-invasively using an inert gas (5% blood soluble N$_2$O, 1% and blood insoluble SF$_6$,) rebreathing system (Innocor, Innovision, Odense, Denmark). Stroke volume is then calculated as cardiac output ÷ heart rate, whilst total peripheral resistance is derived as mean arterial pressure ÷ cardiac output. Forearm and calf blood flows will also be determined as the average of a minimum of four measurements obtained via automated venous occlusion plethysmography (Hokanson AI6, D.E. Hokanson, Inc., Bellevue, WA, USA). 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 (Finometer Pro, Fina-press Medical Systems, Amsterdam, Netherlands). Cardiac baroreflex sensitivity (an index of cardiac autonomic activity) will be determined via the sequence technique using software provided by Finapres Medical Systems (PRVBRS, Fina-press Medical Systems, Amsterdam, Netherlands).

*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,
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 18-80 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.