The Effect of Plasma Osmolality and Baroreceptor Loading Status on Postexercise Heat Loss Responses

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We examined the separate and combined effects of plasma osmolality and baroreceptor loading status on postexercise heat loss responses. Nine young males completed a 45-min treadmill exercise protocol at 58±2% VO\textsubscript{2peak} followed by a 60-min recovery. On separate days, participants received 0.9% NaCl (ISO), 3.0% NaCl (HYP) or no infusion (natural recovery) throughout exercise. In two additional sessions (no infusion), lower-body negative (LBNP) or positive (LBPP) pressure was applied throughout the final 45-min of recovery. Local sweat rate (LSR; ventilated capsule: chest, forearm, upper back, forehead) and skin blood flow (SkBF; laser-Doppler flowmetry: forearm, upper back) were continuously measured. During HYP, upper back LSR was attenuated from end-exercise to 10-min of recovery by ~0.35±0.10 mg·min\textsuperscript{-1}·cm\textsuperscript{-2} and during the last 20-min of recovery by ~0.13±0.03 mg·min\textsuperscript{-1}·cm\textsuperscript{-2} while chest LSR was lower by 0.18±0.06 mg·min\textsuperscript{-1}·cm\textsuperscript{-2} at 50 min of recovery when compared to natural recovery (all \(P<0.05\)). Forearm and forehead LSR were not affected by plasma hyperosmolality during HYP (all \(P>0.28\)), which suggests regional differences in the osmotic modulation of postexercise LSR. Furthermore, LBPP application attenuated LSR by ~0.07-0.28 mg·min\textsuperscript{-1}·cm\textsuperscript{-2} during the last 30-min of recovery at all sites except the forehead when compared to natural recovery (all \(P<0.05\)). Relative to natural recovery, forearm and upper back SkBF were elevated during LBPP, ISO and HYP by ~6-10% by the end of recovery (all \(P<0.05\)). We conclude that 1) hyperosmolality attenuates postexercise sweating heterogeneously among skin regions, and 2) baroreceptor loading modulates postexercise SkBF independently of changes in plasma osmolality without regional differences.

**Keywords**: thermoregulation, exercise, osmoreceptors, hydration, heat loss
INTRODUCTION

Dehydration associated with heat stress (i.e., a progressive loss of body water through sweating without adequate fluid replacement) is known to cause marked impairments in cardiovascular and thermoregulatory control (14, 30). Dehydration is associated with a state of both hypovolemia and hyperosmolality, and therefore simultaneously implicates both baroreceptor unloading (via reductions in blood volume) and osmoreceptor activation. However, the vast majority of studies have considered these effects separately with respect to the regulation of the heat loss responses (i.e., skin blood flow and sweating). Hyperosmolality alone exerts a central modulation of thermoregulatory activity via increases the core temperature onset threshold for both sweating and cutaneous vasodilation during whole body passive heat stress (2, 11, 24, 31, 36) and exercise (8) without affecting the thermosensitivity of the response. Moreover, hypovolemia simulated with lower body negative pressure (LBNP) application during passive heat stress can independently delay the onset threshold for cutaneous vasodilation with no effect on sweating (24). Hypovolemia induced via reductions in blood volume during exercise (i.e., through profuse sweating) can reduce the thermosensitivity of sweating (7) as well as peak forearm blood flow (8, 28).

To our knowledge, only two studies have assessed the combined effects of hyperosmolality and hypovolemia on the heat loss responses, and these have been limited to passive heat stress (16, 24). Examining solely the skin blood flow response, Ito et al. (16) demonstrated greater reductions in forearm vascular conductance when graded levels of LBNP were applied in a hyperosmotic state compared to an isosmotic state. The study by Lynn et al. (24) demonstrated a parallel response such that the attenuation of skin blood flow during hyperosmolality or LBNP alone was exacerbated when their effects were combined.
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Additionally, the study revealed that reductions in sweat rate were primarily mediated by hyperosmolality (24).

Taken together, these findings underline the important role that changes in plasma volume and osmolality may play in the regulation of heat loss responses during heat stress. To date however, little is known about the consequences of exercise-induced changes in plasma volume and osmolality (as induced by profuse sweating during exercise) on the control of postexercise heat loss responses, and therefore core temperature regulation. Such knowledge is important given the large number of studies that have repeatedly shown that heat loss is rapidly suppressed following dynamic exercise to near baseline levels within ~20-min, consequently evoking persistent elevations in core and muscle tissue temperatures for up to 2 hours (22, 39). This disturbance in postexercise thermoregulatory function has been attributed to nonthermal sensory receptor modulation of skin blood flow and sweating (21). In particular, baroreceptor unloading has been shown to have an important influence in the attenuation of skin blood flow, whereas its influence on sweating remains controversial (9, 18, 27). However, the extent to which exercise-induced changes in plasma volume and osmolality may have a role in the modulation of postexercise heat loss responses remains unclear.

Thus, the purpose of this study was to examine the separate and combined effects of plasma osmolality and baroreceptor loading status on postexercise skin blood flow and sweating. It was hypothesized that 1) hyperosmolality, but not baroreceptor unloading, would independently exacerbate the postexercise suppression of sweating, and 2) an additive suppressive effect of hyperosmolality and baroreceptor unloading would be observed for skin blood flow such that it would be lowest during the application of LBNP. Based on evidence suggesting heterogeneous patterns of the local thermoeffector responses to nonthermal stimuli (7,
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8, 41), a secondary objective was to characterize the regional variation of these responses.
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METHODS

Ethical approval

The experimental protocol was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and is in accordance with the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to their participation in the study.

Participants

The minimum required sample size was determined using data from Lynn et al. (24). Using an effect size of 1.1 calculated from the pooled mean data for the difference in the mean body temperature threshold at which the onset of sweating occurred during heat exposure between isosmotic (36.77 ± 0.36°C) and either hyperosmotic (37.19 ± 0.32°C) or hyperosmotic + LBNP (37.25 ± 0.42°C), we found that the sample size for determining a main effect using a repeated measures analysis of variance (R-ANOVA) with a power of 95% and an α error probability of 0.05 was seven participants. Nine young healthy males volunteered for this study. Exclusion criteria included a history of smoking as well as any respiratory, metabolic, renal, or cardiovascular disease. All participants reported being physically active (participating in 2-5 structured physical activity sessions per week for ≥30 min each). Their characteristics (mean ± standard deviation) are as follows: age, 21 ± 1 years; height, 175 ± 5 cm; body mass, 78.19 ± 5.29 kg; body surface area, 1.94 ± 0.07 m²; and peak oxygen uptake (VO₂peak), 50 ± 6 mL·kg⁻¹·min⁻¹.
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Experimental design

Participants volunteered for an initial screening visit followed by a preliminary session. They subsequently returned for five experimental sessions performed in a randomized order. All sessions were conducted on different days and separated by a minimum of 48 h. Prior to each visit, participants were instructed to refrain from consuming alcohol and engaging in strenuous exercise for 24 h, as well as taking caffeine and non-steroidal anti-inflammatory medications for 12 h. Participants were encouraged to drink water throughout the day before coming to the laboratory to ensure euhydration. They were instructed to eat a light breakfast no more than 2 h before their arrival. Hydration status was verified using urine specific gravity of a urine sample provided by the participant at the start of the preliminary session and each of the five experimental sessions. Participants had to achieve a cut-off value of ≤1.015. This was selected based on the urine specific gravity ranges corresponding to different hydration levels as established by Sawka et al. (1). In this Position Stand, individuals are considered mildly dehydrated at USG values >1.020.

Screening session. During this session, participants were informed of the study protocol and familiarized with the equipment. Body height, mass, and surface area as well as VO2peak were also determined. Body height was measured with a stadiometer, while body mass was obtained (± 0.01 kg) using a digital weight scale platform (Model CBU150X, Mettler Toledo, Scherzenbach, Switzerland) with a weighing terminal (Model IND560, Mettler Toledo). Body surface area was subsequently calculated using body height and mass (6). To determine VO2peak, participants performed a progressive incremental treadmill (Woodway Desmo H treadmill, Waukesha, Wisconsin, USA) protocol to exhaustion. Participants were asked to run at a comfortable pace (~10 km·h⁻¹) while the incline was increased by 2% every minute. Breath-by-
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Breath oxygen uptake was measured by an automated gas analyzer (Medgraphics Ultima, Medical Graphics Corp., St Paul, MN, USA).

Preliminary session. This session was included to establish the magnitude of total body fluid loss for each individual associated with the exercise protocol. Participants were asked to complete 45-min of brisk treadmill walking (60% of VO_{2peak}) in a thermal chamber (Can-Trol Environmental Systems, Markham, ON, Canada) set at 35°C and 30% relative humidity while wearing a loosely fitted poncho covering the torso only. While the room was set to 30% relative humidity, the poncho was employed to reduce the water vapour pressure gradient between the skin and the impermeable layer which helped to maximize sweat production, and therefore total body fluid loss. To ensure that the appropriate intensity was maintained, oxygen consumption was monitored throughout exercise by measuring expired oxygen and carbon dioxide concentrations (AMETEK model S-3A/1 and CD3A, Applied Electrochemistry, Pittsburgh, PA, USA). Speed and incline changes were carefully recorded for each participant such that the exercise protocol could be replicated to induce a similar body water loss in the five experimental sessions (see below). A measurement of nude body mass was obtained prior to and immediately following treadmill exercise to determine total body fluid loss.

Experimental sessions. For each experimental session, participants reported to the laboratory at the same time of day. Upon arrival, they were instructed to provide a urine sample and to change into shorts and running shoes. A nude body mass was obtained after euhydration was verified using urine specific gravity (1). An 18-gauge venous catheter was inserted in the antecubital fossa vein and connected to a Luer Lock extension [LifeShield™ Microbore Extension Set with removable Clave™ connector and locking spin collar, Non-DEHP (Hospira Inc., Lake Forest, IL, USA)] while participants rested supine. They were then instrumented in a
thermoneutral room and transferred to a thermal chamber set at 35°C and 30% relative humidity. After 15-min of upright seated baseline resting, participants donned a poncho and replicated the 45-min treadmill exercise protocol performed in the preliminary session. Noteworthy, the left arm was stabilized at the level of the heart throughout the treadmill exercise in order to prevent extravasation of the venous catheter. Following exercise, participants were immediately transferred without being wiped dry (~5-min) to an upright-seated pressure box sealed at the level of the iliac crests using custom-made, loose fitting neoprene shorts. Thereafter, participants were re-instrumented for the measurement of blood pressuring using a Finometer and remained seated for 60-min of recovery. At the end of the experimental session, nude body mass and a urine sample were obtained.

This study consisted of five experimental conditions. In conditions #1 and #2, participants received either an isotonic (0.9% NaCl, ISO) or hypertonic (3.0% NaCl, HYP) infusion of saline (warmed to near body temperature) for the duration of the exercise protocol (45-min) to replace total body fluid loss as measured during the preliminary session. In conditions #3 and #4, participants received no infusion and they endured either lower body positive (+40 mmHg, LBPP) or negative (-20 mmHg, LBNP) pressure applied continuously for the final 45-min of the recovery period. Lastly, in condition #5, participants received no infusion and no pressure (0 mmHg with circulating air) (natural recovery). To standardize between conditions, circulating air was applied during ISO and HYP in the same manner as in natural recovery.

The volume of saline infused during the ISO and HYP conditions was calculated from the total body fluid lost during the preliminary session (average across participants: 0.81 ± 0.13 kg) for each participant. The volume of saline required to maintain plasma volume to near baseline
levels (i.e., to counteract the fluid lost through sweating) was based on volumetric proportions of 0.9% and 3.0% saline previously used in other studies to induce similar plasma volume changes in each infusion condition (31, 36). This resulted in the infusion of 0.65 ± 0.09 L (ISO) and 0.31 ± 0.06 L (HYP), equivalent to 80.8 ± 4.4 % and 38.1 ± 5.1 % of the total body fluid loss during exercise, respectively. Given that the volume of saline differed for each participant, the constant infusion rates were adjusted accordingly (average across participants: 0.190 ± 0.026 and 0.088 ± 0.015 mL·min⁻¹·kg⁻¹ of body mass for ISO and HYP conditions, respectively).

Measurements

A general purpose thermocouple temperature probe (Mallinckrodt Medical Inc., St-Louis, MO, USA) was inserted ~40 cm past the nostril and into the esophagus for the measurement of esophageal temperature. Thermocouples (Concept Engineering, Old Saybrook, CT, USA) were attached to six skin sites for the measurement of skin temperature. Mean skin temperature was calculated using six skin temperatures weighted to the regional proportions as determined by Hardy and Du Bois (15): upper back 21%, chest 21%, biceps 19%, quadriceps 9.5%, hamstrings 9.5%, and front calf 20%. Temperature data were collected at 15-s intervals with an HP Agilent data acquisition module (Model 34970A; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and simultaneously displayed with LabVIEW software (Version 7.0, National Instruments, Austin, TX, USA) and recorded in spreadsheet format on a desktop computer.

Local sweat rate was measured at the chest, forearm, upper back, and forehead with a ventilated plastic capsule affixed at each site using adhesive rings, topical skin glue (Collodion HV, Mavidon Medical Products, Lake Worth, FL, USA), and surgical tape. Anhydrous nitrogen gas was passed through each capsule at a rate of 1 L·min⁻¹ over the skin surface. Long vinyl
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tubes connected the gas tank (positioned in the chamber) to the sweat capsule so that the gas
temperature was equilibrated to room temperature (~35˚C) before reaching the sweat capsule.
Capacitance hygrometry (Model HMT333, Vaisala, Helsinki, Finland) was used to measure
water content from the effluent air. Local sweat rate was calculated every 5-s as follows:

\[
\text{Local sweat rate} = \frac{\left( \frac{r_{\text{out}} \cdot P_{\text{H}_2\text{O}} \cdot f \cdot T_{\text{cap}} \cdot k}{100} \right) - \left( \frac{r_{\text{in}} \cdot P_{\text{H}_2\text{O}} \cdot f \cdot T_{a} \cdot k}{100} \right)}{A} \quad (\text{mg·min}^{-1}·\text{cm}^{-2})
\]

where \( r_{\text{in}} \) and \( r_{\text{out}} \) are the relative humidity of air entering and leaving the capsule (%), \( P_{\text{H}_2\text{O}} \) is
the partial pressure of water vapour of air entering the capsule if 100% saturated (mmHg), \( f \) is the
flow rate through the rotameter (l·min^{-1}), \( k \) is the water vapour gas constant (3.464 mmHg·l·g^{-1}·K^{-1}), \( T_{a} \) and \( T_{\text{cap}} \) are the temperature of air entering and leaving the capsule (K), and \( A \) is the
surface area of skin under the capsule (2.8 cm^2) (38).

Skin blood flow (perfusion units) was measured at the forearm and upper back using
laser-Doppler flow probes (PeriFlux System 5000, Perimed AB, Stockholm, Sweden) with an
integrated 7-laser array (Model 413, Perimed AB) that were each housed in a local heating
element (PF5020 Temperature Unit, Perimed AB) and affixed to the skin. Noteworthy, the
forearm was stabilized at the level of the heart throughout the baseline, exercise, and recovery
periods. At the end of each experimental session, both skin blood flow sites were heated to 42˚C
for ~10 min followed by an additional ~20 min at a temperature of 44˚C until a plateau was
observed for at least 2-3 min (maximum skin blood flow). Skin blood flow was calculated as a
percentage of the maximal skin blood flow response measured during the local heating to 44˚C.
Given that dry heat loss is primarily dependent on absolute blood flow to the skin and that our
study focus is on the recovery period, skin blood flow data are presented as a percent decrease
from the end of exercise to clearly delineate differences in the postexercise suppression of the
response.
Mean arterial pressure was measured during baseline and recovery from the beat-to-beat recording of the left middle finger arterial pressure waveform via the volume-clamp method (29) using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). Prior to each measurement period, physiocal criteria (40) were used to calibrate the finger arterial size to the point where the finger cuff air pressure equals the finger arterial blood pressure. With the left arm supported at heart level, the brachial artery pressure reconstruction (12, 13) was calibrated with an upper arm return-to-flow systolic pressure detection (3). Blood pressure was verified by manual auscultation with the arm stabilized at the level of the heart using a validated mercury column sphygmomanometer (Baumanometer Standby Model, WA Baum Co, Copiague, NY, USA) after each Finometer calibration period (i.e., at baseline within 5 min postexercise). Blood pressure was also measured via manual auscultation with the arm stabilized at the level of the heart at every 5-min during exercise and at the end of the maximal skin blood flow protocol, and calculated as mean arterial pressure (diastolic blood pressure plus one-third of pulse pressure).

Heart rate was recorded continuously and stored every 15-s using a Polar-coded wearlink transmitter, Polar RS400 interface, and Polar Trainer 5 software (RS400, Polar Electro, Kempele, Finland). Urine-specific gravity was assessed in duplicate from the urine samples obtained at the start and end of each experimental session using a handheld total solids refractometer (Model TS400, Reichter, Inc., Depew, NY, USA).

Plasma volume changes from baseline and plasma osmolality were determined from five venous blood samples (~8 mL each) drawn at end of baseline, end-exercise, as well as prior to the start (15-min of recovery), midway through (37.5-min of recovery), and at the end (60-min of recovery) of the 45-min period of pressure application to the lower legs. These recovery time points were selected to obtain a representation of plasma volume changes during the pressure
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application period. Participants had been seated upright for a minimum of 60-min prior to obtaining the baseline blood sample. The end-exercise sample was obtained within the final 1-min of exercise (i.e., participants were briskly walking upright at this time). Following a <5-min transition period from the treadmill to the pressure box, participants had been seated for 10-, 32.5-, and 55-min for blood samples obtained at 15-, 37.5-, and 60-min of recovery, respectively. Venous blood samples were collected without stasis into K2 EDTA™ and Serum™ vacutainers (BD Vacutainer, Franklin Lakes, NJ, USA) for the determination of plasma volume and plasma osmolality, respectively. The K2 EDTA™ blood samples were immediately analyzed for hemoglobin (Hb) concentration and hematocrit (Hct) ratio. Hematocrit and hemoglobin concentrations were determined using the Coulter method (Coulter AC·T diff 2 analyzer; Beckman Coulter, Miami, FL, USA). Changes in plasma volume from baseline levels were estimated from changes in hemoglobin and hematocrit using the method of Dill & Costill (5). Serum™ blood samples sat for 20 min to allow for full coagulation before being centrifuged at ~3300 rpm for 10 min. The plasma aliquots were transferred into plastic collection vials for the measurement of plasma osmolality. Plasma samples were initially stored at -20°C for 24 h and subsequently at -80°C until measurement. All samples of plasma osmolality were analyzed by freezing point depression based on procedures detailed by Osmometer (Advanced Instruments; Norwood, MA, USA).

Data and statistical analysis

Values for osmolality and the percent change in plasma volume were obtained by averaging duplicate measurements. All other values were obtained by averaging measurements performed over a 5-min period during baseline, during the last 5-min of exercise (except for mean arterial
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pressure, which represents one manual auscultation measurement at the end of exercise), and over the final 5-min of each 10-min interval throughout the 60-min recovery.

Mean arterial pressure, heart rate, mean skin and esophageal temperatures and sweat rate were analyzed using a two-way repeated-measures analysis of variance (2R-ANOVA) with the factors of condition (five levels: natural recovery, LBPP, LBNP, ISO and HYP) and time (8 levels: baseline, end of exercise, and every 10-min of recovery). A 1R-ANOVA with the repeated factor of condition (five levels) was used to analyze skin blood flow measured at baseline and at the end of exercise. The percent decrease in skin blood flow from the end of exercise was analyzed using a 2R-ANOVA with the factors of condition (five levels) and time (six levels: every 10-min recovery). Plasma osmolality and the percent change in plasma volume from baseline were analyzed using a 2R-ANOVA with the factors of condition (five levels) and time (five levels: baseline, end of exercise, and 15-, 37.5-, and 60-min of recovery). Urine specific gravity was analyzed using a 2R-ANOVA with the factors of condition (five levels) and time (two levels: pre- and post-trial). Finally, pre-session body mass was analyzed using a 1R-ANOVA with the repeated factor of condition (five levels). When a significant interaction or main effect was observed, post-hoc comparisons were carried out using paired samples $t$ tests.

Due to having multiple experimental conditions, adjustments for multiple comparisons were not performed and comparisons were limited to those with the natural recovery condition. For all analyses, differences were considered significant when $P \leq 0.05$. All statistical analyses were performed using the statistical software package SPSS 22.0 for Windows (SPSS Inc. Chicago, IL, USA). All values are presented as means ± standard error unless otherwise indicated. Due to technical difficulties, esophageal temperature as well as upper back and forehead sweat rate are presented for eight participants and chest sweat rate is presented for seven participants.
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RESULTS

Hydration status

Pre-nude body mass was similar between conditions (natural recovery, 77.78 ± 5.67; LBPP, 78.35 ± 5.29; LBNP, 78.17 ± 5.35; ISO, 78.22 ± 5.58; HYP, 77.89 ± 5.70 kg; \( P = 0.50 \)). In addition, a main effect of time \( (P < 0.01) \), but no main effect of condition \( (P = 0.91) \) or interaction \( (P = 0.07) \) was measured for urine-specific gravity. Specifically, participants were similarly hydrated at the start and relatively less hydrated at the end of each experimental condition (average urine-specific gravity increase across conditions: 0.009 ± 0.002; all \( P < 0.01 \)).

Plasma volume changes and plasma osmolality

Plasma volume changes. An interaction of time and condition was detected for the percent change in plasma volume \( (P < 0.01; \) Fig. 1a). The infusion of saline maintained plasma volume similar to baseline levels at the end of exercise (ISO: -0.9 ± 1.1; HYP: -0.6 ± 1.3%; \( P > 0.05 \)) whereas it was decreased during natural recovery (-8.9 ± 1.1%), LBPP (-8.9 ± 1.2%) and LBNP (-9.1 ± 0.9%; all \( P < 0.01 \)). Moreover, plasma volume in both ISO and HYP was greater compared to the natural recovery condition throughout recovery by ∼4.7 ± 0.8% (all \( P < 0.01 \)). The application of LBNP during recovery exacerbated the loss of plasma volume such that a 5.4 ± 0.8% greater reduction was observed relative to natural recovery (both \( P < 0.01 \)). On the other hand, the application of LBPP facilitated the restoration of plasma volume such that it was increased by 3.4 ± 1.6% relative to natural recovery by the end of recovery, although not significantly \( (P > 0.05) \).

Plasma osmolality. There was an interaction of time and condition measured for osmolality \( (P < 0.01; \) Fig. 1b). Plasma osmolality was similar between conditions at baseline
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(average across conditions: 288 ± 1 mmol·kg of solvent\(^{-1}\); \(P > 0.35\)). In all conditions, osmolality was increased at the end of exercise and throughout the recovery period relative to baseline (all \(P < 0.01\)). The infusion of 3.0% NaCl increased osmolality further (303 ± 1 mmol·kg of solvent\(^{-1}\)) at end-exercise compared to natural recovery (294 ± 1 mmol·kg of solvent\(^{-1}\)), and osmolality remained elevated in HYP by ∼6 ± 1 mmol·kg of solvent\(^{-1}\) relative to natural recovery for the duration of the recovery period (all \(P < 0.01\)). No differences in osmolality were detected in the LBPP, LBNP and ISO conditions compared to natural recovery (all \(P > 0.10\)).

Hemodynamic responses

Mean arterial pressure. An interaction of condition and time was detected for mean arterial pressure (\(P < 0.01\); Fig. 2a). Mean arterial pressure was similar between conditions at baseline until 10 min of recovery inclusively (all \(P > 0.13\)). Mean arterial pressure at 10 min postexercise was similar to baseline in all conditions (all \(P > 0.10\)). Thereafter, the application of LBPP resulted in an elevation of mean arterial pressure by 10 ± 2 mmHg relative to natural recovery starting from 20 min and lasted until the end of the recovery period (\(P < 0.02\)). Mean arterial pressure in ISO was elevated relative to natural recovery at 50 min postexercise (\(P < 0.05\)); however, no differences were detected in the LBNP and HYP conditions relative to natural recovery (all \(P > 0.10\)).

Heart rate. There was an interaction of condition and time measured for heart rate (\(P < 0.01\); Fig. 2b). While heart rate did not differ between conditions at baseline, the infusion of saline attenuated end-exercise heart rate relative to natural recovery (all \(P \leq 0.05\)). In addition, heart rate recovered more rapidly in ISO and with LBPP application such that it was reduced compared to natural recovery at 20 min until the end of recovery by 7 ± 2 and 13 ± 2 bpm,
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respectively (all $P \leq 0.04$). In contrast, LBNP application caused an elevation in heart rate by $8 \pm 3$ bpm relative to natural recovery of by the end of recovery (all $P < 0.03$). Heart rate was not different from natural recovery in HYP throughout recovery (all $P > 0.10$).

Temperature responses

Esophageal temperature. There was no interaction of condition and time detected for esophageal temperature ($P = 0.37$, Fig. 3a). However, a main effect of time was detected such that esophageal temperature was increased by $\sim 1.7 \pm 0.1 ^\circ C$ relative to baseline at the end of exercise and remained elevated by $\sim 0.9 \pm 0.1 ^\circ C$ at the end of recovery (all $P < 0.01$).

Mean skin temperature. An interaction of time and condition was measured for mean skin temperature ($P < 0.01$; Fig. 3b) such that mean skin temperature was similar between conditions from baseline until 30 min of recovery inclusively (all $P > 0.05$). However, the infusion of 3.0% NaCl resulted in an elevation of mean skin temperature by $\sim 0.3 \pm 0.1 ^\circ C$ relative to natural recovery from 40 min until the end of recovery ($P \leq 0.03$).

Heat loss responses

Sweating. An interaction of condition and time was detected for sweat rate measured at the chest, forearm, and upper back (all $P < 0.01$), but not at the forehead ($P = 0.15$; Fig. 4). Sweat rate was similar between conditions during baseline at all skin sites (all $P > 0.05$). During the ISO condition, sweat rate measured at the end of exercise was augmented by $0.20 \pm 0.08$ mg·min$^{-1}$·cm$^{-2}$ relative to natural recovery at the chest site only ($P = 0.05$). However, sweat rate was reduced during ISO relative to natural recovery from 30 to 50 min of recovery at the chest, and at 40 min of recovery at the upper back (all $P \leq 0.05$). During the HYP condition, upper back sweat
rate was lower at the end of exercise compared to natural recovery ($P = 0.02$), and it remained attenuated at 10 min as well as from 40 to 60 min of recovery by $0.35 \pm 0.11$ and $0.13 \pm 0.04$ mg·min$^{-1}$·cm$^{-2}$, respectively (all $P < 0.02$). Moreover, chest sweat rate was reduced by $0.18 \pm 0.06$ mg·min$^{-1}$·cm$^{-2}$ at 50 min postexercise during HYP compared to natural recovery ($P < 0.05$ at 50 min). Forearm and forehead sweating were unaffected by HYP ($P > 0.28$). The application of LBPP caused a reduction in sweat rate relative to natural recovery from 30 min to the end of the recovery period by $0.24 \pm 0.08$ mg·min$^{-1}$·cm$^{-2}$ at the chest, $0.12 \pm 0.03$ mg·min$^{-1}$·cm$^{-2}$ at the forearm, and $0.22 \pm 0.05$ mg·min$^{-1}$·cm$^{-2}$ at the upper back (all $P \leq 0.05$). In contrast, LBNP application did not impact sweat rate at any site throughout recovery (all $P > 0.16$).

**Skin blood flow.** A significant interaction of condition and time was detected for the percent decrease in skin blood flow from the end of exercise measured at the forearm and upper back (both $P < 0.03$; Fig. 5). The decrease in forearm skin blood flow was attenuated during the LBPP and HYP conditions for the final 30 min of recovery as well as during ISO at 40 and 60 min of recovery compared to natural recovery (all $P \leq 0.05$). In addition, the postexercise decrease in skin blood flow at the upper back was blunted during LBPP from 20 min to the end of recovery (all $P < 0.01$), as well as during both ISO and HYP at 60 min of recovery compared to natural recovery (both $P \leq 0.04$).
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DISCUSSION

To the best of our knowledge, this is the first study to examine the separate and combined effects of plasma osmolality and baroreceptor loading status on postexercise sweating and skin blood flow. Consistent with our first hypothesis, hyperosmolality as induced by the infusion of 3.0% saline attenuated sweating at the upper back and to a lesser extent at the chest. However, it did not impact forearm or forehead sweating, which may suggest regional heterogeneity in the osmotic modulation of postexercise sweating. Interestingly, inducing baroreceptor loading via 0.9% saline infusion or prolonged LBPP application resulted in the attenuation of sweat rate from 30 to 60 min of recovery at all sites but the forehead. Lastly, in support of our second hypothesis, baroreceptor loading with LBPP application and to lesser extent the infusion of saline maintained both forearm and upper back skin blood flow at elevated levels from 20 to 60 min of recovery; albeit no effect of plasma osmolality was observed.

Sweating

Until now, a role for osmoreceptors in the postexercise suppression of sweating had not been explored. However, it is well established that recovery from exercise is associated with a state of hyperosmolality (and therefore, activation of osmoreceptors) (4) and that hyperosmolality negatively influences sweating during passive heat stress (2, 16, 24, 31, 36) and exercise (8). A novel extension in the current study is that an increase in plasma osmolality of ~12 mmol·kg of solvent⁻¹ above baseline levels attenuated upper back sweat rate by ~25% by end-exercise relative to natural recovery (a plasma osmolality increase of only ~6 mmol·kg of solvent⁻¹), and this suppression persisted into the 60-min recovery despite core temperature elevations of ≥0.9˚C above baseline. Therefore, even under conditions that elicit an elevated thermal drive for
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sweating, nonthermal modulation of sweating via osmoreceptors can override thermal influences. Noteworthy, the hyperosmotic-induced reduction of sweating was heterogeneously distributed such that sweating was mainly suppressed at the upper back and to a lesser extent at the chest while there were no changes at the forearm or forehead. Thus, hyperosmolality has a greater effect on the eccrine sweat glands of the torso, which is known as a region of high sweat output (32, 33) and therefore may implicate a modulation of whole body heat loss. While this would ultimately attenuate whole body heat loss during and following exercise, core temperatures during HYP were comparable to natural recovery (i.e., ~0.9°C above baseline by the end of recovery). However, these data indicate that the level of sweating during HYP was reduced for the same core temperature, implying that hyperosmolality alters the relationship between core temperature and sweating during and following exercise.

Postexercise baroreceptor unloading associated with pronounced cardiovascular adjustments has been held primarily responsible for the abrupt suppression of sweating following exercise. However, there remains conflicting evidence regarding the role for baroreceptors in modulating postexercise sweating (18, 27). We show in the present study that while LBNP application exacerbated the state of baroreceptor unloading compared to natural recovery as evidenced by the reflex elevation in heart rate (Fig. 2b) and the greater reduction in plasma volume (Fig. 1a), there were no differences in sweat rate relative to natural recovery. This finding is supported by previous reports that also employed prolonged application of negative, positive, or no pressure to the lower limbs (18, 27).

An unexpected result is that the application of LBPP consistently attenuated sweating at the chest, forearm, upper back and, although statistically insignificant, the forehead (by ~30%) in the final 30 min of recovery. Noteworthy, these findings appear consistent with the recent study
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by McGinn et al. (27) such that forearm sweating was reduced (although not statistically different) during prolonged LBPP application by comparable levels (~0.2 mg·min$^{-1}$·cm$^{-2}$) relative to natural recovery. The mechanism underlying these findings is currently unknown. However, while a subjective index of thermal sensation was not measured, participants in the present study anecdotally reported greater comfort during LBPP application. Considering that eccrine sweating can be modulated by the level of emotional and/or perceived stress (25, 26), it is plausible that sweat rate was reduced during LBPP application as a result of an attenuated level of mental stress. Further, this observation in our study as well as in the study by McGinn et al. (27) is only reported in the final 30 min of recovery wherein core temperature had recovered to ~37.5°C. Therefore, the level of emotional/perceived stress may have a temperature-dependent influence on postexercise sweating. Future studies are warranted to explore the potential role for emotional/perceived stress in the control of postexercise thermoeffector responses and its implications for core temperature regulation.

Skin blood flow

Numerous reports have consistently demonstrated that hyperosmolality independently increases the onset threshold for cutaneous vasodilation during passive heat stress (2, 24, 31, 36) and exercise (8, 37) without altering the relationship between core temperature and cutaneous vasodilation. We are the first to show that osmolality does not modulate the suppression of postexercise skin blood flow given that it was maintained at similar levels during ISO and HYP conditions, during which plasma osmolality was independently modulated (i.e., the increase in osmolality was twofold greater during HYP compared to ISO). On the other hand, these data cannot eliminate a role for osmoreceptors given that plasma osmolality was above baseline levels.
in all conditions indicating activation of the osmoreceptors. As a result, the possibility remains that the threshold level for osmoreceptor-mediated control of postexercise skin blood flow was surpassed in all conditions of the current study.

The role of baroreceptors in modulating postexercise skin blood flow is well established (21). Consistent with this notion, our findings suggest that baroreceptor loading status is an important nonthermal mediator in the suppression of postexercise skin blood flow. This is supported by our observation that baroreceptor loading, as induced by the infusion of saline or by LBPP application, maintained skin blood flow at elevated levels compared to natural recovery. Conversely, postexercise skin blood flow was unaffected by the sustained application of LBNP, which may be explained by a temperature-dependent influence of baroreceptor loading status as proposed by McGinn et al (27). In that study, reductions in postexercise cutaneous vascular conductance during the prolonged application of LBNP were only observed following a second bout of high intensity cycling wherein core temperature was ≥38.0°C, which is higher than the core temperature at which LBNP application began in the current study (i.e., ≤37.6°C). Furthermore, McGinn et al (27) reported substantial postexercise hypotension whereas this was not observed in the current study. Previous reports have demonstrated that changes in postexercise blood pressure regulation impact the control of postexercise skin blood flow (19, 20), and would also in part explain the disparate findings between studies. Finally, it is noteworthy that the enhanced skin blood flow during LBPP application, ISO, and HYP was sufficient to counteract the parallel attenuations of sweat rate such that core temperature recovered similarly in all conditions.
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**Limitations**

Given that we included only young males, the findings of the current study cannot be generalized to other populations groups such as females and/or older adults who display an impaired capacity to dissipate heat (10, 23, 35). In particular, both sex and ageing have been shown to independently alter the osmotically-driven release of vasopressin, a hormone implicated in fluid regulation, such that the concentration of vasopressin is reduced for a given level of plasma osmolality in females and older adults when compared to males and young adults, respectively (17, 34). These differences in fluid regulation suggest differences may also exist in the osmoreceptor-mediated control of thermoeffector activity. While there are no sex-related differences in the osmoreceptor modulation of sweating and skin blood flow during passive heat stress (2), it remains to be elucidated whether sex- and age-related differences exist during and following exercise. In addition, it should be noted that the lack of statistical differences in esophageal temperature despite differences in heat loss responses between conditions may have been confounded by the inherent vast differences in fluid distribution between conditions and therefore heat transfer between body tissues. Lastly, while postexercise skin blood flow was unaffected by baroreceptor unloading at esophageal temperatures ≤37.6°C, this response may have differed at a greater level of hyperthermia (i.e., >38°C), as was observed by McGinn et al (27). Thus, a role for baroreceptor unloading in attenuating postexercise skin blood flow should not be discounted, particularly in the context of high heat stress combined with severe dehydration.

**Perspectives and Significance**

Several reports have attempted to delineate the relative contributions of nonthermal factors (e.g., baro-, metabo-, metachoreceptors/muscle pump, and central command) to the suppression of
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Postexercise heat loss, with the vast majority of studies focusing on the role for baroreceptors (21). Our findings reveal an osmotically-induced suppression of postexercise sweating that is capable of overriding elevated levels of hyperthermia (i.e., core temperature elevation >0.9°C). Furthermore, although sweating was affected at regions of high sweat output (i.e., the upper back and chest), it is unclear whether this would translate to a modulation of whole body heat loss postexercise. While this response would serve to mitigate total body fluid loss during progressive dehydration in order to promote the maintenance of blood volume and blood pressure, this comes at the expense of thermoregulatory function as reductions in sweat rate impair the dissipation of heat. Future research is warranted using direct whole-body calorimetry to assess whether hyperosmolality associated with dehydration would induce meaningful changes in whole body heat loss that would impact body heat storage, particularly during prolonged and/or intermittent work in the heat when individuals are at greatest risk for dehydration and thermal strain. In this context, factors that may alter skin wettedness (e.g., prolonged exercise, increased humidity, changes in air velocity and skin temperature) and therefore the sweating response to hyperosmolality should also be considered in future studies.

In summary, we show that plasma hyperosmolality attenuated sweating from the end of exercise throughout 60-min of recovery. This effect was greatest at the torso such that the upper back and chest sites were most susceptible to increases in plasma osmolality. However, this response cannot be fully attributed to osmoreceptors alone given that baroreceptor loading via saline infusion or LBPP application also attenuated sweating in the mid-to-late stages of recovery (i.e., ≥30 min). In contrast, baroreceptor loading modulated skin blood flow in the mid-
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to late stages of recovery (i.e., ≥20 min) similarly at the forearm and upper back regions and
independent of changes in plasma osmolality.
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Disclosures

None to declare.
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REFERENCES


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Figure Legends

Figure 1: Changes in plasma volume relative to baseline (panel A, n=9) and plasma osmolality (panel B, n=9) measured at baseline, end-exercise (End Ex), as well as at 15-, 37.5- and 60-min of recovery (corresponding to the start, midpoint, and end of a 45-min period of lower body pressure application) during the following conditions: natural recovery, lower body positive pressure (LBPP), lower body negative pressure (LBNP), infusion of 0.9% NaCl (ISO), and infusion of 3.0% NaCl (HYP). Start of pressure application at 15-min into recovery indicated by down arrow. Values presented as mean ± standard error. *HYP, †ISO and ‡LBNP significantly different from natural recovery (P < 0.01).

Figure 2: Mean arterial pressure (panel A, n=9) and heart rate (panel B, n=9) measured at baseline, end-exercise (End Ex), and every 10-min of recovery during the following conditions: natural recovery, lower body positive pressure (LBPP), lower body negative pressure (LBNP), infusion of 0.9% NaCl (ISO), and infusion of 3.0% NaCl (HYP). Pressure application began at 15-min into recovery (indicated by down arrow) and lasted for the remaining 45-min of recovery. Values presented as mean ± standard error. *HYP, †ISO, ‡LBNP, and §LBPP significantly different from natural recovery (P ≤ 0.04).

Figure 3: Esophageal (panel A, n=8) and mean skin (panel B, n=9) temperatures measured at baseline, end-exercise (End Ex), and every 10-min of recovery during the following conditions: natural recovery, lower body positive pressure (LBPP), lower body negative pressure (LBNP), infusion of 0.9% NaCl (ISO), and infusion of 3.0% NaCl (HYP). Pressure application began at 15-min into recovery (indicated by down arrow) and lasted for the remaining 45-min of recovery. Values presented as mean ± standard error. *HYP significantly different from natural recovery (P ≤ 0.03).

Figure 4: Sweat rate at the chest (panel A, n=7), upper back (panel B, n=8), forearm (panel C, n=9), and forehead (panel D, n=8) measured at baseline, end-exercise (End Ex), and every 10-min of recovery during the following conditions: natural recovery, lower body positive pressure (LBPP), lower body negative pressure (LBNP), infusion of 0.9% NaCl (ISO), and infusion of 3.0% NaCl (HYP). Pressure application began at 15-min into recovery (indicated by down arrow) and lasted for the remaining 45-min of recovery. Values presented as mean ± standard error. *HYP, †ISO, and §LBPP significantly different from natural recovery (P ≤ 0.05).

Figure 5: Percent change in skin blood flow (SkBF) relative to the end of exercise at the forearm (panels A n=9) and upper back (panels B, n=9) measured every 10-min of recovery during the following conditions: natural recovery, lower body positive pressure (LBPP), lower body negative pressure (LBNP), infusion of 0.9% NaCl (ISO), and infusion of 3.0% NaCl (HYP). Pressure application began at 15-min into recovery (indicated by down arrow) and lasted for the remaining 45-min of recovery. Values presented as mean ± standard error. *HYP, †ISO, and §LBPP significantly different from natural recovery (P ≤ 0.05).
Figure 2

**A**
- Mean Arterial Pressure (mmHg)
- *Natural recovery*
- LBPP
- LBNP
- ISO
- HYP

**B**
- Heart Rate (bpm)
- *Natural recovery*
- LBPP
- LBNP
- ISO
- HYP
**Figure 3**

**A**

- **Esophageal Temperature (˚C)**
  - Baseline
  - End Ex
  - Recovery (min)
  - Natural recovery
  - LBPP
  - LBNP
  - ISO
  - HYP

**B**

- **Mean Skin Temperature (˚C)**
  - Baseline
  - End Ex
  - Recovery (min)
  - Natural recovery
  - LBPP
  - LBNP
  - ISO
  - HYP

*Note: The diagram shows the changes in esophageal and mean skin temperature over recovery time with different conditions marked as Natural recovery, LBPP, LBNP, ISO, and HYP.*
Figure 4

A-B: Comparison of sweat rate (mg min⁻¹ cm⁻²) during recovery for different conditions:
- **Natural recovery**
- **LBPP**
- **LBNP**
- **ISO**
- **HYP**

C-D: Sweat rate (mg min⁻¹ cm⁻²) for different body areas:
- Chest SR
- Upper back SR
- Forearm SR
- Forehead SR

Recovery (min)