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14. ABSTRACT
Hemorrhage is a leading cause of deaths on the battlefield. An understanding of the mechanisms and modulators of coagulopathy under conditions soldiers currently experience on the battlefield is important for improved treatment of the hemorrhaging soldier. The global objective of this project tests the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis). During the current funding period we completed the objectives in specific aim 1A (test the hypothesis that passive heat stress alters hemostatic function during simulated hemorrhage) and are well on our way towards completing the objectives of aim 1B (Dehydration during exercise in the heat alters hemostatic function during simulated hemorrhage). For Aim 1A, subjects completed four visits (familiarization, a passive heat stress, a normothermic control, and a time control). For Aim 1B, each subject will likewise complete four visits (familiarization, exercise without dehydration, and two exercise with dehydration trials). TEG based assays have been obtained while plasma-based assays for Aim 1A are currently being run in at USAISR. We anticipate the plasma based assays for Aim 1B will be run by USAISR in the Fall of 2014 through early Winter of 2015.

15. SUBJECT TERMS
hemorrhage, hyperthermia, heat stress, coagulopathy, hemostasis.

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1. Introduction

Worldwide, trauma is the cause of 1 in 10 deaths, with 30-40% of trauma deaths being due to hemorrhage. Hemorrhage is also a leading cause of death on the battlefield. An understanding of the mechanisms and modulators of coagulopathy under conditions soldiers often experience on the battlefield is important to improve medical treatment of the hemorrhaging soldier. The global objective of this project is to test the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis), resulting in compromised ability to survive a hemorrhagic injury. This objective will be accomplished by evaluating the following Specific Aims: 1A) Passive heat stress alters hemostatic function during simulated hemorrhage. 1B) Dehydration during exercise-induced hyperthermia alters hemostatic function during a subsequent simulated hemorrhage. 2) Heating a hemorrhaging individual who is not hypothermic is detrimental to blood pressure control, cerebral perfusion, and hemostatic function. A secondary objective of this work is to evaluate the effectiveness of two pre-hospital devices that are designed to provide the caregiver information regarding the hemorrhagic status of an individual. This project will provide the Department of Defense with valuable information resulting in improved medical treatment of soldiers who have experienced a hemorrhagic injury while in hyperthermic environmental conditions.

2. Keywords

Hemorrhage
Hyperthermia
Dehydration
Hemostasis
Exercise
Heat stress
Environment
Pre-hospital
Triage

3. Overall Project Summary

The global objective of this project tests the hypothesis that environmental and physiological conditions, often experiences on the battlefield, alters hemodynamic and hemostatic function. A secondary objective is to evaluate the capabilities of two devices to detect cardiovascular reserve during a simulated hemorrhagic challenge.

4. Key Research Accomplishments During the Prior 12 Months:

- We identified that heat stress itself, as well as dehydration associated with an exercise bout in hyperthermic environmental conditions, does not alter the capability of the Flashback technologies CRI device to monitor compensatory reserve during a progressive simulated hemorrhagic challenge (see Figure 1). Primary deliverable: The CRI device could be used under the imposed environmental conditions.

- We completed the project to identify the effects of skin surface cooling and heating during a hemorrhagic insult on the capacity to withstand that insult in otherwise normothermic individuals. We found that neither the average time to pre-syncope, nor

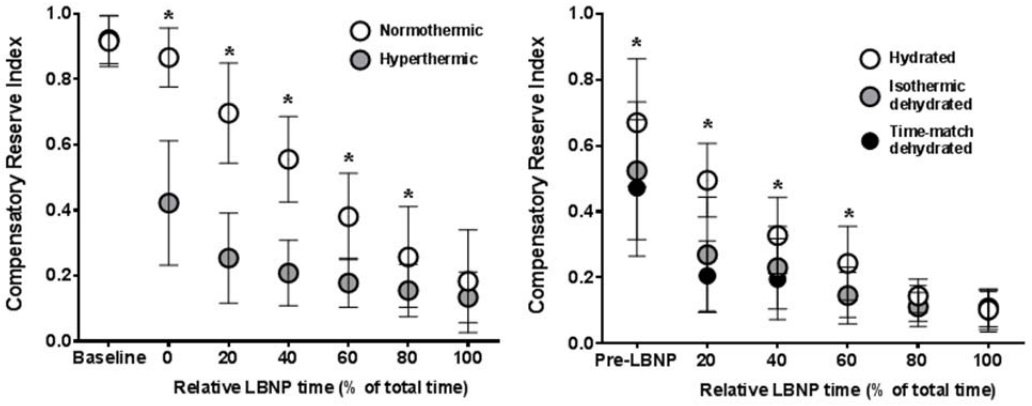

Figure 1 The compensatory reserve index during progressive lower body negative pressure (LBNP) to pre-syncope. The left panel presents data during whole-body passive heat stress (hyperthermic) and a normothermic time-control period. The right panel presents data following exercise during which: i) fluid losses were replaced (hydrated), ii) fluid losses were not replaced and exercise lasted until the same increase in core temperature as hydrated (isothermic dehydrated), and iii) fluid losses were not replaced and exercise lasted the same duration as hydrated (time-match dehydrated). Data are plotted as a function of relative time, expressed as percentage of total LBNP time. 0% depicts data immediately prior to LBNP, 100% depicts pre-syncope. *Significantly ($P \leq 0.05$) different from hyperthermic (right panel) or from both dehydrated conditions (right panel).

the simulated hemorrhagic level at pre-syncope, were different between thermal conditions (see Figure 2). However, mean arterial blood pressure was elevated during the cool trial while it was lower during the warm trial (see Figure 3). Despite this similar increase in arterial blood pressure, middle cerebral artery blood velocity decreased to a similar extent from baseline to pre-syncope, regardless of the thermal conditions applied.

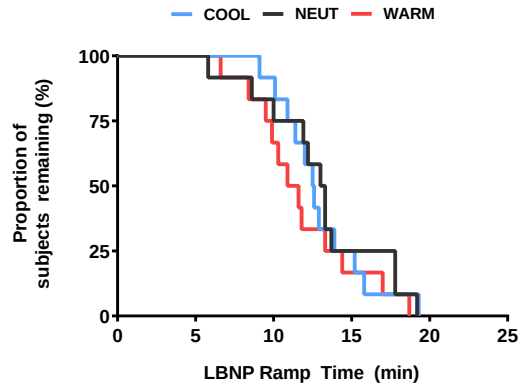

Figure 2 Proportion of subjects who tolerated the LBNP ramp protocol for the indicated duration for the COOL (blue), NEUT (black), and WARM (red) trials. There were no differences in tolerance time between trials.

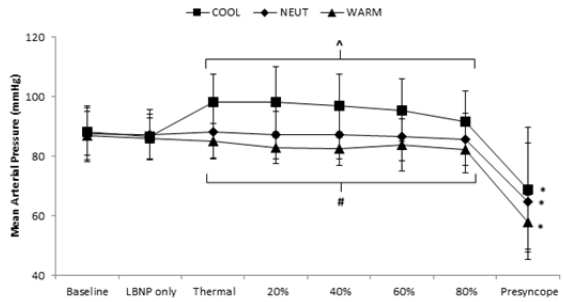

Figure 3 Mean (± SD) arterial pressure for Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60% and 80% of LBNP ramp, and Presyncope; ^significantly greater than Baseline for the COOL condition; #significantly less than Baseline for the WARM condition; *significantly less than Baseline and LBNP ramp.

It is notable that the compensatory reserve index was able to identify hemorrhagic status regardless of the applied thermal condition (see Figure 4).

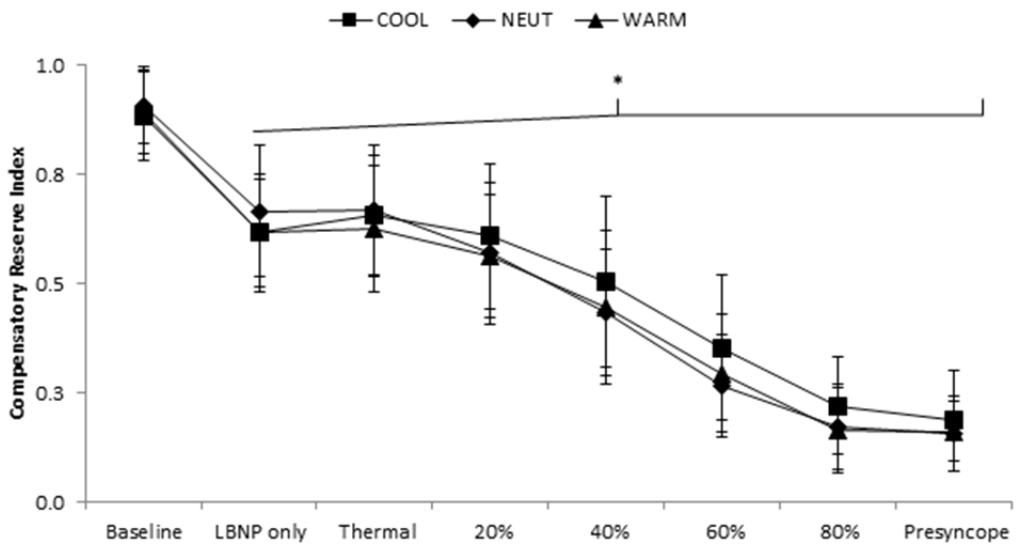

Figure 4 Mean (± SD) compensatory reserve index Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60% and 80% of LBNP ramp, and Presyncope; *significantly less than Baseline for all thermal conditions.

5. Conclusion:

We continue to be very active as we wrap up this project. The obtained findings clearly demonstrate that the capacity of the Flashback Technologies CRI device to accurately track hemorrhagic state remains effective following exercise-induced dehydration as well as when skin surface cooling and heating are applied during a simulated hemorrhagic insult. These data suggest that this pre-hospital diagnostic device could be used to assist in identifying the hemorrhagic status of a soldier in the evaluated thermal conditions. Furthermore, we found that in otherwise normothermic individuals, relatively mild skin surface cooling and heating are not beneficial nor detrimental during a progressive hemorrhagic insult. Thus, it is unlikely that mild heating of a normothermic individual who is injured will be detrimental to their hemorrhagic status.

We continue to work with the US Army to have the coagulation data analyzed and reduced. We are hopeful that this will be completed within the next ~4 months such that the results from that work could be written up, submitted and published. We will also work with Flashback technologies to process the obtained data through their revised algorithms. During the ensuing ~12 months we also plan to evaluate the effectiveness of the Flashback CRI device to detect compensatory reserve status while an individual is exposed to an environmental cooling stimulus. This will be accomplished upon the modification of our environmental chamber such that it could achieve a cooling state (e.g., 0° F) that would affect finger blood flow and thus may affect the capability of the CRI device to determine compensatory reserve. This project will be similar to the cooling project outlined in Figure 2-4 but an environmental cooling challenge will be imposed rather than mild skin surface cooling. However, this project is conditional upon

approval to use carry-over funds to upgrade the environmental chamber to achieve that level of cooling. Finally, we will work with the reviewers to publish the two manuscripts that are currently in revision pertaining to the projects illustrated in the figures above.

6. Publications, Abstracts, and Presentations:

1. Lay Press: None
2. Peer-Reviewed Scientific Journals supported in full or in part by this grant.
    - Lucas, A.I.R., J Pearson, Z.J. Schlader, C.G. Crandall. Cardiopulmonary and arterial baroreceptor unloading during passive hyperthermia does not contribute to hyperthermia-induced hyperventilation. *Exp Physiol* 100:1309-1318, 2015

    - Schlader, Z.J., D. Gagnon, E. Rivas, V.A. Convertino, C.G. Crandall. Fluid restriction during exercise in the heat reduces tolerance to progressive central hypovolemia. Exp Physiol 100:926-934, 2015

    - Schlader, Z.J., T.E. Wilson. C.G. Crandall. Mechanisms of orthostatic intolerance during heat stress. *Autonomic Neuroscience: Basic and Clinical* (in press).

    - Poh, P.Y.S., D. Gagnon, S.A. Romero, V.A. Convertino, B. Adams-Huet, C.G Crandall. Hemodynamic stability to surface warming and cooling during sustained and continuous stimulated hemorrhage in humans. *Shock* (in revision).

    - Gagnon, G, Z.J. Schlader, A. Adams, E. Rivas, J. Mulligan, G.Z. Grudic, V.A. Convertino, C.G. Crandall. The compensatory reserve index during simulated hemorrhage following passive heat stress and exercise-induced dehydration *Shock* (in revision).

3. Invited Articles: None
4. Abstracts
    - "Hemodynamic responses to cooling and warming during a continuous simulated hemorrhage ramp." Paula Y. S. Poh, Daniel Gagnon, Steven A. Romero, Victor A. Convertino and Craig G. Crandall (Presented at the Military Health System Research Symposium)

Presentations:

- C.G. Crandall "Thermal and Vascular Physiology Laboratory: From Clinic to Battlefield" Center for Environmental and Respiratory Health Research. University of Oulu, Finland, January 22, 2016

- C.G. Crandall Cardiovascular responses to heat stress: implications in health and disease" Department of Biological Sciences, University of North Texas, Denton, TX January 29, 2016.

7. Inventions, patents, and licenses:

Nothing to Report

8. Reportable Outcomes:
See item 5 Conclusions above.

9. Other Achievements
Paula Poh completed her doctoral dissertation from the University of Illinois Urbana-Champaign with the bulk of that project originating from this DOD grant. She continued in my laboratory as a post-doctoral student while she worked on the project outlined above addressing the effects of skin cooling/warming on a continuous simulated hemorrhagic challenge. She effectively ran that project. Thus, in addition to the results and deliverables originating from this work, an under-representative minority student (Pacific Islander heritage) was able to receive outstanding training while completing the projects outlined above.

Additionally, the aforementioned projects provided the biomedical research training opportunities for the following individuals:

Dan Gagnon, Ph.D. (post-doctoral fellow)
Steven Romero, Ph.D. (under-represented minority post-doctoral fellow)
Paula Poh, MS. (under-represented minority post-doctoral fellow)
Ken Koda, MD, Ph.D. (Visiting professor from Japan)
Matthew Cramer (post-doctoral fellow)
Christian Ramirez (under-represented minority undergraduate student)

10. References
None

11. Appendix
The following manuscripts are included in the Appendix

- Schlader, Z.J., D. Gagnon, E. Rivas, V.A. Convertino, **C.G. Crandall**. Fluid restriction during exercise in the heat reduces tolerance to progressive central hypovolemia. Exp Physiol 100:926-934, 2015.

- Lucas, A.I.R., J Pearson, Z.J. Schlader, **C.G. Crandall**. Cardiopulmonary and arterial baroreceptor unloading during passive hyperthermia does not contribute to hyperthermia-induced hyperventilation. *Exp Physiol* 100:1309-1318, 2015.

Exp Physiol 100.8 (2015) pp 926–934

Research Paper

Fluid restriction during exercise in the heat reduces tolerance to progressive central hypovolaemia

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New Findings

- **What is the central question of this study?**
  Interactions between dehydration, as occurs during exercise in the heat without fluid replacement, and hyperthermia on the ability to tolerate central hypovolaemia are unknown.
- **What is the main finding and its importance?**
  We show that inadequate fluid intake during exercise in the heat can impair tolerance to central hypovolaemia even when it elicits only mild dehydration. These findings suggest that hydration during physical work in the heat has important military and occupational relevance for protection against the adverse effects of a subsequent haemorrhagic injury.

This study tested the hypothesis that dehydration induced via exercise in the heat impairs tolerance to central hypovolaemia. Eleven male subjects (32 ± 7 years old, 81.5 ± 11.1 kg) walked (O_2 uptake 1.7 ± 0.4 l min^{-1}) in a 40°C, 30% relative humidity environment on three occasions, as follows: (i) subjects walked for 90 min, drinking water to offset sweat loss (Hydrated, $n = 11$); (ii) water intake was restricted, and exercise was terminated when intestinal temperature increased to the same level as in the Hydrated trial (Isothermic Dehydrated, $n = 11$); and (iii) water intake was restricted, and exercise duration was 90 min (Time Match Dehydrated, $n = 9$). For each trial, tolerance to central hypovolaemia was determined following exercise via progressive lower body negative pressure and quantified as time to presyncope. Increases in intestinal temperature prior to lower body negative pressure were not different ($P = 0.91$) between Hydrated (1.1 ± 0.4°C) and Isothermic Dehydrated trials (1.1 ± 0.4°C), but both were lower than in the Time Match Dehydrated trial (1.7 ± 0.5°C, $P < 0.01$). Prior to lower body negative pressure, body weight was unchanged in the Hydrated trial (−0.1 ± 0.2%), but was reduced in Isothermic Dehydrated (−0.9 ± 0.4%) and further so in Time Match Dehydrated trial (−1.9 ± 0.6%, all $P < 0.01$). Time to presyncope was greater in Hydrated (14.7 ± 3.2 min) compared with Isothermic Dehydrated (11.9 ± 3.3 min, $P < 0.01$) and Time Match Dehydrated trials (10.2 ± 1.6 min, $P = 0.03$), which were not different ($P = 0.19$). These data indicate that inadequate fluid intake during exercise in the heat reduces tolerance to central hypovolaemia independent of increases in body temperature.

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Introduction

Haemorrhage, and subsequent central hypovolaemia and cardiovascular decompensation, is a leading cause of death in both civilian and military settings (Bellamy, 1984; Kauvar & Wade, 2005). Many individuals who are at risk for a haemorrhagic injury often undertake physical work in hot conditions [e.g. soldiers (Carter *et al.* 2005), miners (Brake & Bates, 2002) and firefighters (Colburn *et al.* 2011)], which renders them hyperthermic (i.e. elevated skin and internal temperatures) and, due to sweat loss, dehydrated (i.e. a hypovolaemic and hyperosmotic state). Notably, hyperthermia (Schlader & Crandall, 2014), dehydration (Frey *et al.* 1994) and physical work (i.e. exercise; Lacewell *et al.* 2014) can independently impair tolerance to central hypovolaemia.

Hyperthermia reduces tolerance to central hypovolaemia due, at least partly, to hyperthermia-induced decreases in central blood volume (Crandall *et al.* 2008) and cerebral perfusion (Wilson *et al.* 2006; Brothers *et al.* 2009; Nelson *et al.* 2011), together with attenuated increases in peripheral resistance during such a challenge (Crandall *et al.* 2010; Ganio *et al.* 2012; Pearson *et al.* 2013). Dehydration decreases the ability to withstand central hypovolaemia via similar mechanisms, such as an attenuated capacity to maintain central blood volume (Frey *et al.* 1994), stroke volume (Convertino, 1993; Frey *et al.* 1994) and cerebral perfusion (Carter *et al.* 2006; Romero *et al.* 2011) during a central hypovolaemic challenge, as well as alterations in baroreflex control of blood pressure (Convertino & Baumgartner, 1997; Charkoudian *et al.* 2003). Finally, exercise appears to impair tolerance to central hypovolaemia due to reductions in baroreflex sensitivity (Piepoli *et al.* 1993) and an attenuated ability to increase peripheral resistance during such instances (Halliwill *et al.* 1996; Davis & Fortney, 1997).

Our laboratory and others have investigated interactions between many of these factors on tolerance to central hypovolaemia. For instance, we identified that passively induced hyperthermia (i.e. elevated skin and internal temperatures) in combination with dehydration (1.6% body weight loss) further compromises tolerance to central hypovolaemia relative to hyperthermia during which dehydration was prevented with intravenous fluids (Lucas *et al.* 2013). We have also shown that hyperthermia impairs lower body negative pressure (LBNP) tolerance to a similar extent whether induced via exercise or passive heat stress when skin temperatures are similar between trials (Pearson *et al.* 2014). Furthermore, Davis & Fortney (1997) have identified that fluid ingestion following exercise in a moderate environment improved cardiovascular responses during central hypovolaemia, which is suggestive of improved tolerance. These studies generally support the premise that exercise together with dehydration and hyperthermia may impair tolerance to central hypovolaemia. However, interactions between dehydration, at the levels that occur during physical work in the heat without fluid replacement (i.e. drinking), and hyperthermia on the ability to tolerate central hypovolaemia are unknown. The purpose of this study, therefore, was to test the hypothesis that fluid restriction and accompanying dehydration during an exercise task performed in the heat, which is common to many occupational demands, impairs tolerance to central hypovolaemia. The testing of this hypothesis will provide important information regarding the prevention, treatment and care of individuals at risk of haemorrhagic injury and who perform physical work in the heat (e.g. soldiers, firefighters and miners). Thus, the information obtained has direct implications for policy and practices regarding fluid consumption in many recreational and occupational settings.

Methods

Subjects

Eleven healthy, physically active men participated in this study. The subject characteristics were as follows (means ± SD): age 32 ± 7 years; height 183 ± 10 cm; weight 81.5 ± 11.1 kg; and peak oxygen uptake 3.8 ± 1.0 l min^{-1}. All subjects were non-smokers, not taking medications and were free of any known cardiovascular, metabolic, neurological or psychological diseases. Each subject was fully informed of the experimental procedures and possible risks before giving informed, written consent. The protocol and consent were approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center at Dallas and Texas Health Presbyterian Hospital of Dallas. This study also conformed to the standards set by the latest revision of the Declaration of Helsinki.

Subjects visited the laboratory on four (or three; see below) occasions. Visit 1 was a screening trial, during which subjects underwent a peak exercise test using methods previously described in our laboratory (Ganio *et al.* 2014). The remaining visits involved the experimental trials, which are described in detail below. These trials were separated by at least 8 weeks, but completed at the same time of day (within a subject). For these trials, subjects arrived at the laboratory euhydrated (confirmed via urine specific gravity and plasma osmolality; see Table 1) and having refrained from strenuous exercise, alcohol and caffeine for a period of 24 h. Experimental testing was conducted throughout the calendar year in Dallas, TX, USA and, as a result, heat acclimatization status was not controlled.

Table 1. Thermal and hydration indices pre-exercise and at end of exercise in the heat

	Hydrated		Isothermic Dehydrated		Time Match Dehydrated	
Parameter	Pre-exercise	End of exercise	Pre-exercise	End of exercise	Pre-exercise	End of exercise
Exercise time (min)	—	90 ± 0	—	50 ± 19*†	—	90 ± 0
Intestinal temperature (°C)	37.0 ± 0.2	38.1 ± 0.3[a]	37.0 ± 0.2	38.1 ± 0.4[a]†	36.9 ± 0.2	38.6 ± 0.4[a]*
Δ Intestinal temperature (°C)	—	1.1 ± 0.4	—	1.1 ± 0.4†	—	1.7 ± 0.5*
Mean skin temperature (°C)	35.5 ± 0.4	34.7 ± 1.0[a]	35.3 ± 0.7	35.2 ± 0.9	35.7 ± 0.5	34.8 ± 0.8[a]
Heart rate (beats min^{-1})	89 ± 13	127 ± 13[a]	88 ± 11	136 ± 17[a]	87 ± 13	144 ± 20[a]*
Urine specific gravity	1.013 ± 0.007	—	1.013 ± 0.010	—	1.011 ± 0.005	—
Δ Body weight (kg)	—	−0.1 ± 0.2	—	−0.8 ± 0.4*†	—	−1.7 ± 0.5*
Δ Body weight (%)	—	−0.1 ± 0.2	—	−0.9 ± 0.4*†	—	−1.9 ± 0.6*
Δ Plasma volume (%)	—	−5.9 ± 3.3	—	−8.1 ± 3.4*†	—	−11.2 ± 2.6*
Plasma osmolality (mosmol kg^{-1})	288 ± 6	286 ± 5[b]	288 ± 2	293 ± 3[a]*	288 ± 3	295 ± 4[a]*

Values are means ± SD. Trials are as follows: Hydrated, $n = 11$; Isothermic Dehydrated, $n = 11$; and Time Match Dehydrated, $n = 9$. Δ indicates change from pre-exercise; *significantly different from the Hydrated trial ($P \leq 0.017$); †significantly different from the Time Match Dehydrated trial ($P \leq 0.002$); [a]significantly different from pre-exercise within the trial ($P \leq 0.002$); and [b]significantly different from pre-exercise within the trial ($P = 0.051$).

Instrumentation and measurements

Approximately 60 min prior to any experimental testing, each subject swallowed a telemetry pill (HQ Inc., Palmetto, FL, USA) for the measurement of intestinal temperature. Mean skin temperature was measured as the weighted average of six thermocouples attached to the following locations: abdomen (14%), calf (11%), chest (22%), lower back (19%), thigh (14%) and upper back (19%). Heart rate was continuously recorded from an ECG (GE Healthcare, Little Chalfont, UK) interfaced with a cardiotachometer (CWE, Ardmore, PA, USA). Urine specific gravity was measured in duplicate using a refractometer (PAL-10S; Atago Inc., Bellevue, WA, USA). Body weight was measured using a standard scale (Health o meter Professional Scales, McCook, IL, USA), while oxygen uptake was measured via indirect calorimetry (Parvo Medics, Sandy, UT, USA). During LBNP (see Experimental Protocol), beat-to-beat blood pressure was measured continuously via the Penaz method (Finometer Pro; FMS, Amsterdam, The Netherlands) and confirmed intermittently via auscultation of the brachial artery by electrosphygmomanometry (Tango+; SunTech, Raleigh, NC, USA). Venous blood samples were measured for haemoglobin, haematocrit (both via fluorescent flow cytometry) and plasma osmolality (via osmometry).

Experimental protocol

Following at least 30 min of supine rest in a thermoneutral environment, a baseline blood sample was drawn. Subjects then entered an environmental chamber maintained at 41 ± 1°C, 25 ± 4% relative humidity and exercised on a treadmill with a fan placed in front of them that produced an air velocity of 5 ± 2 m s^{-1}. The speed and gradient of the treadmill were adjusted to elicit 55 ± 3% of peak oxygen uptake (1.7 ± 0.4 l min^{-1}; no differences between trials, $P = 0.560$), which is similar to that typically observed in soldiers while on foot patrol (Buller *et al.* 2010). During exercise, oxygen uptake was measured over a 2–3 min period every 10 min during the first 30 min of exercise, and the speed and gradient were kept constant thereafter. Following thorough removal of sweat that was on the skin surface with a towel, changes in body weight (inclusive of clothing and instrumented equipment) were measured every 15 min throughout exercise.

The three experimental trials comprised different conditions that varied depending on fluid (i.e. water) consumption and exercise duration. (i) Water intake was sufficient to offset sweat losses fully throughout 90 min of exercise (Hydrated). This water was warm (38.6 ± 1.0°C), and the timing of drinking was carefully controlled such that no fluid was permitted within 5 min of measuring intestinal temperature. This prevented water temperature from influencing the measurement of intestinal temperature, which was confirmed by continuously monitoring intestinal temperature throughout the exercise, including during drinking. (ii) Water was withheld throughout exercise, and subjects exercised until they achieved the same increase in intestinal temperature as that occurring in the Hydrated trial (Isothermic Dehydrated). (iii) Water was withheld throughout exercise, and subjects exercised for the full 90 min (Time Control Dehydrated). The study was originally designed to compare only the Hydrated and Isothermic Dehydrated trials; however, given that exercise duration was substantially shorter during the Isothermic Dehydrated trial compared with the Hydrated trial (see

Table 1), the Time Control Dehydrated trial was added *post hoc*. As a result, the order of the trials was not randomized. Eleven subjects completed the Hydrated and Isothermic Dehydrated trials, but only nine subjects returned to complete the final, Time Control Dehydrated trial. The characteristics of these nine subjects were as follows: age 34 ± 6 years; height 184 ± 11 cm; weight 83.9 ± 11.0 kg; and peak oxygen uptake 4.0 ± 1.0 l min^{-1}.

Immediately after exercise, while remaining in the same hot environment, subjects were moved to a patient bed and placed in the supine position within the LBNP box, where they were instrumented and underwent progressive LBNP to presyncope, a model that simulates haemorrhage in humans (Cooke *et al.* 2004; Hinojosa-Laborde *et al.* 2014; Johnson *et al.* 2014). All efforts were made to ensure a rapid transition between the end of exercise and the start of LBNP, so as to mimic conditions of a person incurring a haemorrhagic injury during physical work in the heat. As a result, physiological measures were constrained to those that were considered essential for subject safety and data integrity (e.g. blood pressure and heart rate). The transition from end of exercise to the commencement of LBNP was 18 ± 3 min, which was not different between trials ($P = 0.651$). The LBNP commenced at 20 mmHg, with the level of LBNP increasing by 10 mmHg every 3 min until the onset of syncopal signs and symptoms, which included the following: continued self-reporting of feeling faint, sustained nausea, rapid and progressive decreases in blood pressure resulting in sustained systolic blood pressure being <80 mmHg and/or relative bradycardia accompanied with a narrowing of pulse pressure. Notably, every LBNP trial was terminated due to haemodynamically identified syncopal signs. After exercise, the subjects were not allowed to drink fluids at any time. Venous blood samples were drawn pre-exercise (following 30 min supine rest) and immediately prior to LBNP. It should be noted that due to the relatively rapid transition between exercise and LBNP, plasma volume shifts due to changes in posture might not have been complete during the pre-LBNP blood draw (Hagan *et al.* 1978), which may have affected the calculated relative (percentage) changes in plasma volume. This was considered acceptable given that the primary research question involved interactions between exercise, dehydration and LBNP tolerance, while blood measures were used as indices of hydration status that were considered secondary to changes in body weight.

Data and statistical analyses

Thermal and cardiovascular data were collected at 50 Hz via a data acquisition system (MP 150; Biopac Systems Inc., Santa Barbara, CA, USA). With regard to exercise, data were analysed immediately before and at the end of exercise. During LBNP, data were analysed immediately before commencing LBNP (pre-LBNP, 60 s average) and at 20 and 30 mmHg LBNP (60 s average), which were the levels that all subjects completed fully in all trials, upon the attainment of the highest heart rate achieved during the final 2 min of LBNP (peak LBNP, 10 s average; Schlader & Crandall, 2014), and during the final 10 s of LBNP (presyncope). To isolate the effect of LBNP, these data were also analysed as the change (Δ) from pre-LBNP.

The tolerance was quantified as LBNP time, as well as via the cumulative stress index (Levine *et al.* 1991), which is calculated by summing the product of LBNP and the time at each level of LBNP across the trial until the test was terminated (i.e. 20 mmHg × 3 min + 30 mmHg × 3 min, etc.). Percentage changes in plasma volume from pre- to postexercise were estimated using the methods of Dill & Costill (1974).

Data from pre-exercise and the end of exercise, as well as data during LBNP, were analysed using two-way (main effects: trial × time) repeated-measures ANOVA, while data on the change from pre-exercise to the end of exercise and measures of LBNP tolerance were analysed using a one-way repeated-measures ANOVA. Where appropriate, *post hoc* Holm–Sidak pairwise comparisons were made. Data were analysed using SigmaPlot (version 13; Systat Software, Inc., San Jose, CA, USA). *A priori* statistical significance was set at $P \leq 0.05$ and exact P values are reported where possible. All data are reported as mean values ± SD.

Results

Fluid restriction during exercise in the heat

Pre-exercise intestinal and mean skin temperatures, heart rate, urine specific gravity and plasma osmolality were not different between trials ($P \geq 0.261$; Table 1). Exercise duration was 40 ± 7 min shorter during the Isothermic Dehydrated trial compared with both Hydrated and Time Match Dehydrated trials ($P < 0.001$; Table 1). During the Hydrated trial, subjects drank 1257 ± 39 ml of water to offset sweat loss during exercise. The increase ($P < 0.001$) in intestinal temperature during exercise was greatest in the Time Match Dehydrated trial ($P < 0.001$), while, by design, the increase in intestinal temperature was not different between the Hydrated and Isothermic Dehydrated trials ($P = 0.910$; Table 1). Changes in body weight and plasma volume were graded, such that the Hydrated trial had the smallest changes with exercise, the Time Match Dehydrated trial had the greatest changes ($P < 0.001$), and the alterations occurring in the Isothermic Dehydrated trial were in between ($P \leq 0.017$; Table 1). Plasma osmolality increased during exercise in both the Isothermic Dehydrated and Time Match Dehydrated trials ($P < 0.001$), both of which were higher

than the Hydrated trial ($P < 0.001$), during which plasma osmolality decreased from pre-exercise ($P = 0.051$).

Responses to central hypovolaemia postexercise

During the transition from exercise to LBNP, intestinal temperature did not change relative to end-exercise values ($P \geq 0.187$, mean difference -0.1 ± 0.4°C), such that differences between the Time Match Dehydrated trial (38.5 ± 0.5°C) compared with the Hydrated (38.0 ± 0.4°C, $P = 0.013$) and Isothermic Dehydrated trials (38.1 ± 0.3°C, $P = 0.011$) persisted at pre-LBNP. Intestinal temperature in the Hydrated and Isothermic Dehydrated trials remained not different at pre-LBNP ($P = 0.813$). Mean skin temperature increased by 1.4 ± 0.7°C from postexercise to pre-LBNP ($P < 0.001$), but this increase was not different between trials ($P = 0.667$). Not surprisingly, heart rate decreased by 41 ± 15 beats min^{-1} from postexercise to pre-LBNP ($P < 0.001$), but there were no differences between trials ($P = 0.114$).

LBNP tolerance, as expressed via the cumulative stress index, was lower in the Isothermic Dehydrated ($P = 0.031$) and Time Match Dehydrated trials ($P = 0.004$) compared with the Hydrated trial, while there was no difference in tolerance between the Isothermic Dehydrated and Time Match Dehydrated trials ($P = 0.188$; Fig. 1). Likewise, LBNP time to presyncope and the final LBNP stage reached was greater in the Hydrated trial (14.7 ± 3.2 min, 60 ± 10 mmHg) compared with both the Isothermic Dehydrated (11.9 ± 3.3 min, 50 ± 10 mmHg, $P \leq 0.031$) and Time Match Dehydrated trials (10.2 ± 1.6 min, 50 ± 10 mmHg, $P \leq 0.019$), while there were no differences in these measures between the Isothermic Dehydrated and Time Match Dehydrated trials ($P \geq 0.188$).

Mean arterial pressure decreased and heart rate increased throughout LBNP ($P < 0.001$); however, these changes were not statistically different between trials (trial × time interaction, $P \geq 0.207$; Fig. 2).

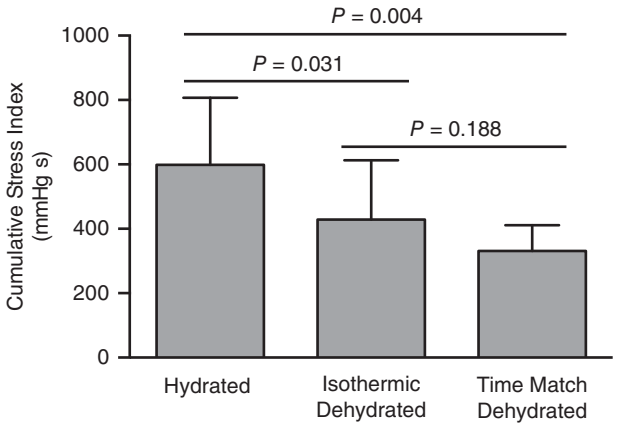

Figure 1. Lower body negative pressure (LBNP) tolerance, expressed as the cumulative stress index, following exercise in a hot environment during which: (i) water was ingested to offset sweat losses (Hydrated, $n = 11$); (ii) water was withheld, and exercise was terminated upon the same increase in intestinal temperature relative to the Hydrated trial (Isothermic Dehydrated, $n = 11$); and (iii) water was withheld, but exercise duration was the same as that occurring during the Hydrated trial (Time Match Dehydrated, $n = 9$)
Data are mean values ± SD. Main effect of trial: $P = 0.004$. Exact P values are reported for all comparisons.

Discussion

This study tested the hypothesis that fluid restriction during exercise in the heat impairs tolerance to central hypovolaemia and that this impairment is exacerbated with further dehydration and increases in body temperature. In support of this hypothesis, LBNP tolerance was compromised by fluid restriction when increases in internal temperature were similar (Fig. 1, see Hydrated *versus* Isothermic Dehydrated). In contrast to our hypothesis, however, additional dehydration (a further 1.0 ± 0.8% body weight loss) and hyperthermia (a further 0.5 ± 0.4°C increase in intestinal temperature), which occurred by matching exercise time, did not further compromise LBNP tolerance (Fig. 1, see Isothermic Dehydrated *versus* Time Match Dehydrated). The precise mechanisms underlying these alterations in LBNP tolerance are not readily apparent from the present study. However, it is clear that fluid restriction during exercise did not differentially affect the blood pressure or heart rate responses prior to or up to the point of presyncope during LBNP (Fig. 2). Overall, these data suggest that inadequate fluid intake during exercise in the heat can impair tolerance to central hypovolaemia even when it elicits only mild dehydration (~1% body weight loss). Moreover, further dehydration and increases in body temperature elicited by the time matched condition had minimal impact.

Exercise-induced dehydration and tolerance to central hypovolaemia

Exercise (Lacewell *et al.* 2014) and dehydration (Frey *et al.* 1994) can independently impair tolerance to central hypovolaemia, while fluid ingestion following 60 min of exercise in a moderate environment (20°C) partly alleviates cardiovascular strain during LBNP (e.g. increases in heart rate, reductions in stroke volume; Davis & Fortney, 1997). The present study extends these findings by demonstrating that fluid restriction during exercise in the heat impairs tolerance to central hypovolaemia

independent of the magnitude of hyperthermia (Fig. 1). Changes in heart rate and blood pressure, both before and during LBNP, do not provide insights regarding the basic haemodynamic mechanisms of this impairment (Fig. 2). However, based on a similar study (Davis & Fortney, 1997), it can be speculated that dehydration reduced blood volume and probably attenuated the magnitude of increases in peripheral resistance. Together, these responses are likely to have compromised stroke volume (Convertino, 1993) and, ultimately, cardiac output during LBNP, which would result in an earlier precipitous drop in blood pressure and, probably, cerebral perfusion, when dehydrated.

Hyperthermia, exercise-induced dehydration and central hypovolaemia tolerance

Resarchers in our laboratory have demonstrated that passive heating-induced hyperthermia (i.e. skin temperatures of ∼38°C, increases in intestinal temperature of ∼1.5°C), in the absence of dehydration, impairs LBNP tolerance and that dehydration (i.e. ∼1.6% reduction in body weight) accompanying this passive heat stress exacerbates this impairment (Lucas *et al.* 2013). We have also observed that hyperthermia (i.e. ∼1.2°C increase in intestinal temperature) impairs LBNP tolerance to a similar extent whether induced via exercise or passive heat

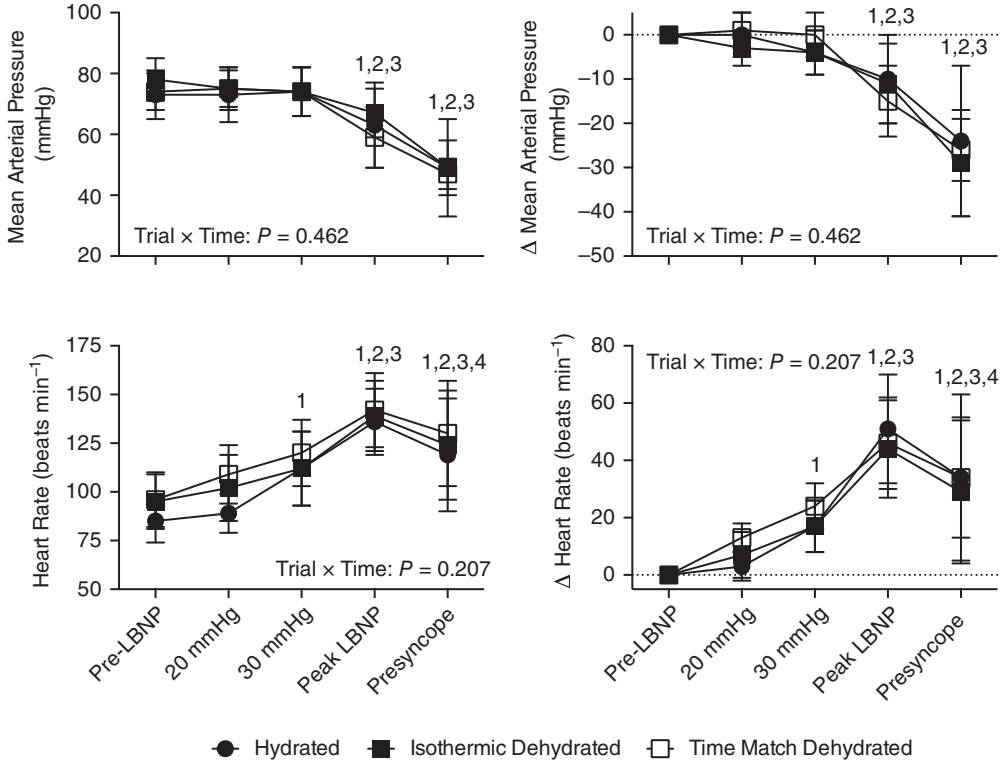

Figure 2. Absolute values (left panels) and the change (Δ; right panels) from immediately prior to lower body negative pressure (pre-LBNP) in mean arterial pressure (top panels) and heart rate (bottom panels) at pre-LBNP, 20 mmHg LBNP, 30 mmHg LBNP, upon the attainment of the highest heart rate achieved during the final 2 min of LBNP (peak LBNP) and at presyncope
LBNP to presyncope was undertaken following exercise in a hot environment during which: (i) water was ingested to offset sweat losses (Hydrated, $n = 11$); (ii) water was withheld, and exercise was terminated upon the same increase in intestinal temperature as the Hydrated trial (Isothermic Dehydrated, $n = 11$); and (iii) water was withheld, but exercise duration was the same as that occurring during the Hydrated trial (Time Match Dehydrated, $n = 9$). Data are mean values ± SD. It should be noted that peak LBNP and presyncope occurred at different absolute levels of LBNP during each trial. There were no trial × time interactions for any comparisons (P values for these interactions are reported). Changes over time, independent of trial, are indicated as follows: (1) different from pre-LBNP ($P \leq 0.006$); (2) different from 20 mmHg ($P < 0.001$); (3) different from 30 mmHg ($P \leq 0.044$); and (4) different from peak LBNP ($P \leq 0.010$).

stress when skin temperatures are not different between these trials (Pearson *et al.* 2014). With this background, we hypothesized that further dehydration (~1.9% reduction in body weight) and hyperthermia (~1.7°C increase in intestinal temperature) induced by exercising for 90 min without fluid consumption would further reduce LBNP tolerance in the presence of moderate skin temperatures (~35°C). Against our expectations, LBNP tolerance was not further reduced by this additional strain (Fig. 1, see Isothermic Dehydrated *versus* Time Match Dehydrated). These findings may be explained by a 'basement effect', such that mild dehydration associated with 50 min of exercise without fluid ingestion may have already reduced LBNP tolerance to such a point where further reductions would be unlikely. In support of this contention, 33% of subjects (three of nine) underwent LBNP for a slightly longer duration (by 2.4 ± 1.2 min) during the Time Match Dehydrated trial compared with the Isothermic Dehydrated trial. Notably, these three subjects were among the four shortest LBNP durations during the Isothermic Dehydrated trial. This suggests that the deleterious impact of mild dehydration may have exerted an effect sufficient in magnitude to mask any further impairments induced by additional dehydration and increases in body temperature. Alternatively, it is possible that further dehydration induced during the time matched conditions was not severe enough to observe further reductions in the ability of the body to tolerate central hypovolaemia. The time matched condition elicited an overall decrease in body weight of only ~2%, and it therefore remains unknown whether greater dehydration (e.g. 3–4% body weight loss) would further compromise tolerance to central hypovolaemia. Nonetheless, the findings of the present study collectively suggest that the additional effects of moderate dehydration (i.e. ~2% body weight loss) and slightly greater increases in internal temperature (~0.5°C) elicited by the time matched condition on LBNP tolerance are small. Therefore, mild exercise-induced dehydration that can occur during exercise in the heat impairs tolerance to central hypovolaemia, and slightly greater elevations in internal temperature and dehydration do not exacerbate this impairment.

Considerations

Due to the nature of the study design and the *post hoc* addition of the Time Match Dehydrated trial, we were unable to randomize the order of the trials. Although a potential limitation, this would appear unlikely given that LBNP tests in the present study were conducted ~8 weeks apart. For instance, it has been demonstrated that repeated LBNP tests in the same individuals conducted within 30 min of each other (Convertino & Sather, 2000) and as long as 1 year apart (Convertino, 2001) produce nearly identical levels of LBNP tolerance. Nevertheless, it must be acknowledged that the lack of randomization may have, at least partly, masked the magnitude of the effect of dehydration on LBNP tolerance.

This study evaluated the impact of hydration status on the ability to tolerate central hypovolaemia immediately following exercise in the heat, in an attempt to mimic conditions of a person incurring a haemorrhagic injury during physical work in a hot environment. Thus, in order to promote the translation of these findings to such circumstances, the time between the end of exercise and commencement of LBNP was made as short as possible, and instrumentation was minimized to collect only those data most important for subject safety and data integrity. As a result, the present study provides little insight regarding the mechanisms for the present observations. Nevertheless, given that blood pressure is the product of cardiac output and vascular resistance, it is likely that mild dehydration reduced the duration that cardiac output and/or vascular resistance could be sufficiently regulated to maintain blood pressure before reaching presyncope and a precipitous drop in blood pressure. Thus, LBNP tolerance may have been impaired during dehydration via reductions in blood/plasma volume and/or an attenuated ability to increase vascular resistance [e.g. via reductions in baroreflex sensitivity (Charkoudian *et al.* 2003)]. As a result, venous return was likely to be lower when dehydrated for a given level of LBNP, which reduced stroke volume and resulted in an inability to maintain cardiac output adequately. Importantly, accurate measures of cardiac output throughout LBNP are required in order to discern the haemodynamic mechanisms underlying impairments in tolerance to central hypovolaemia with dehydration.

Perspectives and significance

The present study suggests that individuals performing physical work (e.g. exercise) in a hot environment will be likely to succumb earlier to a haemorrhagic injury if they fail to maintain hydration adequately prior to the insult. Importantly, the level of dehydration capable of achieving this end is relatively mild. Furthermore, slightly larger increases in internal temperature accompanied by further dehydration (to the extent assessed in the present study) do not further impair haemorrhagic tolerance. Thus, the present study has identified that even mild dehydration reduces the time line to commence treatment during a haemorrhagic injury. Such insight is important because early recognition of a haemorrhagic injury and the initiation of treatment is vital to survival after such an injury (Bellamy, 1984; McNicholl, 1994). Clearly, the results from the present study demonstrate that hydration strategies performed in operational settings that require physical work in the heat are important to protect against the adverse effects of a subsequent haemorrhagic injury.

Conclusions

The present study demonstrates that, compared with conditions when sweat losses are fully offset by drinking water, LBNP tolerance is lower when fluid is restricted during exercise in the heat and that this reduced tolerance is independent of the magnitude of hyperthermia. Furthermore, slightly greater levels of dehydration and increases in internal temperature do not further reduce LBNP tolerance. These data demonstrate that mild dehydration associated with fluid restriction during exercise in the heat can have a profound impact in impairing the ability to withstand central hypovolaemia, as occurs during a haemorrhagic injury, and that further dehydration and increases in body temperature (to the extent assessed in the present study) do not further compromise such tolerance.

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Additional information

Competing interests

None declared. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Author contributions

All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed as authors. Furthermore, all authors are accountable for all aspects of the work, ensuring questions related to accuracy or integrity have been appropriately investigated and resolved. Conception or design of the work: Z.J.S., V.A.C. and C.G.C. Acquisition, analysis or interpretation of data: Z.J.S., D.G., E.R. and C.G.C. Drafting or revising critically for important intellectual content: Z.J.S., D.G., E.R., V.A.C. and C.G.C. All authors approved the final version of the manuscript.

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Research Paper

Cardiopulmonary and arterial baroreceptor unloading during passive hyperthermia does not contribute to hyperthermia-induced hyperventilation

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New Findings

- **What is the central question of this study?**
  Does baroreceptor unloading during passive hyperthermia contribute to increases in ventilation and decreases in end-tidal carbon dioxide during that exposure?
- **What is the main finding and its importance?**
  Hyperthermic hyperventilation is not mitigated by expanding central blood volume and reloading the cardiopulmonary baroreceptors via rapid saline infusion or by reloading the arterial baroreceptors via phenylephrine administration. The absence of a reduction in ventilation upon reloading the baroreceptors to pre-hyperthermic levels indicates that cardiopulmonary and arterial baroreceptor unloading with hyperthermia is unlikely to contribute to hyperthermic hyperventilation in humans.

This study tested the hypothesis that baroreceptor unloading during passive hyperthermia contributes to increases in ventilation and decreases in end-tidal partial pressure of carbon dioxide (P_{ET,CO_2}) during that exposure. Two protocols were performed, in which healthy subjects underwent passive hyperthermia (increasing intestinal temperature by ∼1.8°C) to cause a sustained increase in ventilation and reduction in P_{ET,CO_2}. Upon attaining hyperthermic hyperventilation, in protocol 1 ($n = 10$; three females) a bolus (19 ± 2 ml kg^{-1}) of warm (∼38°C) isotonic saline was rapidly (5–10 min) infused intravenously to restore reductions in central venous pressure, whereas in protocol 2 ($n = 11$; five females) phenylephrine was infused intravenously (60–120 μg min^{-1}) to return mean arterial pressure to normothermic levels. In protocol 1, hyperthermia increased ventilation (by 2.2 ± 1.7 l min^{-1}, $P < 0.01$), while reducing P_{ET,CO_2} (by 4 ± 3 mmHg, $P = 0.04$) and central venous pressure (by 5 ± 1 mmHg, $P < 0.01$). Saline infusion increased central venous pressure by 5 ± 1 mmHg ($P < 0.01$), restoring it to normothermic values, but did not change ventilation or P_{ET,CO_2} ($P > 0.05$). In protocol 2, hyperthermia increased ventilation (by 5.0 ± 2.7 l min^{-1}, $P < 0.01$) and reduced P_{ET,CO_2} (by 5 ± 2 mmHg, $P < 0.01$) and mean arterial pressure (by 9 ± 7 mmHg, $P < 0.01$). Phenylephrine infusion increased mean arterial pressure by 12 ± 3 mmHg ($P < 0.01$), restoring it to normothermic values, but did not change ventilation or P_{ET,CO_2} ($P > 0.05$). The absence of a reduction in ventilation upon reloading the cardiopulmonary and arterial baroreceptors to

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Experimental Physiology

pre-hyperthermic levels indicates that baroreceptor unloading with hyperthermia is unlikely to contribute to hyperthermic hyperventilation in humans.

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Introduction

Hyperthermic hyperventilation is well documented in humans and is associated with high skin and body core temperatures (T_{core}, in excess of +1.0°C; Cabanac & White, 1995; Fujii *et al.* 2008*b*). Given the relatively minor contribution of respiratory heat loss with respect to human heat balance, the mechanism(s) and/or the physiological relevance of hyperthermic hyperventialtion in humans are unclear. Previous mechanisms proposed to contribute to this response in humans include selective brain cooling (White, 2006), heightened chemoreceptor sensitivity (Fujii *et al.* 2008*a*) and increases in cutaneous vasodilatation (Hayashi *et al.* 2009). However, the effectiveness of respiratory heat dissipation in the absence of cooling devices (i.e. face fanning or nasopharyngeal coolant spray) challenges the validity of selective brain cooling (Nybo & Secher, 2011). Furthermore, chemoreceptors contribute little to hyperthermic hyperventilation (Fujii *et al.* 2008*a*), and enhanced skin vasodilatation via heat acclimatization does not alleviate hyperthermic hyperventilation (Fujii *et al.* 2012). Rapid skin surface cooling (excluding the head) restores P_{ET,CO_2} and presumably reverses hyperventilation during severe hyperthermia (Lucas *et al.* 2010). However, it is unknown whether this is due to a cold-induced pressor response and subsequent loading of the baroreceptors (Wilson *et al.* 2007*a*).

Hyperventilation is also triggered by hypotension in normothermic (Convertino *et al.* 2009; Thomas *et al.* 2009; Stewart *et al.* 2011) and hyperthermic conditions (Pearson *et al.* 2013). For example, pharmacological unloading and loading of the baroreceptors, via decreasing (by ∼18 mmHg) and increasing arterial blood pressure (by ∼8 mmHg), causes ventilation to increase (by 9.7 ± 2.4 l min^{-1}) and decrease (by 5.1 ± 1.1 l min^{-1}), respectively (Stewart *et al.* 2011). The physiological significance of such a response may be the capacity of the respiratory pump to increase venous return and cardiac filling; that is, increasing respiration and subsequent generation of a more negative intrathoracic pressure aids cardiac filling and increases cardiac output as well as arterial and central venous pressures (CVP; Kilburn & Sieker, 1960; Moreno *et al.* 1967; Conway, 1975). Therefore, increased respiratory pump activation may be a protective mechanism triggered to optimize cardiac filling in conditions of central hypovolaemic hypotension (Convertino *et al.* 2009).

Hyperthermia and associated heat-dissipation mechanisms also reduce cardiac filling pressure, central blood volume and mean arterial pressure (MAP), consequently unloading the cardiopulmonary and arterial baroreceptors (Rowell *et al.* 1969; Wilson *et al.* 2007*b*; Ganio *et al.* 2011). Thus, prolonged hyperthermia-related hypotension and the corresponding unloading of baroreceptors may contribute to hyperthermic hyperventilation. However, the influence of baroreceptor unloading on hyperthermic hyperventilation has not been examined. In the present study, therefore, we tested the following hypothesis: cardiopulmonary baroreceptor unloading and arterial baroreceptor unloading during passive hyperthermia contribute to increases in ventilation and decreases in end-tidal partial pressure of carbon dioxide (P_{ET,CO_2}) during that exposure.

Methods

Two protocols were undertaken for this study. For protocol 1, 10 subjects participated (three females; age, 29 ± 5 years; height, 177 ± 10 cm; and weight, 75.5 ± 12.2 kg). For protocol 2, 11 subjects participated (five females; age, 26 ± 5 years; height, 178 ± 12 cm; and weight, 71.3 ± 14.9 kg). Subjects were not taking medications, were free of any known cardiovascular, metabolic or neurological diseases and were non-smokers. As only within-subject comparisons were performed (see '*Data collection and statistics*' section), menstrual cycle phase was recorded but not controlled for in female subjects. Subjects were asked to abstain from exercise and alcohol for 24 h before testing, as well as caffeine for 12 h. Each subject was fully informed of the experimental procedures and possible risks before giving informed, written consent, but subjects were not informed of the proposed hypothesis. Both protocols and the informed consent were approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center at Dallas and Texas Health Presbyterian Hospital of Dallas, and all procedures conformed to the standards set by the *Declaration of Helsinki*.

Instrumentation

For both protocols, subjects were dressed in a long-sleeved and long-legged, two-piece, tube-lined perfusion suit (Med-Eng, Ottawa, ON, Canada), enabling the control of

skin temperature and T_{core} via the temperature of the water perfusing the suit. Measurements of T_{core} were derived from a telemetry temperature pill swallowed ∼2 h before the onset of data collection (HQ Inc., Palmetto, FL, USA). Whole-body mean skin temperature was measured from the weighted average of six thermocouples attached to the skin with porous adhesive tape on the calf (11%), thigh (14%), abdomen (14%), chest (22%), lower back (19%) and upper back (20%; Taylor *et al.* 1989). Expired air was sampled via a facemask attached to a two-way valve (Hans Rudolf, Inc., Shawnee, KS, USA). Ventilatory parameters (ventilation, tidal volume and breathing rate) were measured (body temperature and pressure saturated) using an automated gas analysis system (TrueOne 2400; Parvo-Medics, Sandy, UT, USA), with values recorded over 15 s epochs. The P_{ET,CO_2} was sampled from the mask and measured using a capnograph (9004 Capnocheck® Plus; Smiths Medical International Ltd, Watford, UK). Heart rate was collected from an ECG signal (Agilent, Munich, Germany) interfaced with a cardiotachometer (1000 Hz sampling rate; CWE, Ardmore, PA, USA). Beat-to-beat arterial blood pressure was measured and reconstructed to give brachial artery pressure via finger-cuff photoplethysmography (Finometer Pro; FMS, Amsterdam, The Netherlands or NexFin HD; BMEYE BV, Amsterdam, The Netherlands).

Experimental protocol 1

This protocol was performed to determine whether reloading primarily the cardiopulmonary baroreceptors and increasing CVP would attenuate hyperthermic hyperventilation. In eight of the 10 subjects, a peripherally inserted central venous catheter was advanced into the superior vena cava via the basilic vein. Positioning of the central venous catheter was confirmed by the following observations: (i) the distance that the catheter was advanced relative to the subject's height; (ii) adequate pressure waveforms; and (iii) an appropriate rapid rise and fall in pressure during a Valsalva and Müller manoeuvre, respectively. The central venous catheter was connected to a pressure transducer and zeroed at the position of the mid-axillary line. This catheter was used for continuous measurement of CVP.

Following instrumentation, subjects rested in the supine position for a minimum of 30 min, while water at 34°C circulated through the suit. After ∼20 min of wearing the facemask (ensuring steady-state ventilatory responses), normothermic baseline thermal, haemodynamic and respiratory measures were obtained. To minimize participant's discomfort, the facemask was removed after these normothermic measurements. Subjects were then passively heated by circulating water at ∼49°C through the suit. Between 10 and 15 min into the passive heating phase, the facemask was re-attached, and ventilation and P_{ET,CO_2} were monitored for at least 20 min before hyperthermia measurements were taken (42 ± 13 and 33 ± 9 min in protocols 1 and 2, respectively). After T_{core} had increased (1.9 ± 0.5°C) and there was a consistent increase in ventilation, associated with a ∼5 mmHg reduction in P_{ET,CO_2}, 19 ± 2 ml kg^{-1} warmed (∼38°C) isotonic saline was rapidly administered over 6.9 ± 2.1 min through a separate catheter placed in an antecubital vein, as this rate and volume are sufficient to return CVP to pre-hyperthermic pressures (Crandall *et al.* 1999). The duration of the saline infusion differed between subjects (range, 5 – 10 min). After the infusion and subsequent data collection, skin surface cooling was performed by circulating water at ∼20°C through the water-perfusion suit for 10 min. This method of cooling rapidly decreases the mean skin temperature with little initial effect on T_{core} (see Results).

Experimental protocol 2

This protocol was performed to determine whether reloading primarily the arterial baroreceptors and increasing MAP would attenuate hyperthermic hyperventilation. This protocol was almost identical to that outlined in the previous subsection; however, rather than administering warm saline, phenylephrine (PE; 60–120 μg min^{-1}) was titrated intravenously for 5 min to increase MAP by 12 ± 3 mmHg. In protocol 2, participants lay supine with their lower legs off the end of the bed and their feet on a footstool, so that their knee angle was ∼73 deg. This was done to aid venous pooling and augment hyperthermia-related reductions in MAP. Also, mean skin temperature was gradually returned to pre-hyperthermic stress levels 5 min after the PE infusion ended in order to avoid a potential hypertensive event that would otherwise occur with whole-body cooling in combination with the administered PE.

Data collection and statistics

Data were acquired continuously at 50 Hz throughout the experiment (Biopac, Santa Barbara, CA, USA) and were reduced into the following 1 min periods: immediately before whole-body heating (normothermia); immediately before rapid saline infusion or PE administration (hyperthermia); and during rapid saline and PE infusions. The duration of rapid saline infusion differed between subjects; therefore, the final 5 min of the infusion are presented. All data were statistically analysed using one-way repeated-measures ANOVA with the repeated factor of time (normothermia, hyperthermia and the final 5 min of rapid saline or PE infusions), followed by Tukey-corrected *post hoc* tests when significant differences were identified. Additionally for protocol 1,

Table 1. Thermal, haemodynamic and respiratory parameters during normothermia, hyperthermia, rapid saline infusion and skin surface cooling for protocol 1

Parameter	Normothermia	Hyperthermia	Rapid infusion	Skin cooling
Body core temperature (°C)	37.0 ± 0.3	38.9 ± 0.4*	39.0 ± 0.5*	39.0 ± 0.5*
Mean skin temperature (°C)	34.7 ± 0.2	39.4 ± 0.8*	39.2 ± 0.8*	34.5 ± 0.9†‡
Mean arterial pressure (mmHg)	82 ± 8	79 ± 11	74 ± 8*	74 ± 8*
Heart rate (beats min^{-1})	59 ± 10	113 ± 17*	112 ± 14*	96 ± 13*†‡

*Significantly different from normothermia $P < 0.05$; †significantly different from hyperthermia, $P < 0.05$; and ‡significantly different from rapid infusion, $P < 0.05$. Values are 1 min means ± SD.

the fifth minute of skin surface cooling after the heat stress was analysed and compared using a one-way repeated-measures ANOVA with normothermia, hyperthermia and the final minute of rapid saline infusion. A skin surface cooling time point was not included in protocol 2 analysis on account of the gradual skin surface cooling employed. A linear regression analysis was performed to characterize further the relationship between changes in ventilation and CVP or MAP during rapid saline, skin surface cooling or PE infusion, respectively. Each subject's change scores for ventilation, CVP ($n = 8$) and MAP ($n = 11$) were calculated from the difference between 1 min hyperthermic baseline and rapid saline infusion, skin surface cooling or PE infusion periods. Data were analysed using GraphPad Prism (version 6; GraphPad Software, Inc., La Jolla, CA, USA) with *a priori* statistical significance set at $P \leq 0.05$. All data are reported as mean values ± SD.

Results

Protocol 1

Passive hyperthermia increased T_{core} (by 1.9 ± 0.5°C, $P < 0.01$) and mean skin temperature (by 4.7 ± 0.7°C, $P < 0.01$) and decreased CVP (by 5 ± 1 mmHg, $P < 0.01$; Table 1 and Fig. 1). This was accompanied by an increase in ventilation (by 2.2 ± 1.7 l min^{-1}, $P < 0.01$) and tidal volume (by 0.4 ± 0.3 litres, $P = 0.04$), together with a reduction in P_{ET,CO_2} (by 4 ± 3 mmHg, $P = 0.04$; Fig. 2) when compared with normothermia. Rapid infusion of 19 ± 2 ml kg^{-1} saline increased CVP (by 5 ± 1 mmHg, $P < 0.01$) but did not change ventilation ($P = 0.70$) or P_{ET,CO_2} ($P = 0.98$) relative to pre-infusion hyperthermic values. The T_{core} and mean skin temperature values were not different between hyperthermia and rapid infusion ($P > 0.05$).

Skin surface cooling after the heat stress lowered mean skin temperature (by 4.7 ± 1.5°C, $P < 0.01$) but did not change T_{core} ($P = 0.99$) from rapid infusion values. With skin surface cooling, CVP remained 5 ± 1 mmHg higher ($P < 0.01$) than pre-infusion hyperthermic values. Notably, skin surface cooling returned tidal volume ($P = 0.98$) and ventilation ($P = 0.52$) to values similar to those in normothermia, but P_{ET,CO_2} remained slightly depressed (by 4 ± 2 mmHg, $P = 0.01$; Fig. 2. There was no association between increasing CVP and ventilation with either rapid saline infusion ($r^2 = 0.06$, $P = 0.55$; Fig. 3*A*) or skin surface cooling ($r^2 = 0.05$, $P = 0.61$; Fig. 3*B*).

Protocol 2

Passive hyperthermia increased T_{core} (by 1.8 ± 0.5°C, $P < 0.01$), increased mean skin temperature (by 6.0 ± 0.7°C, $P < 0.01$) and decreased MAP (by 9 ± 7 mmHg, $P < 0.01$; Table 2 and Fig. 4). This was accompanied by an increase in ventilation (by 5.0 ± 2.7 l min^{-1}, $P < 0.01$) and a reduction in P_{ET,CO_2} (by 5 ± 2 mmHg, $P < 0.01$). Relative

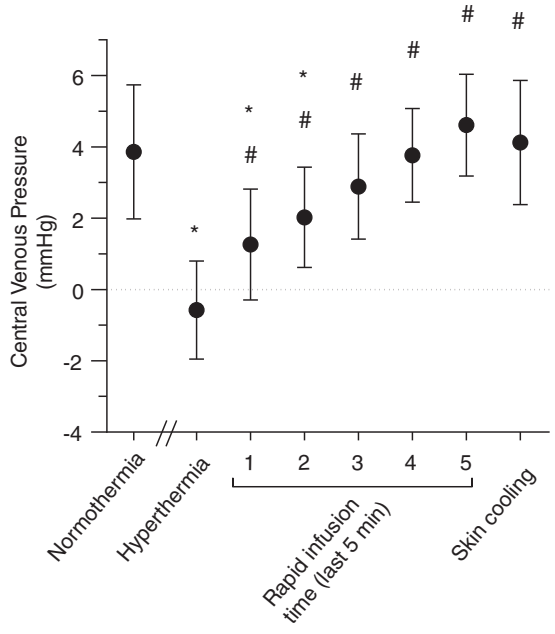

Figure 1. Central venous pressure ($n = 8$) immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the final 5 min of rapid saline infusion while hyperthermic
*Significantly different from normothermia, $P < 0.05$; and #significantly different from hyperthermia, $P < 0.05$.

to pre-infusion hyperthermia, PE elevated MAP (by 12 ± 3 mmHg, $P < 0.01$), but did not change ventilation ($P = 0.66$) or P_{ET,CO_2} ($P = 0.66$; Fig. 5). The T_{core} or mean skin temperature did not change from hyperthermic values during the PE infusion ($P = 0.93$). There was a weak association between PE-induced increases in MAP and changes in ventilation ($r^2 = 0.33$, $P = 0.07$; Fig. 6).

Discussion

This is the first study to examine whether cardiopulmonary or arterial baroreceptor unloading contributes to hyperthermic hyperventilation. The novel findings from this study are that hyperthermic hyperventilation is not mitigated by (i) expanding central blood volume and reloading the cardiopulmonary baroreceptors via rapid saline infusion, and (ii) reloading the arterial baroreceptors via PE administration. The absence of a reduction in ventilation during these perturbations indicates that cardiopulmonary or arterial baroreceptor unloading coincident with hyperthermia is unlikely to contribute to hyperthermic hyperventilation.

In the present study, participants' hyperthermic hyperventilatory response following baroreceptor reloading varied in both protocols 1 and 2. In protocol 1, ventilation decreased to some extent in four of the eight participants with rapid infusion (Fig. 3*A*). Likewise, in protocol 2,

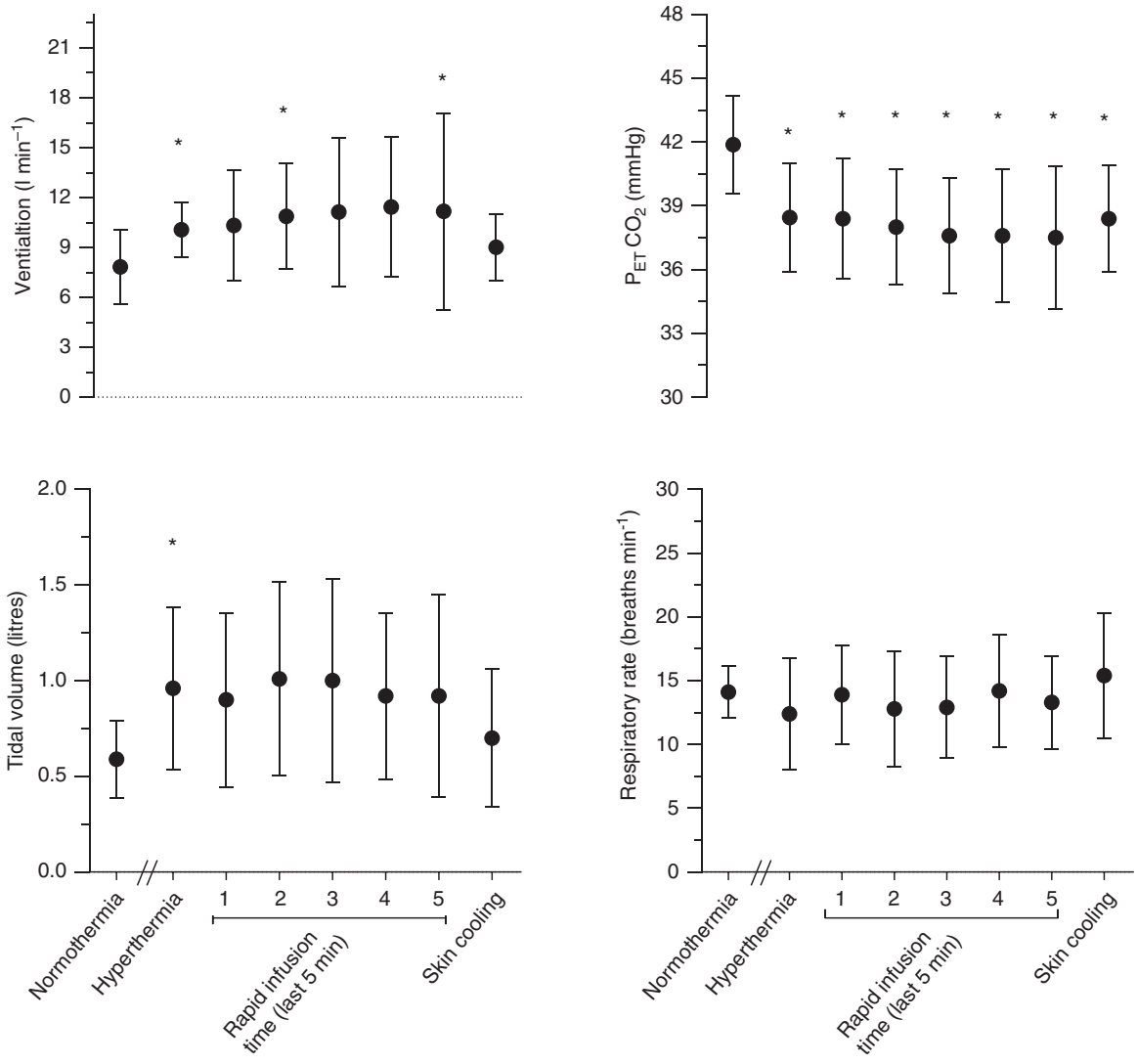

Figure 2. Respiratory responses immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the final 5 min of rapid saline infusion while hyperthermic
*Significantly different from normothermia, $P < 0.05$.

ventilation decreased in seven of the 11 participants with PE infusion (Fig. 6). Thus, it may be that reloading the cardiopulmonary or the arterial baroreceptors attenuates hyperthermic hyperventilation in some individuals. Nevertheless, this interparticipant variation may also be due to various behavioural ventilatory influences (i.e. modulators largely unaffected by the homeostatic regulation of arterial blood gas tension; Shea, 1996). Interparticipant variation for the onset of hyperthermic hyperventilation has been reported previously (Fujii *et al.* 2008*b*). There also appear to be intraparticipant differences according to the type of hyperthermic stimulus, with passive hyperthermia inducing a greater hyperventilatory response in comparison to exercise (Fujii *et al.* 2008*b*). Thus, behavioural ventilatory control elements may prevail over hyperthermic physiological ventilator drivers in some individuals. Interestingly, PE-induced arterial baroreceptor reloading tended to be associated with a decrease in ventilation ($r^2 = 0.33$). It has previously been shown that a bolus PE infusion decreases ventilation in normothermic conditions (Stewart *et al.* 2011). Thus, the presence of a ventilatory baroreflex may remain while an individual is hyperthermic; however, it remains unlikely that cardiopulmonary or arterial baroreceptor unloading contributes to hyperthermic hyperventilation in general.

Notably, rapid skin surface cooling restores P_{ET,CO_2} during severe hyperthermia (T_{core} increased by 2°C) combined with lower body negative pressure (15 mmHg; Lucas *et al.* 2010). Likewise, in the present study, skin surface cooling reduced tidal volume and ventilation from hyperthermic and rapid infusion values (Table 1). Rapid skin cooling elicits a pressor response whereby peripheral and visceral arteries constrict and central venous and right and left ventricular filling pressures increase (Wilson *et al.* 2007*a*,*b*). However, findings from the present study indicate that this pressor response is unlikely to contribute to reductions in hyperthermic hyperventilation with rapid skin cooling, as baroreceptor reloading alone did not attenuate hyperthermic hyperventilation. Given

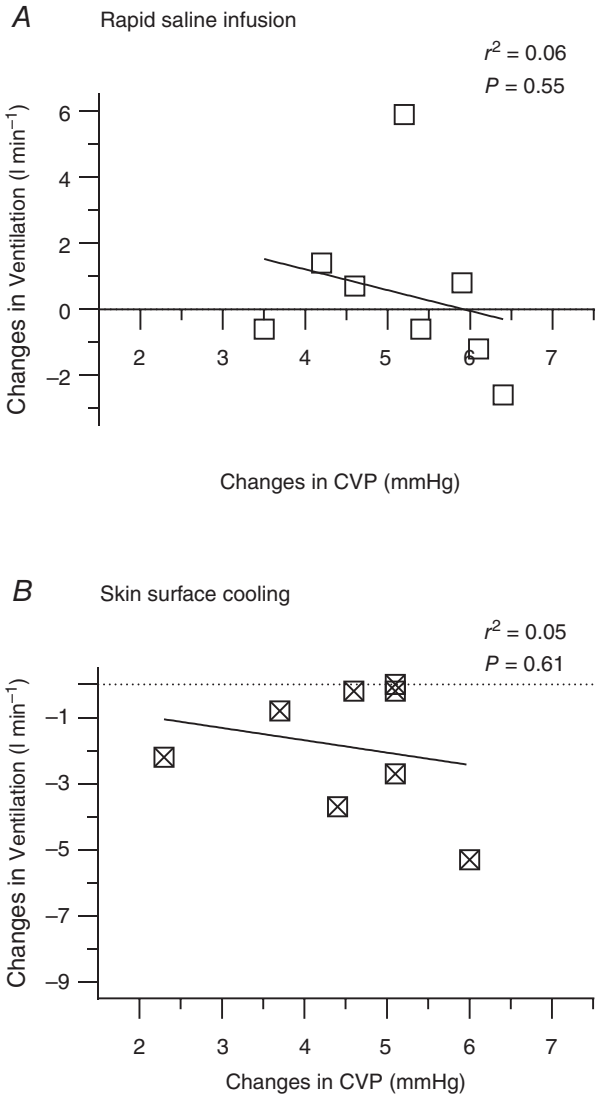

Figure 3. The relationships between changes in ventilation and central venous pressure (CVP) following rapid saline infusion (relative to hyperthermia; *A*) and following skin surface cooling (relative to hyperthermia; *B*)
Data are individual responses to rapid saline infusion ($n = 8$).

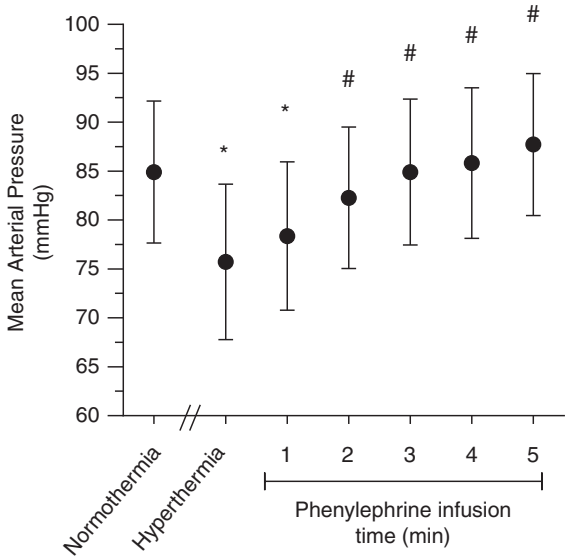

Figure 4. Mean arterial pressure immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the first 5 min of phenylephrine infusion
*Significantly different from normothermia, $P < 0.05$; and #significantly different from hyperthermia, $P < 0.05$.

Table 2. Thermal and haemodynamic paramters during normothermia, hyperthermia and the fifth minute of phenylephrine infusion for protocol 2

Parameter	Normothermia	Hyperthermia	PE infusion
Body core temperature (°C)	36.9 ± 0.2	38.7 ± 0.4*	38.9 ± 0.5*
Mean skin temperature (°C)	33.9 ± 0.5	39.9 ± 0.5*	39.8 ± 0.5*
Heart rate (beats min^{-1})	56 ± 10	107 ± 16*	95 ± 15*†

*Significantly different from normothermia, $P < 0.05$; and †significantly different from hyperthermia, $P < 0.05$. Values are 1 min means ± SD.

that hyperthermic hyperventilation is associated with high T_{core} and skin temperatures (Cabanac & White, 1995; Fujii *et al.* 2008*b*), it is possible that high thermoafferent activity from both the core and the skin may drive hyperthermic hyperventilation. Animal studies have shown that stimulation of hypothalamic thermosensitive neurons increases neural activity of the ventral respiratory group and, consequently, ventilation (Boden *et al.* 2000; Tryba & Ramirez, 2003). If this is the case, presumably rapid skin cooling reduces hyperthermic thermoafferent activity and, subsequently, some of the stimulus for hyperventilation. However, such

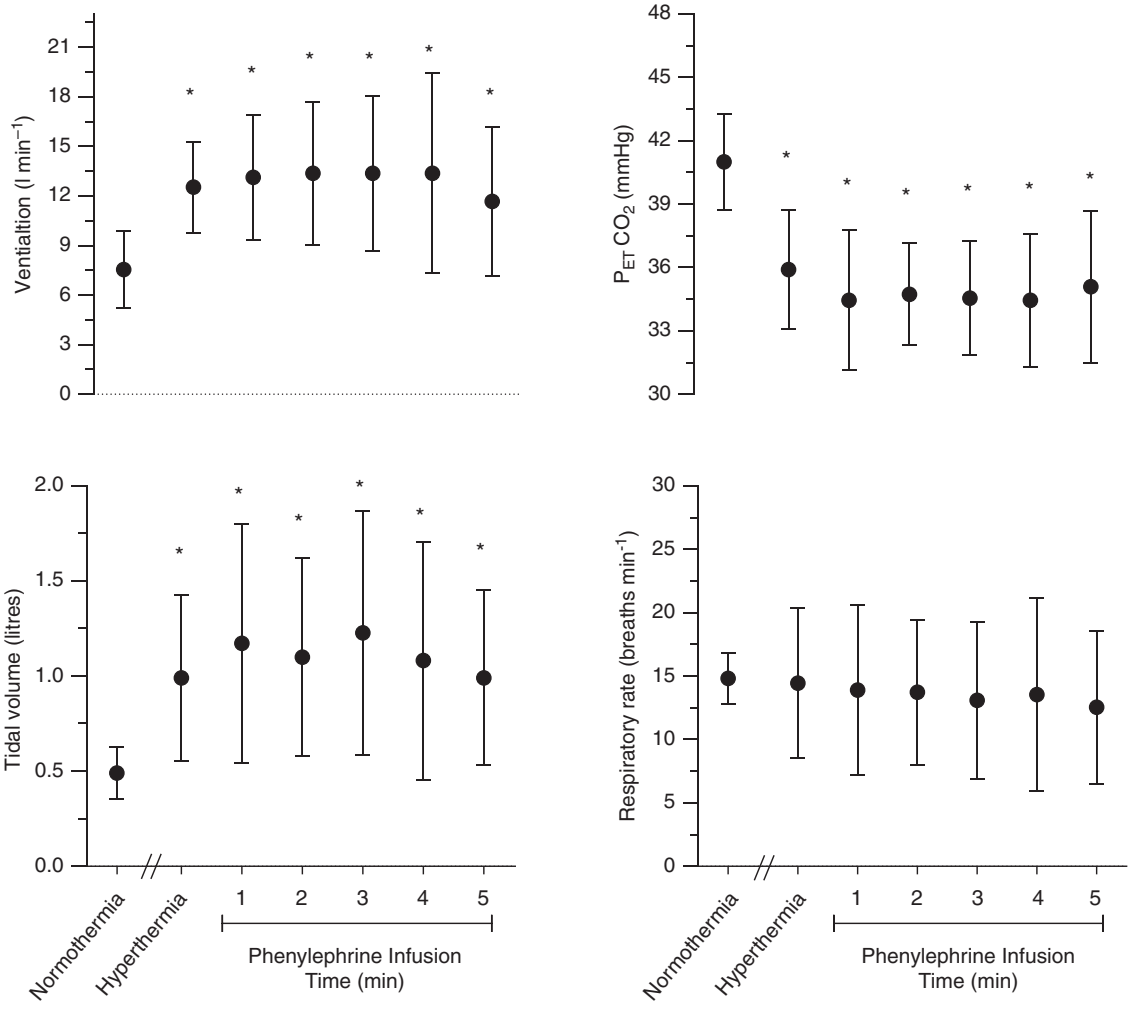

Figure 5. Respiratory responses immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the first 5 min of phenylephrine infusion
*Significantly different from normothermia, $P < 0.05$.

a relationship between thermoafferent neural activity and ventilation has not been examined directly in humans. Alternatively, subjective relief from high skin temperatures with rapid skin cooling may also underlie reductions in hyperthermic hyperventilation. Hyperventilation can be associated with highly arousing negative emotions (Boiten *et al.* 1994), such as might be elicited from the great thermal discomfort accompanying elevated skin and body core temperatures. Thus, hyperthermic hyperventilation may not serve a physiological purpose, but rather is a response to a state of considerable thermal discomfort, anxiety and/or arousal. If this is the case, the relatively high cutaneous contribution to subjective thermal comfort may explain, in part, why rapid skin surface cooling reduces hyperventilation while T_{core} remains elevated (Frank *et al.* 1999). It may also explain the interparticipant variation in hyperthermic hyperventilation, as individuals with more experience and/or resilience (both physiologically and psychologically) to hyperthermia (for example, owing to habitual exercise in warm environments) may be better able to manage thermal discomfort and any resulting hyperthermic hyperventilation. Thus, perhaps hyperthermic hyperventilation indicates when an individual is reaching their psychophysiological hyperthermic limit.

Technological considerations

Central venous pressure was measured in eight of the 10 subjects. As there is no reason to believe that CVP would respond differently during rapid saline infusion in the two subjects who refused the CVP catheter, we deemed it justifiable to include their data within the analyses.

In both protocols, rapid saline infusion and administration of PE were initiated when T_{core} was very high (39.0 and 38.9°C, respectively). It is possible that such high T_{core} and accompanying thermal discomfort dominated any potential baro-mediated ventilatory response. However, this magnitude of hyperthermia was necessary because hyperventilation was a prerequisite for testing our hypotheses. For the present study, we considered that a consistent increase in ventilation, associated with a ∼5 mmHg reduction in P_{ET,CO_2}, would be indicative of hyperthermic hyperventilation. We anticipated that this degree of hyperthermic hyperventilation would occur at a T_{core} of 38–38.5°C based on previous studies (Cabanac & White, 1995; Fujii *et al.* 2008*b*). However, participants in the present study did not show a consistent hyperventilation until they reached a higher T_{core}, indicative of the intraparticipant variability of this response (Fujii *et al.* 2008*b*). A possible explanation for this observation may be the facemask familiarization used in the present protocol. Each subject wore the facemask for at least 20 min prior to normothermic and hyperthermic data collection, thus reducing the likelihood of 'artificially' triggering hyperventilation and affecting the T_{core} threshold for hyperthermic hyperventilation via application of the facemask.

Rapid skin surface cooling after heat stress increased P_{ET,CO_2} by 2 mmHg, but that value remained below the normothermic baseline, whereas at similar T_{core} Lucas *et al.* (2010) found that P_{ET,CO_2} increased by 7 mmHg with skin surface cooling. This difference is likely to be due to

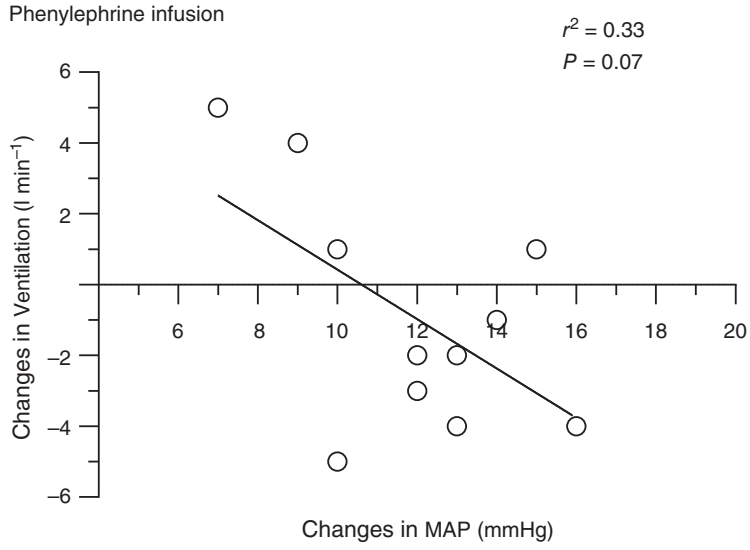

Figure 6. The relationships between changes in ventilation and Mean arterial pressure (MAP) following phenylephrine infusion (relative to hyperthermia)
Data are individual responses to PE infusion (n = 11).

the differences in the water temperature perfusing the suit, with use of 15°C water as opposed to the 20°C water in the present study, determining that a more modest cooling stimulus elicited a smaller reduction in hyperthermic hyperventilation. In the present study, this was designed to avoid overloading the central vascular space and unsafely increasing central venous and arterial pressure, given the volume-loaded state of these individuals following saline infusion. For the same reason, skin temperature was lowered slowly in protocol 2; hence, no skin surface cooling data are presented. This further highlights the impact of reducing skin temperature on ventilator responses.

Implications

Findings from the present study further highlight the importance of reducing high skin temperatures in hyperthermic individuals. Acute baroreceptor loading alone does not appear to circumvent hyperventilatory-induced hypocapnia, which affects cerebral perfusion owing to the cerebral vasoconstriction associated with reductions in arterial P_{CO_2} (Kety & Schmidt, 1948). However, this and previous studies indicate that lowering high skin temperatures in hyperthermic individuals attenuates hyperventilation (Lucas *et al.* 2010). This has ramifications for avoiding syncope and maintaining consciousness during a hyperthermic, hypotensive challenge.

Also, findings from the present study further support the intraparticipant variability associated with hyperthermic hyperventilation (Fujii *et al.* 2008*b*). This degree of variability coupled with a possible role of thermal discomfort in passive hyperthermic hyperventilation seem to indicate a psychophysical influence on hyperthermic hyperventilation. If this is the case, it may be that mental preparation or habituation, as has been observed in the cold (Croft *et al.* 2013), can affect an individual's hyperthermic hyperventilatory threshold, which perhaps explains the variability of this response reported in the literature. However, a psychophysical effect has not been formally established to date.

Conclusion

In the present study, rapid saline infusion and administration of PE successfully elevated CVP and MAP, respectively, ameliorating hyperthermia-related unloading of these baroreceptors. Despite this, ventilation and P_{ET,CO_2} did not change from pre-infusion hyperthermic values. These findings strongly indicate that hyperthermic hyperventilation is not affected by cardiopulmonary or arterial baroreceptor unloading coincident with hyperthermia.

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Additional information

Competing interests

None declared.

Author contributions

All authors contributed to the conception and design of the experiment, collection, analysis and interpretation of data and writing the manuscript. All authors read and approved the final manuscript.

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