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14. ABSTRACT

Hemorrhage is a leading cause of deaths on the battlefield. An understanding of the mechanisms and modulators of blood pressure control and coagulopathy under conditions soldiers currently experience on the battlefield is important for improved treatment of the hemorrhaging soldier. The global objective of this project tested the hypothesis that environmental and physiological conditions a soldier experiences on the battlefield alters hemodynamic and hemostatic function (i.e., coagulation and fibrinolysis). To that end, we investigated these important questions using three unique experimental conditions. The specifics of the experimental protocols and their outcomes are included in the body of the progress report and the accompanying publications. We found that the applied conditions did not adversely affect hemostatic function, but tolerance to simulated hemorrhage was generally impaired when individuals were heat stressed. Finally, an index of cardiovascular compensatory reserve was unaffected by the applied environmental and physiological conditions, suggesting that this pre-hospital assessment provides valuable information regarding the hemorrhagic status of individuals under the applied conditions.

15. SUBJECT TERMS
hemorrhage, hyperthermia, heat stress, coagulopathy, hemostasis, compensatory reserve, dehydration

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Table of Contents

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 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. BODY

The global objective of this project tests the hypothesis that environmental and physiological conditions, often experiences on the battlefield, alters hemodynamic and hemostatic function. The following are the indicated statement of work and the outcome of that work:

Tasks proposed to be accomplished during year 1

1) Obtain IRB approvals from the Department of Defense, University of Texas Southwestern Medical Center, and Texas Health Presbyterian Hospital of Dallas for the proposed studies. **COMPLETED**
2) Purchase the TEG5000 units and train the staff in their use **COMPLETED**
3) Accomplish objectives outlined in specific aim 1A. These studies will investigate the effects of passive heat stress on markers of hemostatic function during simulated hemorrhage. **COMPLETED**
4) Analyze and interpret data obtained during the experiments outlined in specific aim 1A. **COMPLETED**

Description of the outcome of work accomplished under Aim 1A (Year 1 of the statement of work).

Aim 1A tests the specific hypothesis that passive heat stress alters hemostatic function during simulated hemorrhage. The protocol requires subjects to visit the laboratory on two occasions, separated no fewer than 60 days, owing to the amount of blood drawn for each trial relative to the duration needed for the body to replenish that blood. During one visit the subjects are passively heat stressed sufficient to increase internal temperature ~1.2 °C. Upon achieving this temperature, subjects are exposed to progressive simulated hemorrhage via lower-body negative pressure (LBNP) until the onset of syncopal symptoms. During the other visit, subjects remain normothermic for a comparable time relative to the aforementioned heat stress trial, after which

they undergo the same LBNP challenge. The order of exposure to these challenges is randomized.

By design, heat stress increased internal temperature prior to LBNP by 1.22±0.05 °C. Tolerance to graded LBNP was reduced by ~50% when subjects were heat stressed relative to normothermic ($P<0.001$). Data were obtained at normothermic baseline, during heating or normothermic time control just prior to LBNP, throughout LBNP inclusive of presyncope, and during recovery.

We found that although tissue oxygen saturation (from the Reflectance Medical CareGuide 1100) decreases throughout LBNP induced simulated hemorrhage, the magnitude of this reduction was greater while subjects were normothermic relative to heat stressed (see Figure 2 of (4) in the appendix). In that referenced paper, we concluded that either the CareGuide 1100 does not accurately track tissue oxygen saturation during simulated hemorrhage while subjects were heat stressed, or the magnitude of the reduction in tissue saturation during this hemorrhagic insult is attenuated when individuals were heat stressed.

In that same cohort, we evaluated the combined effects of passive heat stress and simulated hemorrhage on the Compensatory Reserve Index (CRI; Flashback Technologies). Those results are published in the following manuscript (1), which is included in the appendix. Heat stress itself (i.e., prior to a hemorrhagic insult) reduced CRI by upwards to 50% (see figure 3 of the referenced manuscript). Subsequently, the simulated hemorrhagic challenge further decreased compensatory reserve while subjects were both normothermic and heat stressed, with the rate of reduction in CRI being similar between these thermal conditions. The compensatory reserve

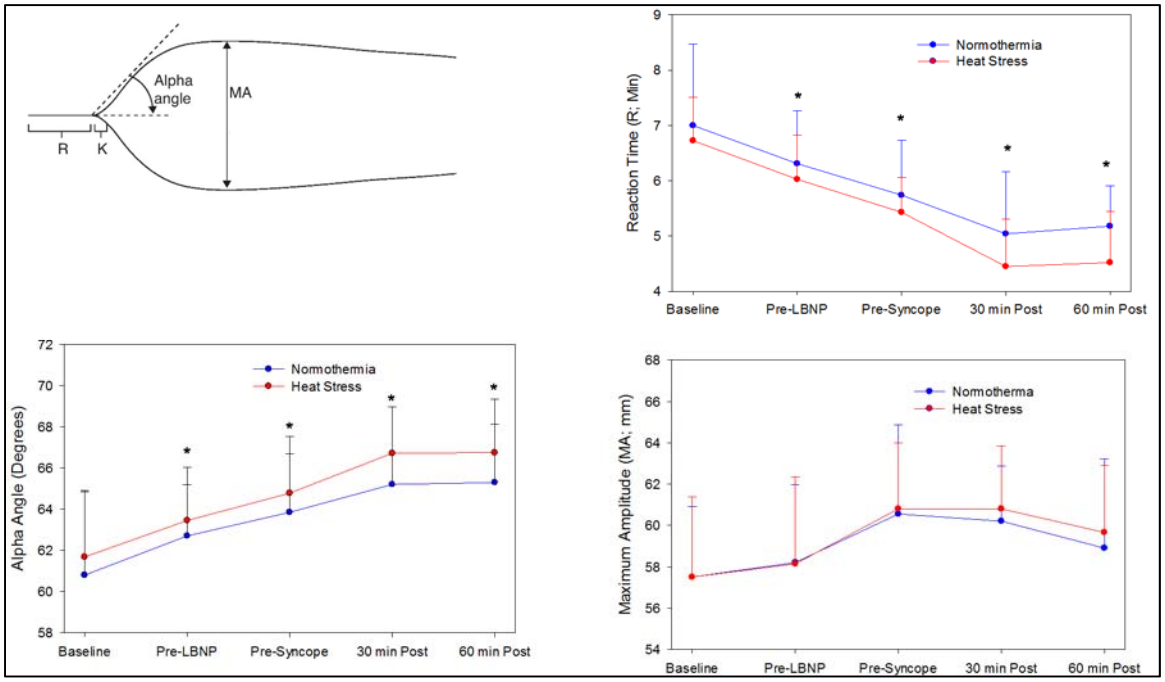

Figure 1, Upper left panel: Typical response obtained by thromboelastography. Upper right panel: Reaction time (R) throughout the protocol between normothermic and heat stressed conditions. Lower left panel: Alpha Angle throughout the protocol between normothermic and heat stressed conditions. Lower right panel: Maximum amplitude (MA) the protocol between normothermic and heat stressed conditions. * signifies significant difference from baseline for both the normothermic and heat stress trials.

values at "cardiovascular decompensation" also did not differ between thermal conditions. We concluded that 1) heat stress itself reduces the body's physiological reserve to compensate for central hypovolemia, and 2) the compensatory reserve index appropriately tracks reductions in tolerance to a simulated hemorrhage insult both while individuals are normothermic and heat stressed.

We also completed the analysis of the thromboelastography (TEG) data for this limb (See Figure 1 above). Specifically, blood samples were obtained at the end of baseline rest, prior to LBNP, immediately post-LBNP, at 30 min post-LBNP, and at 60 min post-LBNP. These samples were analyzed to determine coagulation time (R), clot formation time (K), the kinetics of clot development (α angle), and maximum clot strength (MA). For each of these variables, there was a significant effect of time (all $p \leq 0.001$), however these changes did not differ between thermal conditions (time×condition interactions all $p > 0.1$). As such, the data presented were collapsed across both thermal conditions. Overall, R and K decreased to LBNP, while α angle and MA increased. Generally these responses persisted upwards to 60 min post-LBNP. These data show that hyperthermia in combination with a simulated hemorrhagic challenge does not uniquely alter TEG-based markers of hemostasis relative to a comparable challenge when individuals are normothermic.

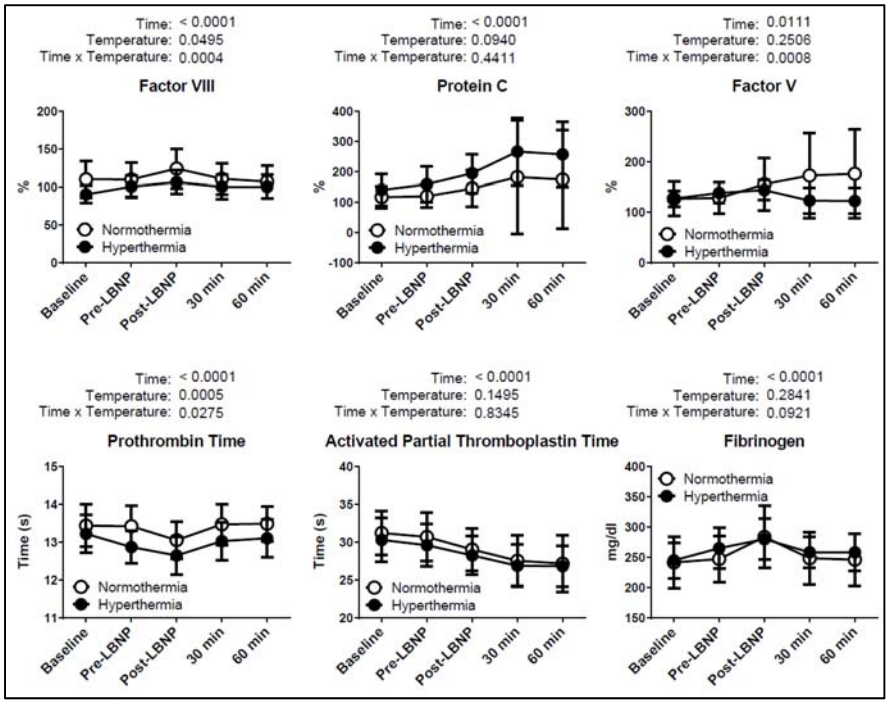

Figure 2: The effect of simulated hemorrhage (LBNP – lower body negative pressure), in both normothermic and heat stressed humans, on the indicated markers of hemostatic function.

Figure 2 illustrates the findings of the various constituents that contribute to hemostasis and how heat stress, with and without simulated hemorrhage alters those responses. Heat stress itself reduced Factor VIII and Prothrombin time, while each of these variables were altered by the simulated hemorrhagic challenge. Moreover, we observed a significant interaction in Factor VIII, Factor V, Prothrombin time such that the magnitude of the observed change to simulated

hemorrhage was influenced by the thermal status of the individual. However, it should be emphasized that the magnitude of the observed changes were relatively minor and well within the range of normative values for these measures.

Tasks proposed to be accomplished during year 2

1) Submit and present work from specific aim 1A to a national meeting. **COMPLETED**
2) Accomplish objectives outlined in specific aim 1B. These studies will investigate the effects of exercise, with and without dehydration, on markers of hemostatic function during simulated hemorrhage. **COMPLETED**
3) Analyze and interpret data obtained during the experiments outlined in specific aim 1A. **COMPLETED**
4) Write technical reports and/or scientific publications to disseminate information obtained from Specific Aims 1A and possibly 1B. **COMPLETED**

Description of the outcome of work accomplished under Aim 1B (Year 2 of the statement of work).

Aim 1B tested the hypothesis that dehydration associated with moderate exercise in hot environmental conditions impairs the capability to tolerate central hypovolemia, as would occur during a hemorrhagic insult. On two occasions subjects exercised on a treadmill in an environmental chamber set at 40 °C (104 °F) and 30% relative humidity. For the first trial, subjects exercised for 90 min while ingesting fluid to offset sweat loss (Hydrated Trial). For the second trial, separated by ~2 months, fluids were withheld throughout the protocol (Dehydrated Trial), while the subjects exercised to the same increase in internal temperature as the first trial. The imposed workload was comparable to a routine foot patrol and was identical between trials. Immediately upon cessation of each exercise trial, subjects were exposed to a simulated hemorrhagic challenge via progressive lower-body negative pressure (LBNP) to pre-syncope. LBNP tolerance. By design, the increase in internal temperature at the end of exercise but prior to LBNP was identical between trials (both 1.2±0.4° C), while body mass was reduced by in the Dehydrated Trial but was unchanged in the Hydrated Trial. LBNP tolerance was reduced by ~25% in the Dehydrated Trial (P=0.03). These results are published in the following papers (1, 3), which are included in the appendix. These data suggest that inadequate fluid intake resulting in relatively mild dehydration during a foot patrol in conditions of elevated environmental temperature may compromise the capacity to withstand central hypovolemia, as would occur during a hemorrhagic insult.

We also evaluated the consequence of dehydration during exercise in the heat on the assessment of compensatory reserve, using Flashback Technology's CRI algorithm. See Figure 3 (right panel) of the following paper for the key findings (1). Those data revealed that dehydration itself reduces CRI by ~20%, while the reduction in the capacity to track compensatory reserve was not altered by hydration status. The results suggest that compensatory reserve index appropriately tracks reductions in tolerance to a simulated hemorrhagic challenge during conditions of exercise with and without accompanying dehydration.

Finally, we evaluated TEG based indices of hemostasis in this protocol to evaluate whether exercise, with and without adequate hydration adversely affect markers of coagulation. Key

findings are illustrated below. While we observed a main effect of time for most of these variables, there was not effect of temperature or a significant interaction between hydration status and time (see Figure 1 for definitions of the various markers).

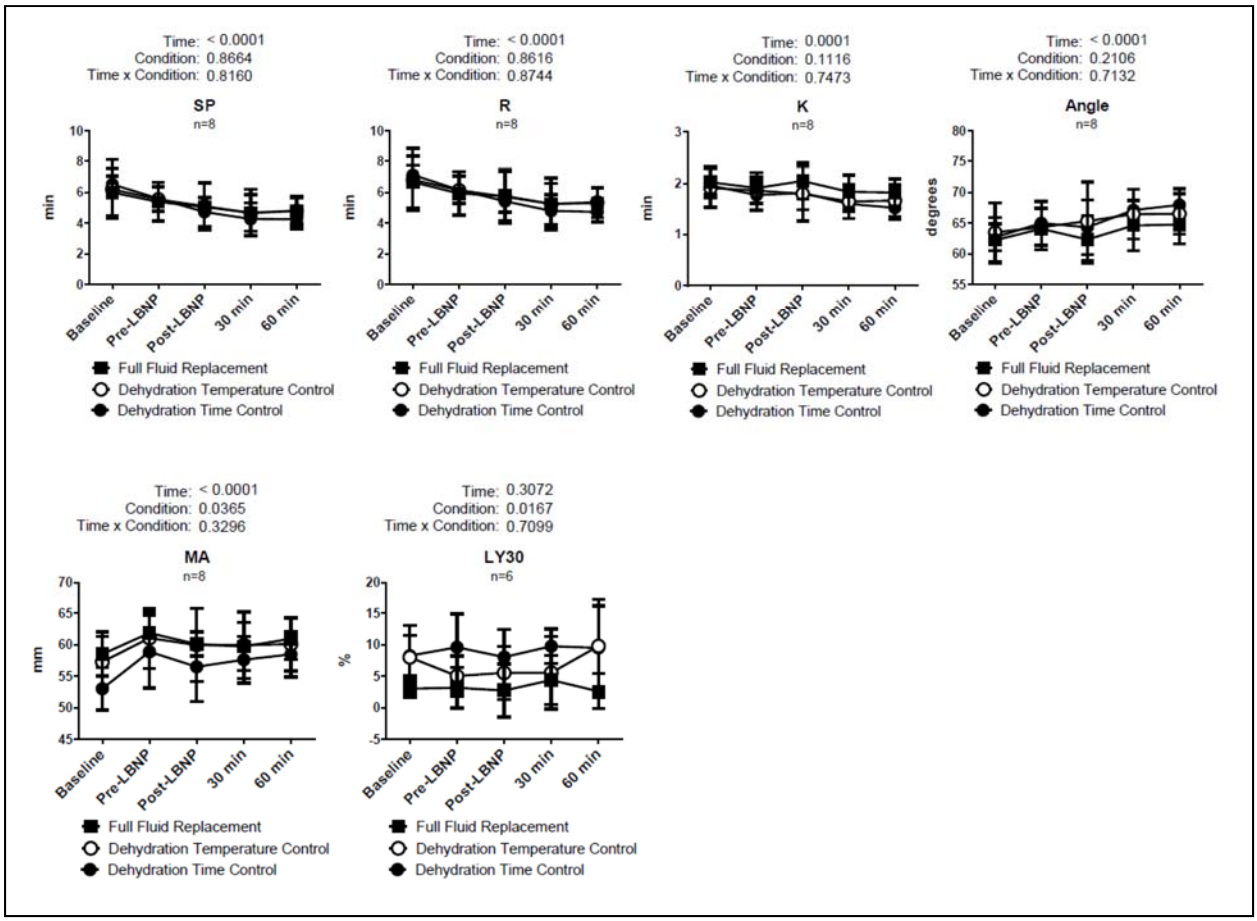

Figure 3: The effect of exercise in the heat, with and without fluid replacement, on thromboelastography based measures of hemostatic function. Full Fluid Replacement: subjects were fully hydrated; Dehydration Temperature Control: subjects dehydrated while they exercised until they achieved the highest core temperature found in the full fluid replacement trial. Dehydration time control: Subjects dehydrated while they exercised for the same duration as the Full Fluid Replacement trial. See Figure 1 for definitions of the thromboelastrography symbols.

Tasks proposed to be accomplished during year 3

1) Submit and present work from specific aim 1b to a national meeting. **COMPLETED**
2) Accomplish objectives outlined in specific aim 2. These studies will identify whether, during a hemorrhagic insult, warming an otherwise normothermic individual is detrimental towards the control of arterial blood pressure, cerebral perfusion, and hemostatic function. The potential beneficial effects of skin surface cooling will also be evaluated on the aforementioned responses. **COMPLETED**
3) Analyze and interpret data obtained during the experiments outlined in specific aim 2. **COMPLETED**
4) Write technical reports and/or scientific publications to disseminate information obtained from Specific Aims 1B and 2. **COMPLETED**

Description of the outcome of work accomplished under Aim 2 (Year 3 of the statement of work).

This objective tested the hypothesis that warming a normothermic individual during a simulated hemorrhagic insult is detrimental to the maintenance of arterial pressure and cerebral perfusion, while skin surface cooling may be beneficial. Subjects underwent a randomized, crossover experimental design performed on 3 separate days. Following 15 min of supine rest, 10 min of 30 mmHg of lower body negative pressure (LBNP) was applied to simulate a mild hemorrhagic challenge. With LBNP continuing, subjects were exposed to whole-body warming (mean skin temperature (Tsk): 36.8 ± 0.4˚C), skin surface cooling (Tsk: 29.7 ± 1.2˚C), or remained thermoneutral (Tsk: 33.4 ± 0.4˚C) for 40 min via a water perfused suit. Hemodynamic and thermal variables were measured continuously, while blood was drawn for assessment of hemostatic function. A significant interaction ($P < 0.001$) suggested that arterial blood pressure during LBNP was dependent on the thermal perturbation applied. Arterial pressure was reduced (-7.3 ± 0.5 mmHg) relative to baseline values during combined warming and simulated hemorrhage ($P < 0.001$), whereas skin surface cooling increased arterial pressure (+3.5 ± 0.9 mmHg) during the hemorrhagic challenge ($P < 0.001$; see Figure 2 below). Finally, arterial pressure (+0.4 ± 2.2 mmHg) was unchanged during LBNP when the subjects remained thermoneutral throughout the trial ($P = 0.90$). Cerebral perfusion responses (ultrasound of the middle cerebral artery) significantly decreased from baseline throughout LBNP (-4.9 ± 0.1 $cm \cdot sec^{-1}$; $P = 0.003$), regardless of the thermal conditions applied. The data obtained during this protocol, as well as a follow-up protocol in which tolerance to a simulated hemorrhagic challenge was assessed under these three thermal conditions, are depicted in the following paper included in the appendix (2). Contrary to our hypothesis, mild heating did not compromise cerebral blood velocity or tolerance to the simulated hemorrhagic insult, relative to when the subjects were thermoneutral or cooled. Of note, none of the applied thermal conditions compromised the ability of compensatory reserved to be indexed through Flashback Technologies CipherSensor device. We concluded that warming an otherwise normothermic hemorrhaging victim is not detrimental to hemodynamic stability, nor is this stability improved with cooling.

3. KEY RESEARCH ACCOMPLISHMENTS

- Upon completion of hemodynamic data analysis for Aim 1A, we identified that heat stress does not alter markers of hemostatic function to a level different relative to simply being supine while normothermic for a similar period of time. Primary deliverable: Information that soldiers who are passively heat stressed are not at a great hemostatic risk following an injury.

- Upon completion of hemodynamic data analysis for Aim 1B, we identified that dehydration during a simulated foot patrol in the heat significantly impairs one's ability to tolerate a hemorrhagic, while hemostatic status is generally unimpaired. Primary deliverable: Information to reinforce the importance of hydration for the soldier given that a relatively small level of dehydration will impair their capability to tolerate a progressive hemorrhagic insult in the heat.

- Upon completion of hemodynamic data analysis for Aim 2, we identified that mild warming can be detrimental to the hemorrhaging soldier given that arterial blood pressure is reduced by this "treatment", while skin surface cooling resulted in slight increases in arterial blood pressure. However, neither of these perturbations significantly affected the magnitude of the reduction in cerebral perfusion that occurred with the mild hemorrhagic insult. Finally, hemostatic status was generally unaffected by these perturbations. Primary deliverable: Information that warming is not beneficial or detrimental to an otherwise normothermic individual experiencing a hemorrhagic insult. Furthermore, mild cooling is also not beneficial or detrimental on hemorrhagic tolerance of an otherwise normothermic individual.

- The assessment of compensatory reserve was unaffected by the applied environmental and physiological conditions. Primary deliverable: We conclude that compensatory reserve can accurately be obtained across these conditions, resulting in improved detection of the hemorrhagic status of a soldier in the pre-hospital setting.

4. REPORTABLE OUTCOMES

Peer-Reviewed Scientific Journals

- Schlader, Z.J. & C.G. Crandall. Normothermic central hypovolemia tolerance reflects hyperthermic tolerance. *Clin Auto Res* 24:119-126, 2014

- Pearson, J., Z.J. Schlader, J Zhao, D. Gagnon, C.G. Crandall. Active and passive heat stress similarly compromise tolerance to a simulated hemorrhagic challenge. *Am J Physiol Reg Comp Physiol* 307:R822-R827, 2014

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- 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 simulated hemorrhage in humans. *Shock* 46 (Suppl 1): 42-49, 2016

- Gagnon, D., Z.J. Schlader, A. Adams, R. Rivas, J. Mulligan, G.Z. Grudic, V.A. Convertino, J.T. Howard, C.G. Crandall. The effect of passive heat stress and exercise-induced dehydration on the compensatory reserve during simulated hemorrhage. Shock 46(Suppl 1): 74-82, 2016

- Pearson, J., R.A.I. Lucas, Z.J. Schlader, D. Gagnon, C.G Crandall. Elevated skin and core temperatures both contribute to reductions in tolerance to a simulated hemorrhagic challenge. Exp Physiol 38:e284-292, 2017

- Gagnon, D., S.A. Romero, H. Ngo, P.Y.S. Poh, C.G. Crandall. Plasma hyperosmolality improves tolerance to combined heat stress and central hypovolemia in humans. Am J Physiol Reg Comp Physiol 312:R273-R280, 2017

Invited Review Articles:

- Schlader, Z.J., T.E. Wilson. C.G. Crandall. Mechanisms of orthostatic intolerance during heat stress. Autonomic Neuroscience: Basic and Clinical 196:37-46, 2016

Abstracts and associated presentations at national meetings:

- Crandall, C.G., Z.J. Schlader, E. Rivas, A.P. Cap, V.A. Convertino. Hyperthermia does not alter TEG-based markers of hemostatic function during simulated hemorrhage in humans. Military Health System Research Symposium. 2013.

- Schlader, Z.J., C.G. Crandall. Heart rate response during hyperthermic lower body negative pressure: implications for tolerance. *Clin Auton Res*: 23: Page 265, 2013.

- Gagnon, D., Schlader, Z.J., Rivas, E., Kern, J.D., Kennedy, N., Cap, A.P., Convertino, V.A., Crandall, C.G. Does hyperthermia alter TEG-based markers of hemostasis during simulated hemorrhage in humans? Med. Sci. Sports Exerc 46(5):108, 2014

- Crandall, C.G., Z.J. Schlader, E. Rivas, D. Gagnon, V.A. Convertino. Dehydration associated with a simulated foot patrol in the heat impairs tolerance to a hemorrhagic insult. MHSRS Conference, August 2014.

- Schlader, Z.J., E. Rivas, N. Kennedy, J.D. Kern, B.R. Soller, V.A. Convertino, C.G. Crandall. Muscle oxygen saturation during hyperthermic central hypovolemia. The Faseb J 28:1104.21, 2014

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- Poh, P.Y.S., D. Gagnon, S.A. Romero, S.J. Petruzzello, V.A. Convertino and C.G. Crandall "Whole-body warming during a simulated hemorrhagic insult compromises arterial blood pressure but not cerebral perfusion." Military Health System Research Symposium, 2015

- Schlader, Z.J., D. Gagnon, E. Rivas, V.A. Convertino, C.G. Crandall "Fluid restriction during exercise in the heat reduces tolerance to central hypovolemia." The Faseb J 29:823.2, 2015.

- Poh, P.Y.S., S.A. Romero, S.J. Petruzzello, V.A. Convertino, C.G. Crandall "Hemodynamic responses to mild warming during simulated mild hemorrhage.". The Faseb J 29:LB714, 2015.

- Pearson, J., R.A.I. Lucas, Z.J. Schlader, D. Gagnon, C.G. Crandall "Elevated core and skin temperatures independently attenuate simulated hemorrhage tolerance." The Faseb J 29:994.18, 2015.

- Poh, P.Y.S., D. Gagnon, S.A. Romero, V.A. Convertino and C.G. Crandall "Hemodynamic responses to cooling and warming during a continuous simulated hemorrhage ramp." The Faseb J 30:1241.2, 2016.

Invited Oral Presentations:

- C.G. Crandall: "Skin, the human radiator: Implications in health and disease" University of Buffalo, February 4, 2015

- C.G. Crandall: "Environmental factors that influence hemorrhage tolerance" Experimental Biology, April 1, 2015

- 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.

- C.G. Crandall "Environmental stressors on the battlefield: perspectives on thermoregulatory and hemorrhage tolerance of the soldier." Honor Award Lecture, Texas ACSM annual meeting. Waco, Texas, February 17, 2017

Other Achievements

Paula Poh was a graduate student from the University of Illinois Urbana-Champaign who was working in my laboratory while we completed the objectives outlined in Aim 2. She was very instrumental in assisting with this project. In fact, this project became her Ph.D. dissertation project, for which she successfully defended on April 9, 2015. Thus, in addition to the results and deliverables originating from this work, she was able to receive outstanding training while

completing the projects outlined above. She currently is a post-doctoral fellow with the US Navy in San Diego.

The aforementioned projects provided research training opportunities for the following individuals:

- Dan Gagnon, Ph.D. (post-doctoral fellow; currently an assistant professor, Montreal Heart Institute Research Centre, University of Montreal)
- Gilbert Moralez, Ph.D. (under-represented minority post-doctoral fellow; currently a post-doctoral fellow in Dr. Crandall's laboratory)
- Hai Ngo, BS (undergraduate student; currently a graduate student in The Netherlands)
- Paula Poh, MS/Ph.D. (graduate student; currently a post-doctoral fellow at the Naval Health Research Center)
- Eric Rivas, MS/Ph.D. (under-represented minority graduate student; soon to be taking an Assistant Professor position at Texas Tech University)
- Steven Romero, Ph.D. (under-represented minority post-doctoral fellow; soon to be taking an Assistant Professor position at the University of North Texas Health Science Center)
- Zachary Schlader, Ph.D. (post-doctoral fellow; currently an assistant professor at the University of Buffalo)

Funding applied for based on work supported by this award
We submitted a grant entitled "Tracking hemorrhagic tolerance under physiological and environmental stressors" that received an outstanding score (1.8). Within weeks of the onset of funding, the money was pulled for reasons I still do not completely understand.

We also submitted a pre-proposal entitled "Impact of environmental and physiological stressors on hemorrhagic tolerance in humans", in association with the following announcement (W81XWH-18-DMRDP-PTCRA). Unfortunately, we were not invited to submit a full proposal.

Patents and licenses applied for and/or issued
None.

5. CONCLUSIONS

The obtained data indicate that passive heat stress (as would be experienced by a gunner on a vehicle, a sniper, or any other condition where a soldier is exposed to hyperthermic environmental conditions and thus is passively heated) does not appreciably alter markers of hemostatic function alone or following a simulated hemorrhagic challenge. These data will be beneficial to those who treat the hemorrhaging soldier in the field by informing them that no modification in hemostasis control is needed when an injured soldier is heat stressed. Data from the second objective strongly suggests that dehydration associated with moderate exercise (as would occur during foot patrols), even when accounting for the magnitude of the increase in internal temperature, will compromise the ability of a soldier to tolerate central hypovolemia as would occur during a hemorrhagic injury. These findings further reinforcement of the importance of hydration as a prophylactic against a possible hemorrhagic insult. With respect to the assessment of compensatory reserve, the obtained findings clearly demonstrate the capacity of the Flashback Technologies device to accurately track

compensatory reserve status during passive heat stress, during exercise heat stress, and during exercise-induced dehydration. 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. Finally, we found that in otherwise normothermic individuals, relatively mild skin surface cooling and heating are not beneficial or detrimental in altering hemorrhagic tolerance. Thus, it is unlikely that mild heating of a normothermic individual who is injured will be detrimental to their hemorrhagic status. However, the effects of mild heating and/or cooling on hemorrhagic tolerance of an otherwise hyperthermic individual (e.g., an individual who is hemorrhaging after exercise-induced hyperthermia) remains unknown.

6. CITED REFERENCES

1. Gagnon D, Schlader ZJ, Adams A, Rivas E, Mulligan J, Grudic GZ, Convertino VA, Howard JT, and Crandall CG. The Effect of Passive Heat Stress and Exercise-Induced Dehydration on the Compensatory Reserve During Simulated Hemorrhage. *Shock* 46: 74-82, 2016.
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7. APPENDICES

PDFs of all manuscripts cited above are included in the appendix.

8. SUPPORTING DATA

None.

SHOCK, Vol. 46, Supplement 1, pp. 74–82, 2016

THE EFFECT OF PASSIVE HEAT STRESS AND EXERCISE-INDUCED DEHYDRATION ON THE COMPENSATORY RESERVE DURING SIMULATED HEMORRHAGE

Daniel Gagnon,[*] Zachary J. Schlader,[†] Amy Adams,[*] Eric Rivas,[‡] Jane Mulligan,[§] Gregory Z. Grudic,[§] Victor A. Convertino,[||] Jeffrey T. Howard,[||] and Craig G. Crandall[*]

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ABSTRACT—Compensatory reserve represents the proportion of physiological responses engaged to compensate for reductions in central blood volume before the onset of decompensation. We hypothesized that compensatory reserve would be reduced by hyperthermia and exercise-induced dehydration, conditions often encountered on the battlefield. Twenty healthy males volunteered for two separate protocols during which they underwent lower-body negative pressure (LBNP) to hemodynamic decompensation (systolic blood pressure <80 mm Hg). During protocol #1, LBNP was performed following a passive increase in core temperature of ~1.2°C (HT) or a normothermic time-control period (NT). During protocol #2, LBNP was performed following exercise during which: fluid losses were replaced (hydrated), fluid intake was restricted and exercise ended at the same increase in core temperature as hydrated (isothermic dehydrated), or fluid intake was restricted and exercise duration was the same as hydrated (time-match dehydrated). Compensatory reserve was estimated with the compensatory reserve index (CRI), a machine-learning algorithm that extracts features from continuous photoplethysmograph signals. Prior to LBNP, CRI was reduced by passive heating [NT: 0.87 (SD 0.09) vs. HT: 0.42 (SD 0.19) units, $P<0.01$] and exercise-induced dehydration [hydrated: 0.67 (SD 0.19) vs. isothermic dehydrated: 0.52 (SD 0.21) vs. time-match dehydrated: 0.47 (SD 0.25) units; $P<0.01$ vs. hydrated]. During subsequent LBNP, CRI decreased further and its rate of change was similar between conditions. CRI values at decompensation did not differ between conditions. These results suggest that passive heating and exercise-induced dehydration limit the body's physiological reserve to compensate for further reductions in central blood volume.

KEYWORDS—Compensatory reserve, dehydration, exercise, heat, hemorrhage

INTRODUCTION

Early detection and intervention are essential for the treatment of hemorrhage—the leading cause of death from trauma in civilian and military settings (1–4). On the battlefield, it is estimated that up to 25% of trauma deaths are potentially survivable with timely and effective intervention, with 85% of these deaths related to hemorrhage (3, 5). Therefore, tools and/or measures that can detect those in greatest need of immediate care have considerable implications for improving the survival of trauma victims.

Heart rate, arterial oxygen saturation, respiration, and blood pressure have long been standard vital signs used to assess the severity of an injury associated with trauma. However, relying upon these variables confounds the early detection of hemorrhage due to physiological compensatory mechanisms that maintain these vital signs to near normal values despite significant blood loss (6, 7). Such compensation may delay treatment until a state of cardiovascular decompensation is reached that culminates in profound hypotension, shock, and potentially death. The integration of all mechanisms that compensate for reductions in central blood volume has been termed compensatory reserve (8). To obtain an estimate of compensatory reserve, the compensatory reserve index was developed using extraction and machine-learning technology (7–10). Compensatory reserve index values are processed from features of the arterial waveform that provide an earlier and more specific marker of reductions in central blood volume compared with traditional vital signs (7, 11–14). For example, receiver operating characteristic (ROC) analysis showed that the compensatory reserve index detects low-volume blood loss following blood donation in human volunteers with greater specificity compared with systolic blood pressure, heart rate, cardiac output, and stroke volume (12). Furthermore, the compensatory reserve index can distinguish individuals with low vs. high tolerance to simulated hemorrhage (9) and tracks acute improvements in tolerance to simulated hemorrhage following intrathoracic pressure therapy (15). Importantly, the compensatory reserve index uses continuous peripheral pulsatile waveforms from a finger pulse oximeter, making it a practical measure in the prehospital setting.

Initial evaluation of the compensatory reserve index as a tool to monitor hemorrhage has necessarily used controlled blood

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draws or simulated hemorrhage. This study expands upon these controlled challenges to include physiological stressors encountered in a battlefield setting. Passive heat stress and exercise-induced dehydration are two conditions often encountered during military activities and in civilian settings (16–18). Both of these conditions reduce human tolerance to reductions in central blood volume (19, 20) and therefore represent ideal scenarios to test the ability of the compensatory reserve index to track compensatory reserve during stressors encountered in the field. The primary purpose of this study was to test the hypothesis that compensatory reserve is reduced by passive heat stress and exercise-induced dehydration, prior to further reductions in central blood volume. A secondary purpose was to test the hypothesis that compensatory reserve decreases further during subsequent reductions in central blood volume and that these conditions do not affect the ability of the compensatory reserve index to predict impending decompensation.

PATIENTS AND METHODS

Subjects

A total of 20 male subjects, recruited from the Dallas/Fort Worth area, volunteered for two separate experimental protocols. For protocol #1, compensatory reserve was evaluated in 12 subjects, their mean (standard deviation) characteristics being: age, 32 (6) y [range: 22–41]; height, 182 (8) cm [range: 140–196]; mass, 83.5 (12.0) kg [range: 69.4–107.6]. For protocol #2, compensatory reserve was evaluated in eight subjects, their characteristics being: age, 35 (6) y [range: 27–44]; height, 184 (12) cm [range: 170–204]; mass, 85.1 (11.1) kg [range: 68.7–99.5]. All subjects were non-smokers, free of any known cardiovascular, respiratory, neurological, or metabolic diseases and not taking any related medications. For both protocols, trials were performed on separate days, at the same time of day within each subject and each trial was separated by a minimum of 8 weeks. Subjects were asked to refrain from strenuous physical activity for 24 h, as well as from caffeine and alcohol for 12 h prior to the experimental visits. The Institutional Review Boards at the University of Texas Southwestern Medical Center and at Texas Health Presbyterian Hospital Dallas approved all procedures and the consent form (STU 122011-011), the latter of which was obtained from all subjects prior to their participation. All studies were performed at the Institute for Exercise and Environmental Medicine in Dallas, TX.

Experimental overview

Compensatory reserve was examined during simulated hemorrhage following whole-body passive heat stress (protocol #1) and exercise in the heat (protocol #2). For protocol #1, a subset of the data investigating tissue oxygen saturation during simulated hemorrhage has been published (21), while a subset of the data investigating the effects of dehydration on tolerance to simulated hemorrhage has been published for protocol #2 (19). Herein, measurements of compensatory reserve obtained as part of these experimental protocols are presented. For both protocols, compensatory reserve was evaluated using the compensatory reserve index during progressive lower body negative pressure (LBNP) to hemodynamic decompensation, a validated model of simulated hemorrhage in humans (22, 23).

Measurements common to both protocols

Core temperature was measured with a telemetric pill (HQ Inc, Palmetto, FL) that was swallowed a minimum of 60 min prior to data collection. Mean skin temperature was measured as the weighted average of six thermocouples attached to the skin surface on the abdomen (14%), calf (11%), chest (22%), lower back (19%), thigh (13%), and upper back (21%). Body mass measurements were obtained with a scale (Health o meter Professional Scales, McCook, IL) accurate to 0.1 kg. Changes in body mass during the protocols were corrected for fluid intake and urine loss. Heart rate was obtained from an electrocardiogram (GE Healthcare, Milwaukee, WI). Continuous blood pressure measurements were obtained noninvasively using photoplethysmography (Finometer Pro, FMS, Amsterdam, The Netherlands). Continuous photoplethysmograph waveforms were recorded from a finger pulse oximeter (Nonin Medical Inc, Plymouth, MN). The CipherOx CRI system (V2.0.1, Flashback Technologies Inc, Boulder, CO) was used to estimate compensatory reserve index values from the recorded photoplethysmograph signals. This novel approach exploits properties of the continuous pulsatile waveforms to estimate the patient's remaining reserve to cardiovascular decompensation. Compensatory reserve index values range between 0 and 1, where 0 represents imminent cardiovascular instability/decompensation and 1 represents maximal capacity for physiological mechanisms to compensate for reductions in central blood volume (7, 9, 10).

Experimental protocol #1

Subjects visited the laboratory on two occasions. Upon arrival, subjects swallowed the telemetric pill before providing a urine sample and weighing themselves nude. Dressed in shorts, subjects were then instrumented for the measurement of heart rate, blood pressure, and mean skin temperature before donning a two-piece tube-lined suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body except for the head, hands, feet, and one forearm. While supine, the subjects were sealed at the waist within a custom made LBNP chamber. A pulse oximeter was placed on one of the subjects' fingers and water maintained at 34°C was circulated through the suit for a baseline period that lasted a minimum of 45 min. After baseline data collection, the subjects underwent either whole-body passive heat stress (''hyperthermic'' condition, HT) or remained normothermic for a time-control period (''normothermic'' condition, NT). All subjects performed both conditions, the order of which was randomized: five subjects performed the NT trial first, seven performed the HT trial first. Whole-body passive heat stress was achieved by circulating 49°C water through the suit until core temperature increased by ~1.2°C, whereas water temperature remained at 34°C during the time-control period which lasted 40 to 60 min. Upon achieving the desired increase in core temperature, or following the time-control period, incremental LBNP to hemodynamic decompensation was performed. The LBNP protocol began at 20 mm Hg, with a 10 mm Hg increase in LBNP every 3 min until decompensation. Criteria for determining decompensation included: continued self-reporting by the subject of feeling faint, sustained nausea, rapid and progressive decrease in blood pressure resulting in a sustained systolic blood pressure <80 mm Hg, and/or relative bradycardia accompanied by a narrowing of pulse pressure.

Experimental protocol #2

Subjects visited the laboratory on four occasions. The first visit consisted of a preliminary session to determine maximum oxygen uptake (VO_2max) using a protocol previously described for our laboratory (24). The remaining visits consisted of the experimental trials. Upon arrival, subjects swallowed the telemetric pill before providing a urine sample and weighing themselves nude. Dressed in shorts and running shoes, subjects were instrumented for the measurement of heart rate, blood pressure, and mean skin temperature before lying in the supine position for a baseline period that lasted a minimum of 30 min at a room temperature of ~24°C. A baseline blood sample was drawn at the end of the baseline period and subjects were subsequently transferred to a climate controlled chamber regulated at a temperature of 41°C and a relative humidity of 25%. Approximately 10 min after entering the chamber, subjects began treadmill exercise with the speed and inclination adjusted to elicit a metabolic heat production equivalent to sustained moderate to heavy military activities (~545 W), such as a dismounted foot patrol (25). Oxygen consumption during exercise averaged ~1.8 L/min [~45% of VO_2max: 4.04 (1.07) L/min, range: 2.59–6.20] whereas metabolic heat production averaged ~540 W across the three conditions. To promote sweat evaporation, a fan was placed in front of the subjects that provided an air velocity of ~5 m/s. Every 15 min during exercise, changes in body mass were measured upon subjects momentarily stepping off the treadmill and drying the skin surface with a towel. Baseline and end-exercise blood pressure measurements were obtained by automated auscultation of the brachial artery (Tango+; SunTech Medical, Morrisville, NC).

The three experimental visits differed in the level of dehydration that was achieved during exercise. During the first visit, subjects exercised for 90 min and fluid loss was replaced by having subjects drink warm (38°C) water administered in aliquots based upon the body mass measurements taken every 15 min (''hydrated'' condition). During the second visit, subjects did not consume any fluid and exercise continued until core temperature increased to the same level as that observed during the hydrated condition (''isothermic dehydrated'' condition). During the third visit, subjects did not consume any fluid and exercised for 90 min (''time-match dehydrated'' condition). Immediately after exercise, subjects remained in the chamber maintained at 41°C and 25% relative humidity and were positioned within a custom-made LBNP chamber. A pulse oximeter was placed on the subject's finger to measure compensatory reserve. Once the transition from the treadmill to the LBNP chamber was complete, a blood sample was drawn following which incremental

Table 1. **Hemodynamics and body temperatures during simulated hemorrhage performed to hemodynamic decompensation following whole-body passive heat stress or a normothermic time-control period (protocol #1)**

	Baseline		Pre-LBNP		Decompensation	
	NT	HT	NT	HT	NT	HT
USG	1.009 (0.007)	1.012 (0.008)	–		–	
Heart rate (beats/min)	57 (8)	59 (7)	60 (10)	101 (16)*	101 (31)	130 (32)*
MAP (mm Hg)	82 (9)	82 (7)	88 (8)	77 (6)*	54 (12)	50 (10)
Tcore (°C)	37.0 (0.2)	36.9 (0.2)	37.1 (0.3)	38.2 (0.2)*	37.3 (0.2)	38.5 (0.2)*
Mean Tsk (°C)	34.0 (0.5)	34.1 (0.4)	34.3 (0.4)	39.0 (1.0)*	34.3 (0.3)	38.8 (1.3)*

Values are mean (standard deviation).
*Significantly different from normothermic ($P \leq 0.05$).
HT indicates hyperthermic; LBNP, lower body negative pressure; MAP, mean arterial pressure; Mean Tsk, mean skin temperature; NT, normothermic; Tcore, core temperature; USG, urine specific gravity.

LBNP to decompensation was performed as described for protocol #1. A final blood sample was drawn immediately after the termination of the LBNP protocol. The venous blood samples were subsequently analyzed for hemoglobin, hematocrit (both via fluorescent flow cytometry), and plasma osmolality (via osmometry). The study was originally designed to compare the dehydrated and isothermic dehydrated conditions. All subjects therefore performed the dehydrated trial first to determine the target increase in core temperature for the isothermic dehydrated trial. However, this approach resulted in a substantially shorter exercise duration during the isothermic dehydrated trial (see the Results section). The time-match dehydrated trial was added *post-hoc* to account for differences in exercise time. The order in which the conditions were performed was therefore not randomized.

Data analysis

Data were collected with data acquisition hardware and software (Biopac Systems Inc, Santa Barbara, CA) at a minimum sampling frequency of 50 Hz. Data were analyzed as a one-group, within-subjects repeated measures design. A 1-min average of the data at each LBNP level was used for analyses. Tolerance to simulated hemorrhage was quantified using the cumulative stress index, calculated by summing the product of LBNP level and the time at each level (e.g. 20 mm Hg × 3 min + 30 mm Hg × 3 min + 40 mm Hg × 1 min = 190 mm Hg × min). For protocol #2, relative changes from baseline in plasma volume were calculated from hemoglobin and hematocrit values (26). Investigators were not blinded to the conditions when performing data analyses.

Statistical analyses

Within each experimental protocol, dependent variables were analyzed using generalized estimating equations (GEE) with compound symmetry covariance structures for longitudinal correlated data analysis of continuous variables (compensatory reserve index, heart rate, and mean arterial pressure) and the dichotomous outcome of decompensation with the repeated factors of LBNP level for each experimental condition (27–29). ROC analysis was conducted by performing GEE repeated measures logistic regression on the dichotomous outcome of decompensation, which was measured at each increment of LBNP (14, 27, 30). The ROC area under the curve (ROC AUC) with 95% confidence intervals was calculated to assess the ability of the compensatory reserve index to predict decompensation under each experimental condition. Within each protocol, tolerance to simulated hemorrhage was analyzed using a Kaplan–Meier curve by plotting the cumulative stress index that was tolerated by each subject. The Kaplan–Meier curves were statistically compared between conditions with a log-rank Mantel–Cox test. For all analyses, the level of significance was set at an alpha of $P \leq 0.05$, with the exception of GEE analysis of longitudinal data across each LBNP level, for which P values were adjusted for multiple comparisons by dividing 0.05 by the number of tests resulting in alpha being set at $P \leq 0.005$ for the normothermic condition, $P \leq 0.007$ for the hyperthermic condition, $P \leq 0.006$ for the hydrated condition, $P \leq 0.007$ for the isothermic dehydration condition, and $P \leq 0.008$ for the time-match dehydration condition. Descriptive statistical analyses were performed using commercially available statistical software (Prism 6, Graphpad Software Inc, La Jolla, CA). GEE procedures for longitudinal correlated data were performed using SAS, version 9.4 (Cary, NC). All variables are reported as mean (standard deviation) unless otherwise indicated.

RESULTS

Experimental protocol #1

Baseline measures were similar between conditions (all $P > 0.10$, Table 1). Whole-body passive heat stress increased mean skin and core temperatures as well as heart rate, whereas it decreased mean arterial pressure and compensatory reserve index relative to the normothermic time-control period (all $P \leq 0.03$). Tolerance to simulated hemorrhage was reduced by heat stress [NT: 920 (718) vs. HT: 254 (177) mm Hg × min, $P < 0.01$], such that the tolerance curve was significantly shifted to the left by heat stress ($P < 0.01$, Fig. 1). Mean arterial pressure did not consistently change during progressive LBNP under the normothermic condition, but did so under the

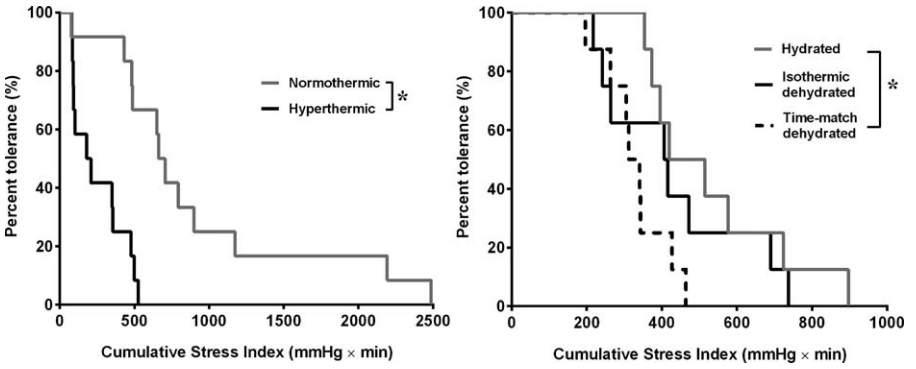

Fig. 1. **Kaplan–Meier curves plotting the percent of individuals who tolerated a given cumulative stress index during simulated hemorrhage.** The left panel presents data during the hyperthermic and normothermic conditions of protocol #1. The right panel presents data during the hydrated, isothermic dehydrated, and time-match dehydrated conditions of protocol #2. Significant ($P \leq 0.05$) difference between the normothermic and hyperthermic curves (left panel) and between the hydrated and time-match dehydrated curves (right panel) based on Log-Rank (Mantel–Cox) test.

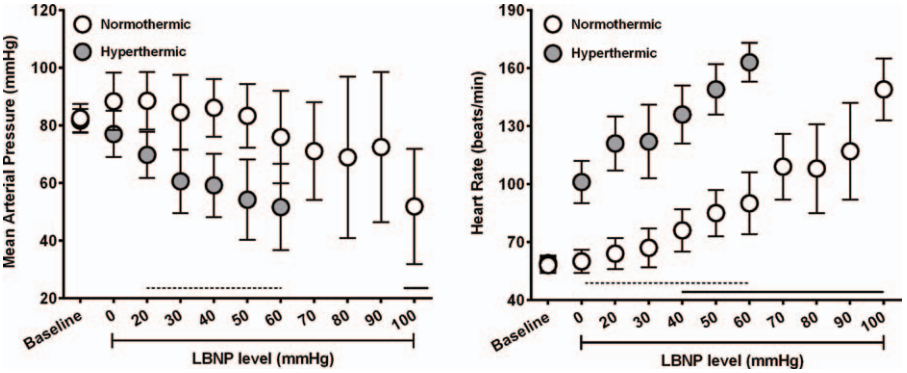

FIG. 2. **Mean arterial pressure (left panel) and heart rate (right panel) during progressive lower body negative pressure (LBNP) to hemodynamic decompensation performed following whole-body passive heat stress (hyperthermic) or a normothermic time-control period (protocol #1).** The data are presented as mean ± 95% confidence intervals and were modeled using generalized estimating equations to account for differences in LBNP level at decompensation. Solid line, significantly different from baseline for normothermic. Dashed line, significantly different from baseline for hyperthermic.

hyperthermic condition (Fig. 2). In contrast, progressive LBNP to decompensation resulted in consistent, time-dependent increases in heart rate under both conditions (Fig. 2). At the point of decompensation (Table 1), heart rate was greater during the hyperthermic condition ($P < 0.01$) whereas mean arterial pressure was similar between conditions ($P = 0.94$). The compensatory reserve index decreased across levels of LBNP during both conditions, although values were lower during the hyperthermic condition (Fig. 3). However, compensatory reserve index was similar between conditions at decompensation [NT: 0.18 (0.16) vs. HT: 0.13 (0.08), $P = 0.33$). Analysis of ROC curves (Fig. 4) and ROC AUC values during normothermic (0.90, 95% CI: 0.82–0.99) and hyperthermic (ROC AUC: 0.78, 95% CI: 0.66–0.90) conditions demonstrate the ability of the compensatory reserve index to predict impending decompensation regardless of thermal status. Although the mean ROC AUC values during the hyperthermic condition were lower relative to the normothermic condition, they were not statistically different between conditions due to the overlapping confidence intervals. The change in CRI as a function of absolute LBNP time did not differ between NT [−0.042 (0.010) units/min] and HT [−0.049 (0.042) units/min, $P = 0.52$]. Individual compensatory reserve index tracings for each condition are presented in Figure 5.

Experimental protocol #2

Baseline measures were similar between conditions (all $P > 0.10$, Table 2). During exercise in the hydrated condition, all subjects exercised for 90 min and fluid replacement maintained body mass ($P = 0.69$, Table 2). Relative to the hydrated condition, exercise duration was shorter and reductions in body mass greater during the isothermic dehydrated condition (both $P \leq 0.01$, Table 2). By design, end-exercise core temperature did not differ between conditions ($P = 0.90$). During the time-match dehydrated condition, all subjects exercised for 90 min. Relative to the hydrated and isothermic dehydrated conditions, body mass loss and core temperature were greater at the end of exercise for the time-match dehydrated trial (both $P \leq 0.01$, Table 2). Following exercise, plasma osmolality was different between conditions ($P \leq 0.01$), being greater during the isothermic and time-match dehydrated conditions relative to hydrated (Table 2). In contrast, changes in plasma volume were not statistically different between conditions ($P = 0.07$, Table 2).

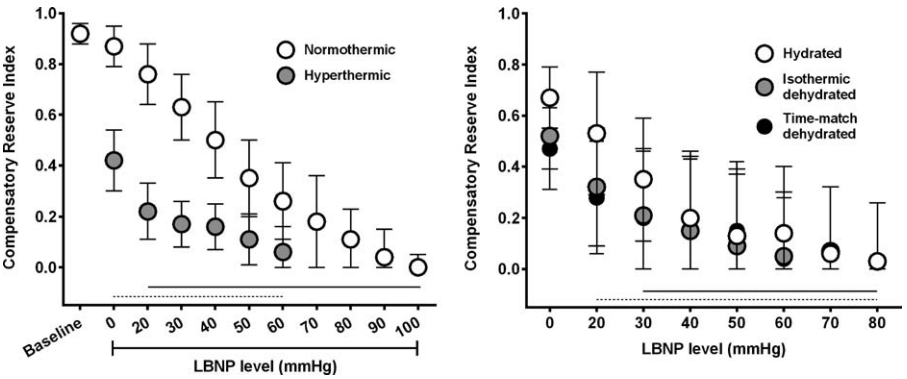

FIG. 3. **The compensatory reserve index during progressive lower body negative pressure (LBNP) to hemodynamic decompensation.** The left panel presents data during the hyperthermic and normothermic conditions of protocol #1. The right panel presents data during the hydrated, isothermic dehydrated, and time-match dehydrated conditions of protocol #2. The data are presented as mean ± 95% confidence intervals and were modeled using generalized estimating equations to account for differences in LBNP level at decompensation. Solid line, significantly different from baseline for normothermic and hydrated. Dashed line, significantly different from baseline for hyperthermic and isothermic/time-match dehydrated.

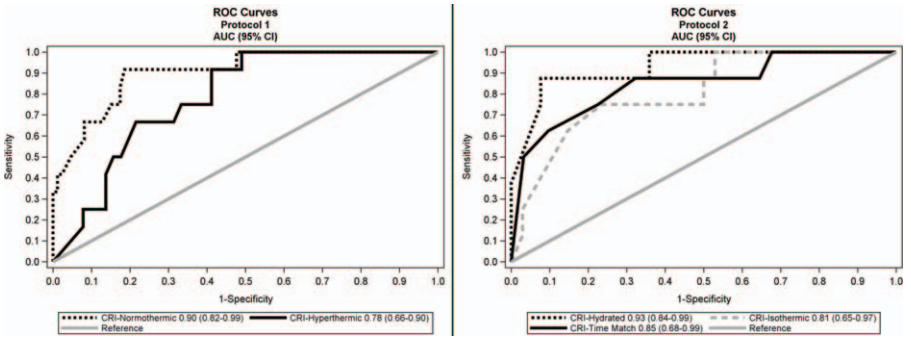

FIG. 4. **Receiver operating characteristic (ROC) curves with area under the curve (AUC) values and 95% confidence intervals for the compensatory reserve index during the normothermic and hyperthermic conditions of protocol #1 (left panel); and during the hydrated, isothermic dehydrated and time-match dehydrated conditions of protocol #2 (right panel).**

The transition from end-exercise to the start of LBNP averaged 19 (3) min and did not differ between conditions ($P = 0.67$). Prior to LBNP, no differences in mean arterial pressure were observed between conditions (Table 2). In contrast, heart rate was greater during the time-match dehydrated condition relative to the hydrated condition ($P \leq 0.01$, Table 2). Furthermore, compensatory reserve index was lower in both dehydrated conditions relative to the hydrated condition (both $P \leq 0.01$). Tolerance to simulated hemorrhage was reduced by dehydration, although differences did not reach statistical significance [hydrated: 532 (193) vs. isothermic dehydrated: 430 (197) vs. time-match dehydrated: 331 (85) mmHg × min, $P = 0.08$]. Nonetheless, the Kaplan–Meier curves were statistically different when comparing all three conditions ($P = 0.04$, Fig. 1). When analyzed separately, a statistical difference was only observed between the hydrated and time-match dehydrated conditions ($P < 0.01$). The tolerance curves for hydrated versus isothermic dehydrated ($P = 0.42$) and isothermic dehydrated versus time-match dehydrated ($P = 0.18$) were not statistically different.

During progressive LBNP, mean arterial pressure remained relatively unchanged until the later stages of LBNP (Fig. 6). In contrast, consistent increases in heart rate (Fig. 6) and decreases in compensatory reserve index (Fig. 3) were observed under all conditions. At decompensation, mean arterial pressure and heart rate (Table 2) as well as compensatory reserve index were similar between conditions [hydrated: 0.10 (0.06) vs. isothermic: 0.11 (0.06) vs. time-match: 0.10 (0.07), $P = 0.98$). Analysis of ROC curves (Fig. 4) and ROC AUC values during hydrated (0.93, 95% CI: 0.84–0.99), isothermic

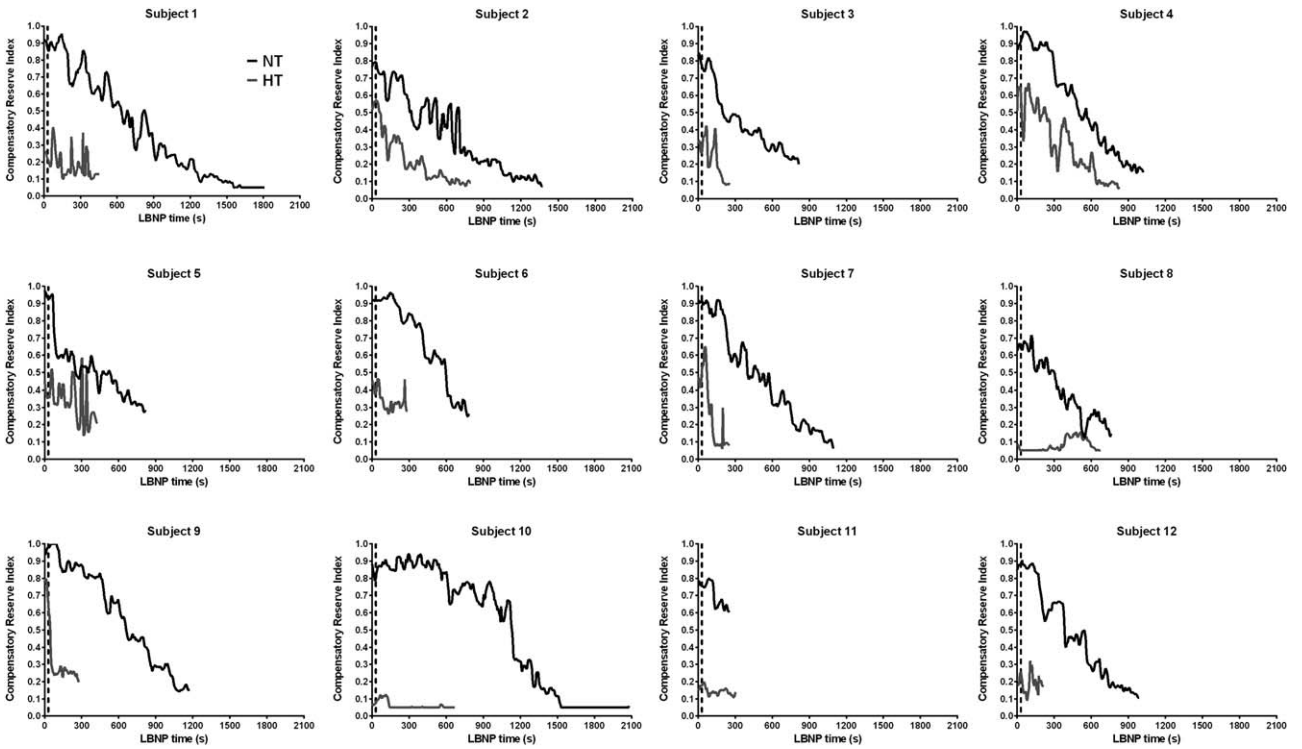

FIG. 5. **Individual compensatory reserve index tracings during progressive lower body negative pressure (LBNP) to hemodynamic decompensation performed following whole-body passive heat stress (HT) and a normothermic (NT) time-control period (protocol #1).** The dashed line indicates the start of LBNP.

TABLE 2. **Measured variables during simulated hemorrhage performed to hemodynamic decompensation following dynamic exercise with and without fluid replacement (protocol #2)**

	Baseline			End-exercise			Pre-LBNP			Decompensation		
	Hydrated	Isothermic	Time-match	Hydrated	Isothermic	Time-match	Hydrated	Isothermic	Time-match	Hydrated	Isothermic	Time-match
USG	1.011 (0.006)	1.013 (0.011)	1.011 (0.006)		—			—			—	
Heart rate (beats/min)	88 (15)	84 (14)	89 (11)	125 (14)	134 (13)	143 (21)	85 (11)	92 (11)	96 (14)	107 (27)	115 (24)	121 (26)
MAP (mm Hg)	86 (5)	83 (5)	86 (2)	86 (9)	85 (7)	87 (5)	74 (8)	78 (7)	77 (5)	42 (15)	47 (9)	42 (7)
Tcore (°C)	37.0 (0.2)	37.0 (0.2)	36.9 (0.2)	38.2 (0.1)	38.2 (0.3)	38.6 (0.5)*,†	38.1 (0.4)	38.0 (0.2)	38.5 (0.5)*,†	38.0 (0.3)	38.2 (0.2)	38.5 (0.4)*,†
Mean Tsk (°C)	35.4 (0.4)	35.4 (0.7)	35.7 (0.6)	34.5 (0.8)	35.0 (0.6)	34.7 (0.8)	35.9 (0.5)	36.6 (0.4)	36.5 (0.4)	35.7 (0.5)	36.3 (0.4)	36.0 (0.5)
Plasma osmolality (mosm/kg)	289 (7)	288 (2)	288 (2)		—		285 (3)	294 (2)*	295 (3)*	288 (3)	294 (4)*	295 (4)*
Δ Plasma volume (%)		—			—		−7.2 (2.4)	−7.6 (3.8)	−11.4 (2.4)	−12.2 (4.2)	−12.8 (5.0)	−16.1 (3.0)
Δ Mass (kg)	—	—	—	−0.1 (0.2)	−0.9 (0.5)*	−1.8 (0.6)*,†	—	—	—	—	—	—
Ex. time (min)	—	—	—	90 (0)	55 (20)*,‡	90 (0)	—	—	—	—	—	—

Values are mean (standard deviation).
*Significantly different from Hydrated ($P \leq 0.05$).
†Significantly different from Isothermic ($P \leq 0.05$).
‡Significantly different from Time-match ($P \leq 0.05$).
ΔMass indicates change in body mass from pre to end-exercise; Ex. time, exercise time; Hydrated, exercise with fluid replacement; Isothermic, exercise without fluid replacement performed until the same increase in core temperature as Hydrated; LBNP, lower body negative pressure; MAP, mean arterial pressure; Mean Tsk, mean skin temperature; Tcore, core temperature; Time-match, exercise without fluid replacement performed for the same duration as Hydrated; USG, urine specific gravity.

dehydrated (0.81, 95% CI: 0.65–0.97), and time-match dehydrated (0.85, 95% CI: 0.68–0.99) conditions demonstrate the ability of the compensatory reserve index to predict impending decompensation regardless of hydration status. Although the mean ROC AUC values during both dehydrated conditions were lower relative to the hydrated condition, these differences were not statistically different due to the overlapping confidence intervals. The change in compensatory reserve index as a function of absolute LBNP time did not differ between conditions [hydrated: −0.043 (0.019) vs. isothermic dehydrated: −0.036 (0.021) vs. time-match dehydrated: −0.038 (0.027) units/min, $P = 0.43$]. Individual compensatory reserve index tracings are presented in Figure 7.

DISCUSSION

The current study examined whether the compensatory reserve index tracks reductions in tolerance to simulated hemorrhage during stressors encountered on the battlefield. The main findings show that passive heat stress and exercise-induced dehydration reduced compensatory reserve prior to the onset of simulated hemorrhage. Furthermore, compensatory reserve index values decreased progressively during simulated hemorrhage and were a strong predictor of impending hemodynamic decompensation based on ROC analysis. These data suggest that the compensatory reserve index appropriately tracks reductions in the physiological reserve to compensate for simulated hemorrhage during whole-body passive heat stress and following exercise-induced dehydration.

The compensatory reserve index has recently been introduced as an innovative clinical tool to continuously and noninvasively monitor the physiological reserve to tolerate central blood volume loss (7–10). Importantly, the compensatory reserve index provides an earlier and more specific indicator of such changes compared with traditional vital signs (9, 11–13). Furthermore, it can be incorporated into standard monitors that generate an arterial waveform, such as a finger pulse oximeter, making it an easy measure to integrate in the prehospital setting. Prior to the current study, however, it remained unknown if the compensatory reserve index appropriately tracks reductions in the physiological reserve to compensate for central blood volume loss during conditions often encountered by individuals at relatively greater risk of hemorrhagic injury (16–18), such as military personnel, firefighters, miners, etc. We therefore examined the effect of two conditions that reduce tolerance to simulated hemorrhage and that are often encountered in a field setting; whole-body passive heat stress and exercise-induced dehydration (19, 20). Both conditions reduced compensatory reserve prior to the onset of simulated hemorrhage (Fig. 3). A reduction in compensatory reserve implies that individuals are closer to the point of hemodynamic decompensation. In other words, a lower compensatory reserve index value indicates that less compensatory reserve is available to tolerate further reductions in central blood volume. The lower compensatory reserve index values prior to simulated hemorrhage during passive heat stress and following exercise-induced dehydration suggest that these conditions already engage physiological responses to compensate for the reduction

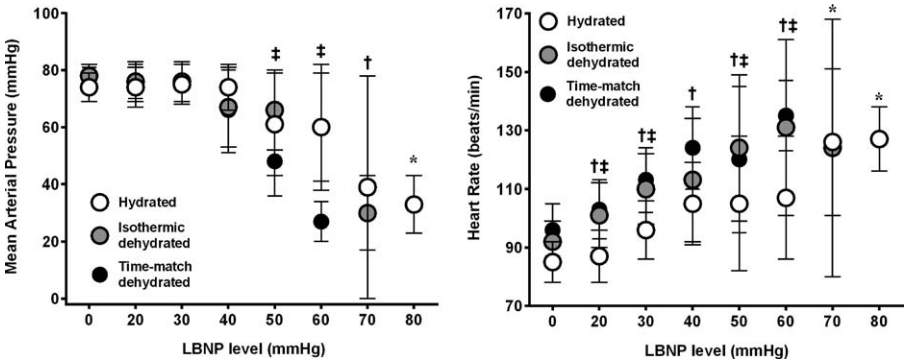

Fig. 6. **Mean arterial pressure (left panel) and heart rate (right panel) during progressive lower body negative pressure (LBNP) to hemodynamic decompensation during the hydrated, isothermic dehydrated, and time-match dehydrated conditions of protocol #2.** The data are presented as mean ± 95% confidence intervals and were modeled using generalized estimating equations to account for differences in LBNP level at decompensation. Significantly different from baseline for hydrated (∗), isothermic dehydrated (†), and time-match dehydrated (‡).

in central blood volume they elicit (31, 32). It is important to note that, on average, compensatory reserve further decreased with subsequent simulated hemorrhage during both conditions. Our observation that compensatory reserve index values at the time of hemodynamic decompensation were similar regardless of condition supports the notion that the onset of hemodynamic decompensation is dictated by the depletion of compensatory reserve. Furthermore, the change in compensatory reserve index as a function of absolute LBNP time was not affected by the experimental conditions tested. Overall, these results suggest that maximal physiological responses to compensate for central blood volume loss are finite, and that reduced tolerance to simulated hemorrhage following passive heat stress and exercise-induced dehydration are due to less reserve available for physiological responses to compensate for further central blood volume loss.

Results from ROC analyses indicate that the compensatory reserve index is a good predictor of impending hemodynamic decompensation, regardless of thermal or hydration status (Fig. 4). Importantly, ROC AUC values during the hyperthermic and dehydrated conditions were not significantly different from those observed during the normothermic and hydrated conditions. These findings suggest that passive heat stress and exercise-induced dehydration do not affect the ability of the compensatory reserve index to predict impending hemodynamic decompensation during simulated hemorrhage. Prior to simulated hemorrhage, compensatory reserve index decreased in every subject during whole-body passive heat stress (Fig. 5). Furthermore, most subjects (10/12) displayed a further reduction in compensatory reserve index during simulated hemorrhage while heat stressed. The lack of further change in compensatory reserve index in 2 of the subjects (#10 and 11) could be related to these subjects having engaged all of their physiological reserve prior to simulated hemorrhage to compensate for the cardiovascular adjustments associated with heat stress. This reinforces the notion that passive heat stress reduces the reserve for physiological compensation during further reductions in central blood volume, therefore resulting in dramatically reduced tolerance time (Fig. 1). In contrast, not all subjects displayed a reduced compensatory reserve index following exercise-induced dehydration (Fig. 7). Interestingly, when compensatory reserve index was not

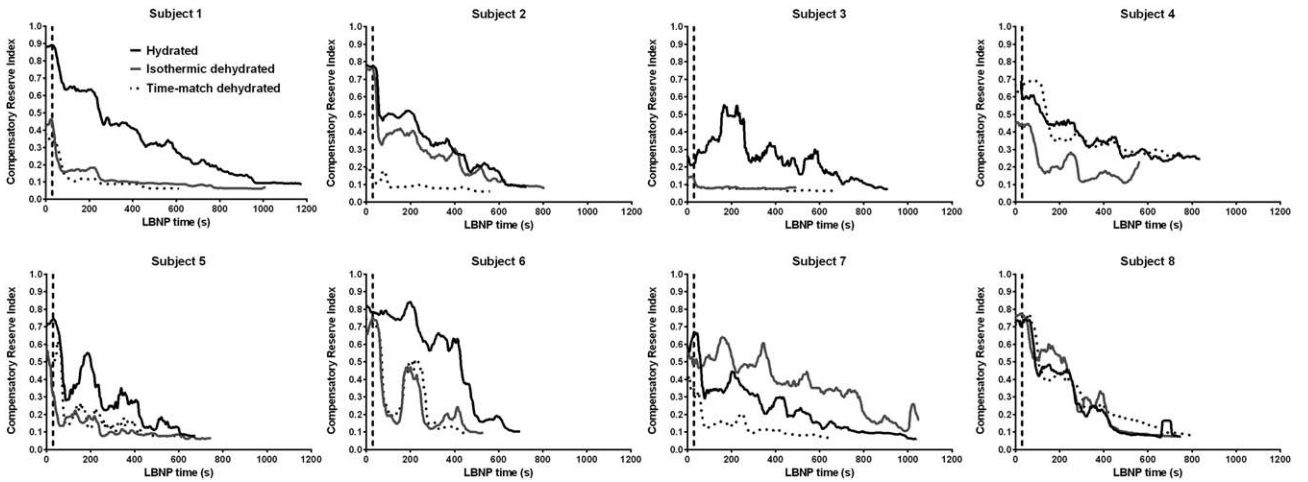

Fig. 7. **Individual compensatory reserve index tracings during progressive lower body negative pressure (LBNP) to hemodynamic decompensation performed following exercise (protocol #2) during which: fluid losses were replaced (hydrated), fluid losses were not replaced and exercise lasted until the same increase in core temperature as hydrated (isothermic dehydrated), and fluid losses were not replaced and exercise lasted the same duration as hydrated (time-match dehydrated).** The dashed line indicates the start of LBNP.

affected by dehydration (isothermic or time-match), tolerance time to simulated hemorrhage was similar to that observed during the hydrated condition (e.g. subjects #2, 4, 8 of Fig. 7). These results highlight interindividual variability in compensatory responses to reductions in central blood volume that were recognized by the compensatory reserve index.

The mechanisms by which heat stress, with or without dehydration, reduces tolerance to simulated hemorrhage are multifactorial (33). The reduction in compensatory reserve with passive heating and exercise-induced dehydration demonstrates the ability of the compensatory reserve index to capture physiological changes that occur with these conditions. The precise factor(s) responsible for reduced compensatory reserve cannot be identified because of individual variations in the integration of physiological mechanisms that compensate for central blood volume loss (34). However, it follows that passive heat stress and exercise-induced dehydration must affect properties of the photoplethysmograph waveforms from which compensatory reserve index is derived (7, 9, 10).

Perspectives

The current results suggest that measurements of compensatory reserve appropriately track reductions in tolerance to simulated hemorrhage following whole-body passive heat stress and exercise-induced dehydration. Previous studies established the compensatory reserve index as an early and specific indicator of reductions in central blood volume under well-controlled environmental and physiological conditions (i.e., at rest, normothermic and hydrated). The current findings therefore extend previous studies to conditions often encountered in the field by individuals who are at relatively greater risk of hemorrhagic injury. The present findings are important if the compensatory reserve index is to be used as a clinical triage tool in the prehospital setting. Furthermore, compensatory reserve index values were obtained from photoplethysmograph signals making the results applicable to a field setting, where enhanced finger pulse oximeters—which are part of first responder medical kits—could be used to obtain compensatory reserve index measurements.

Considerations

It should be considered that hemorrhage was simulated in the current study with the use of progressive LBNP to hemodynamic decompensation. Although this represents a valid model of hemorrhage in humans under normothermic/hydrated conditions (22, 23), it remains unknown if progressive LBNP is an equally valid model of hemorrhage in heat-stressed/dehydrated humans. It should also be considered that the current results are specific to the population (young healthy males) and conditions employed. Analyses were executed within a one-group, within-subject experimental design. The relatively small size and potential for increased variability of compensatory reserve index values due to the physiological stressors examined may have led to underestimates of the true predictive ability of the compensatory reserve index (as measured by ROC AUC). It therefore remains to be determined if the compensatory reserve index adequately tracks changes in the physiological capacity to tolerate central blood volume loss in larger populations inclusive of other demographic groups (females, the elderly, etc.), as well as during other conditions often encountered in a field setting (e.g. altitude/hypoxia, cold stress, energy deprivation, nicotine/caffeine use, etc.). Finally, the current results suggest that the compensatory reserve index adequately tracks reductions in tolerance to simulated hemorrhage following passive heat stress and exercise-induced dehydration. It therefore remains to be determined if countermeasures that improve tolerance to simulated hemorrhage under these conditions (e.g. volume loading, skin-surface cooling, etc.) are paralleled by an improvement in compensatory reserve.

CONCLUSION

In conclusion, we examined the effect of passive heat stress and exercise-induced dehydration on the physiological reserve to compensate for reduced central blood volume, as estimated by the compensatory reserve index. The results show that passive heat stress and exercise-induced dehydration reduce compensatory reserve. However, these conditions do not affect the ability of the compensatory reserve to predict impending hemodynamic decompensation during subsequent simulated hemorrhage. These observations suggest that reduced tolerance to simulated hemorrhage under these conditions is due to less initial reserve to compensate for further central blood volume loss. The results also suggest that the compensatory reserve index appropriately tracks reductions in tolerance to simulated hemorrhage during conditions often encountered in the field by individuals at relatively greater risk of hemorrhagic injury.

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RESEARCH ARTICLE | *Cardiovascular and Renal Integration*

Plasma hyperosmolality improves tolerance to combined heat stress and central hypovolemia in humans

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[1]*Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas and University of Texas Southwestern Medical Center, Dallas, Texas;* [2]*Cardiovascular Prevention and Rehabilitation Centre, Montreal Heart Institute, Montréal, Québec, Canada; and* [3]*Département de pharmacologie et physiologie, Faculté de médecine, Université de Montréal, Montréal, Québec, Canada*

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Gagnon D, Romero SA, Ngo H, Poh PY, Crandall CG. Plasma hyperosmolality improves tolerance to combined heat stress and central hypovolemia in humans. *Am J Physiol Regul Integr Comp Physiol* 312: R273–R280, 2017. First published December 21, 2016; doi:10.1152/ajpregu.00382.2016.—Heat stress profoundly impairs tolerance to central hypovolemia in humans via a number of mechanisms including heat-induced hypovolemia. However, heat stress also elevates plasma osmolality; the effects of which on tolerance to central hypovolemia remain unknown. This study examined the effect of plasma hyperosmolality on tolerance to central hypovolemia in heat-stressed humans. With the use of a counterbalanced and crossover design, 12 subjects (1 female) received intravenous infusion of either 0.9% iso-osmotic (ISO) or 3.0% hyperosmotic (HYPER) saline. Subjects were subsequently heated until core temperature increased ~1.4°C, after which all subjects underwent progressive lower-body negative pressure (LBNP) to presyncope. Plasma hyperosmolality improved LBNP tolerance (ISO: 288 ± 193 vs. HYPER: 382 ± 145 mmHg × min, $P = 0.04$). However, no differences in mean arterial pressure ($P = 0.10$), heart rate ($P = 0.09$), or muscle sympathetic nerve activity ($P = 0.60$, $n = 6$) were observed between conditions. When individual data were assessed, LBNP tolerance improved ≥25% in eight subjects but remained unchanged in the remaining four subjects. In subjects who exhibited improved LBNP tolerance, plasma hyperosmolality resulted in elevated mean arterial pressure (ISO: 62 ± 10 vs. HYPER: 72 ± 9 mmHg, $P < 0.01$) and a greater increase in heart rate (ISO: +12 ± 24 vs. HYPER: +23 ± 17 beats/min, $P = 0.05$) before presyncope. No differences in these variables were observed between conditions in subjects that did not improve LBNP tolerance (all $P \geq 0.55$). These results suggest that plasma hyperosmolality improves tolerance to central hypovolemia during heat stress in most, but not all, individuals.

blood pressure; heart rate; lower-body negative pressure; muscle sympathetic nerve activity

IT IS WELL ESTABLISHED that heat stress profoundly impairs tolerance to central hypovolemia in humans. Although the contributing mechanisms are multifactorial (29), heat-induced hypovolemia is a primary contributing factor (18, 22). However, in addition to reducing blood volume, heat stress is typically accompanied by increased plasma osmolality (1, 17). This raises the question as to whether hyperosmolality has an effect upon tolerance to central hypovolemia during heat stress.

In humans, rapid infusion of hyperosmotic/oncotic solutions (~7% NaCl) is beneficial for the primary resuscitation from severe hypovolemia and hypovolemic shock (20). Moderate salt loading with sodium chloride tablets also improves orthostatic tolerance in individuals with recurrent syncope (4, 13). These beneficial effects are primarily attributed to osmotically mediated fluid shifts into the vascular space. However, a number of observations suggest that hyperosmolality may improve neural and cardiovascular responses to hypovolemia, independently of fluid shifts. In animal models, hyperosmolality causes sympathoexcitation through osmosensitive neurons located in the forebrain circumventricular organs (38, 39). Four weeks of dietary salt loading results in greater vasoconstriction of isolated rat mesenteric resistance arteries to given doses of norepinephrine (33). Finally, hyperosmolality improves cardiac function during resuscitation following hemorrhage in sheep, by stimulating cardiac sympathetic nerve activity (15). In humans, improved orthostatic tolerance with oral salt loading was accompanied by greater forearm vasoconstriction in individuals with recurrent syncope (4). Plasma hyperosmolality also increases resting muscle sympathetic nerve activity (MSNA) and improves the baroreflex control of MSNA (3, 14, 41). These findings are particularly relevant to the present investigation, as the extent to which MSNA increases is an important determinant of tolerance to central hypovolemia in normothermic (5, 32) and hyperthermic (7) conditions.

Overall, current evidence suggests that hyperosmolality stimulates physiological responses that could improve tolerance to central hypovolemia. Hyperosmolality accompanying heat stress may therefore attenuate the detrimental effect of heat-induced hypovolemia during further reductions in central blood volume. The purpose of this study was to examine the effect of plasma hyperosmolality on tolerance to central hypovolemia during heat stress. It was hypothesized that plasma hyperosmolality would improve tolerance to central hypovolemia of heat-stressed humans relative to an iso-osmotic state.

METHODS

Experimental overview. The data presented in this study were collected as part of an experimental protocol designed to examine a separate question (the effect of plasma hyperosmolality on skin sympathetic nerve activity during heat stress), the results of which have been published previously (16). As part of the protocol, skin

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sympathetic nerve activity was examined during passive heating until a core temperature increase of ~0.6°C. For the current study, heating was continued until an increase in core temperature of ~1.4°C, at which time tolerance to central hypovolemia was evaluated by having subjects undergo lower-body body negative pressure (LBNP) to presyncope.

Subjects. Data were collected on 12 healthy subjects (1 female). None of the subjects reported using tobacco products and were free of known cardiovascular, respiratory, neurological, or metabolic diseases, and none were taking medications. Means ± SD of the subject characteristics are the following: age, 31 ± 8 yr (range: 21–47); height, 174 ± 9 cm (range: 154–190); weight, 74.7 ± 14.2 kg (range: 59.2–101.5). The female subject participated between the first and tenth day of her self-reported menses. Subjects were asked to refrain from strenuous physical activity for 24 h, as well as from caffeine and alcohol for 12 h before the experimental visits. They were also asked to eat a light meal and drink water before arriving to the laboratory. The study and informed consent were approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and at Texas Health Presbyterian Hospital Dallas. Written informed consent was obtained from all subjects before their participation.

Measurements. Subjects were dressed in a two-piece, tube-lined suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body, except for the head, hands, feet, and one arm from which measurements were taken. Core temperature was measured by inserting an esophageal probe (Mallinckrodt Medical, St. Louis, MO) to a depth of 40 cm past the nostril. In one subject who could not tolerate the esophageal probe, core temperature was measured with a telemetric pill (HQ, Palmetto, FL) that was swallowed a minimum of 2 h before data collection. Water intake was not allowed after placement of the esophageal probe or ingestion of the telemetric pill. Mean skin temperature was measured as the weighted average of six thermocouples attached to the skin surface on the abdomen, calf, chest, lower back, thigh, and upper back (37).

Cutaneous blood flow was measured on the dorsal side of the uncovered forearm using an integrated laser-Doppler flow probe (Moor Instruments, Wilmington, DE), placed over a microdialysis membrane perfused with iso-osmotic (ISO) saline as previously described (16). Cutaneous vascular conductance was subsequently calculated as the ratio of cutaneous blood flow perfusion units to mean arterial pressure and expressed as a percentage change from pre-LBNP values. Heart rate was obtained from an electrocardiogram (GE Healthcare, Milwaukee, WI) that was interfaced with a cardiotachometer (CWE, Ardmore, PA). Continuous blood pressure measurements were obtained noninvasively from the uncovered arm by finger photoplethysmography (Nexfin, Edwards Life Sciences, Irvine, CA). Multifiber recordings of MSNA were obtained by ultrasound-guided adjustments of a tungsten microelectrode within the radial nerve of the uncovered arm (8). A reference electrode was placed subcutaneously 2–3 cm from the recording electrode. The position of the recording electrode was adjusted until bursts of MSNA were identified based on the following criteria (9, 10): *1*) synchronicity of discharges with pulse rate; *2*) increases in activity during inspiratory apnea, and; *3*) lack of responsiveness to mental or somatosensory stimulation of the innervated region. Nerve signals were amplified, bandpass filtered with a band width of 700–2,000 Hz, and integrated with a time constant of 0.1 s (Iowa Bioengineering, Iowa City, IA).

Plasma hemoglobin (Hemoximeter, OSM3, Radiometer, Copenhagen, Denmark), hematocrit (Adams Microhematocrit II, Becton, Dickinson, Franklin Lakes, NJ), and osmolality (model 3MO plus, Micro-Osmometer, Advanced Instruments, Norwood, MA) were determined in triplicate from venous blood samples drawn into lithium-heparin tubes (BD Vacutainer, Franklin Lakes, NJ). Relative changes in plasma volume from baseline were subsequently calculated from changes in hematocrit and hemoglobin (11).

Experimental protocol. All subjects volunteered for two experimental trials, performed as a counterbalanced and crossover design. Both trials were separated by a minimum of 48 h to avoid physiological adaptation (21). After instrumentation, subjects assumed a supine position on a patient bed and mean skin temperature was clamped at ~33–34°C by adjusting the temperature of the water circulating though the tube-lined suit. An intravenous catheter was inserted into a superficial vein of the arm and a preinfusion blood sample was drawn a minimum of 30 min after the subject assumed the supine posture. An intravenous infusion of either ISO (0.9% NaCl) or hyperosmotic (HYPER, 3.0% NaCl) saline (Baxter, Deerfield, IL) was subsequently initiated and continued for 90 min. Infusion rates for the ISO (0.2 ml·kg body wt^{-1}·min^{-1}) and HYPER (0.125 ml·kg body wt^{-1}·min^{-1}) solutions were chosen based on previous studies showing these rates minimize differences in plasma volume expansion between conditions (2, 24, 30, 34–36).

During the infusion period, skin sympathetic nerve activity was initially obtained to address the primary aim of the previously published study (16). At the end of the infusion period, a blood sample was drawn after which baseline data were collected for 10 min. The temperature of the water circulating through the tube-lined suit was then increased to ~50°C, and whole body heating continued until an increase in core temperature of ~1.2°C was achieved. At this point, the temperature of the water perfusing the suit was reduced to 44–46°C to minimize further increases in core temperature (~0.2°C, for a total increase of ~1.4°C) while the microelectrode was adjusted to obtain a MSNA signal. An appropriate MSNA signal during both conditions could only be obtained in six subjects, as we limited the search to ~10 min. A venous blood sample was then drawn and incremental LBNP to presyncope was performed. The protocol began at 20 mmHg LBNP, with pressure in the chamber subsequently decreasing by 10 mmHg every 3 min until presyncope. Criteria for determining presyncope included continued self-reporting by the subject of feeling faint, sustained nausea, rapid and progressive decrease in blood pressure resulting in a sustained systolic blood pressure <80 mmHg, and/or relative bradycardia accompanied by a narrowing of pulse pressure. Immediately after the release of LBNP, a final venous blood sample was drawn.

Data analysis. Data were collected with data acquisition hardware (MP150, Biopac, Santa Barbara, CA) at a sampling frequency of 50–200 Hz. To account for differences in LBNP tolerance time between conditions (see RESULTS), the data were analyzed as a function of relative LBNP time. To do so, total LBNP time in seconds was divided into increments of 20%. For all variables (except MSNA), data from the last 15 s of each increment were averaged and used for analyses, the exception being that the last 5 s were averaged at presyncope (i.e., 100% of LBNP time). For MSNA data, a 20-s sample was used for analyses at each LBNP increment and at presyncope. This time frame was chosen to best reflect MSNA at presyncope, while keeping a consistent time frame across all time points. Nonetheless, it should be noted that shorter time frames for MSNA analyses increases measurement variability (25). LBNP tolerance was quantified using the cumulative stress index (CSI), calculated by summing the product of LBNP level and absolute time at each level (23).

Statistical analysis. Core and mean skin temperatures, cutaneous vascular conductance, heart rate, mean arterial blood pressure, MSNA, plasma volume, and plasma osmolality were analyzed using a two-way repeated measures analysis of variance with the factors of condition (levels: ISO, HYPER) and relative LBNP time [levels: 0% (pre-LBNP), 20%, 40%, 60%, 80%, 100% (presyncope)]. If a significant condition × LBNP time interaction was observed, a Holm-Sidak post hoc analysis was performed. Heating time and LBNP tolerance were analyzed using paired samples *t*-tests. Comparisons between "responders" and "nonresponders" (see RESULTS) for physical characteristics, LBNP tolerance, plasma volume, and osmolality were per-

formed using independent samples *t*-tests. For all analyses, the level of significance was set at an α of $P \leq 0.05$. Statistical analyses were performed using commercially available statistical software (Prism 6, Graphpad Software, La Jolla, CA). All variables are reported as means ± SD.

RESULTS

All subjects combined. The heating period lasted 64 ± 12 and 66 ± 11 min for the ISO and HYPER conditions, respectively ($P = 0.61$). During the ISO condition, whole body heating increased mean skin temperature from 33.5 to 38.5°C and core temperature by 1.39 ± 0.18°C (from 36.35 to 37.75°C). During the HYPER condition, mean skin temperature increased from 33.5 to 38.3°C and core temperature by 1.39 ± 0.17°C (from 36.42 to 37.81°C). There were no differences between conditions in mean skin and core temperatures throughout subsequent LBNP to presyncope (both $P > 0.30$). Intravenous infusion of hyperosmotic saline increased plasma osmolality and resulted in greater plasma volume expansion compared with the infusion of ISO saline (both $P < 0.01$, Fig. 1). Plasma osmolality and changes in plasma volume remained greater during HYPER, relative to ISO, just before the onset of LBNP (both $P < 0.01$). At the end of LBNP, plasma osmolality remained greater during HYPER ($P < 0.01$), whereas there were no differences between conditions for change in plasma volume ($P = 0.36$). Overall, LBNP tolerance improved during HYPER, expressed as both time (ISO: 553 ± 238 vs. HYPER: 670 ± 164 s, $P = 0.03$) and CSI (ISO: 288 ± 193 vs. HYPER: 382 ± 145 mmHg × min, $P = 0.04$). Despite the improvement in tolerance, there were no differences in mean arterial pressure ($P = 0.10$, Fig. 2), heart rate ($P = 0.09$, Fig. 2), cutaneous vascular conductance ($P = 0.55$, Fig. 2), and MSNA ($P = 0.60$, Table 1) between conditions during the LBNP period. When assessing the individual data (Fig. 3), the CSI of eight subjects improved by 25% or more during HYPER (range: +26 to +180%), whereas it did not change or decreased in four subjects (range: −28% to +5%). We therefore pursued the statistical analyses by separating subjects into "responders" and "nonresponders."

Responders. In eight subjects, LBNP tolerance increased by 25% or more with plasma hyperosmolality (ISO: 177 ± 63 vs. HYPER: 340 ± 117 mmHg × min, $P < 0.01$). In these subjects, the absolute level of LBNP was greater during HYPER, relative to ISO, at 80% of LBNP time (43 ± 5 vs. 31 ± 6 mmHg, $P < 0.01$) and at presyncope (48 ± 9 vs. 39 ± 6 mmHg, $P < 0.01$). Changes in blood pressure and heart rate differed between conditions during LBNP (both $P \leq 0.05$, Fig. 4). During the HYPER condition, mean arterial pressure was greater between 20% and 100% of LBNP time. For heart rate, no individual time points were statistically different according to the post hoc analysis. However, the increase in heart rate from 0 to 80% of LBNP time was greater during HYPER relative to ISO (+23 ± 17 vs. +12 ± 24 beats/min, $P = 0.05$). In contrast, reductions in cutaneous vascular conductance during LBNP did not differ between conditions ($P = 0.41$, Fig. 5). Of the six subjects from whom appropriate MSNA signals were obtained, three were classified as responders. During LBNP, MSNA appeared greater during HYPER in these subjects, although no statistical analyses were performed on these data (Table 1).

Nonresponders. In four subjects, LBNP tolerance either decreased or improved by no more than 5% with plasma hyperosmolality (ISO: 510 ± 171 vs. HYPER: 466 ± 176 mmHg × min, $P = 0.40$). In these subjects, the absolute level of LBNP at a given percentage of LBNP time did not differ between conditions ($P = 0.32$). Blood pressure and heart rate during LBNP were similar between conditions (both $P \geq 0.55$, Fig. 4). Reductions in cutaneous vascular conductance also did not differ between conditions ($P = 0.51$, Fig. 5). In the three nonresponders from whom appropriate MSNA signals were obtained, MSNA did not appear to be affected by plasma hyperosmolality, although no statistical analyses were performed on these data (Table 1).

Responders vs. nonresponders. Of the eight responders, four underwent the ISO trial first and the other four underwent the HYPER trial first. Of the four nonresponders, one underwent the ISO trial first and the other three underwent the HYPER trial first. There were no differences (all $P \geq 0.09$) in physical characteristics between responders (7 males/1 female, age: 34 ± 9 y, height: 173 ± 10 cm, weight: 73 ± 15 kg) and nonresponders (4 males, age: 27 ± 5 y, height: 176 ± 7 cm, weight: 78 ± 16 kg). However, responders displayed lower LBNP tolerance during the ISO condition (177 ± 63 mmHg × min) relative to nonresponders (510 ± 171 mmHg × min, $P < 0.01$). During the HYPER condition, plasma osmolality and relative changes in plasma volume were similar between responders and nonresponders before LBNP (osmolality: 305 ± 3 vs. 305 ± 2 mosmol/kg; plasma volume: +4.4 ± 2.5 vs. +6.7 ± 3.6%, both $P > 0.21$) and immediately following LBNP (osmolality: 307 ± 5 vs. 306 ± 3 mosmol/kg; plasma volume: −7.3 ± 5.9 vs. −10.8 ± 3.4%, both $P > 0.31$).

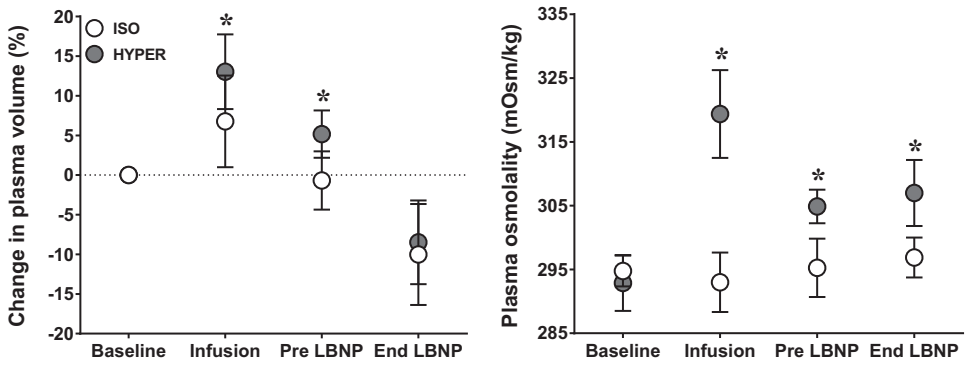

Fig. 1. Relative changes in plasma volume from baseline (*left*) and plasma osmolality (*right*) after 90 min of intravenous infusion (infusion) of either iso-osmotic (ISO) or hyperosmotic (HYPER) saline and a subsequent passive heating period followed by lower-body negative pressure (LBNP) to presyncope. Values are means ± SD for 12 subjects. Data were analyzed with a two-way repeated measures ANOVA. Pre-LBNP: values immediately before the LBNP protocol. End-LBNP: values immediately after the LBNP protocol. *$P \leq 0.05$ between conditions.

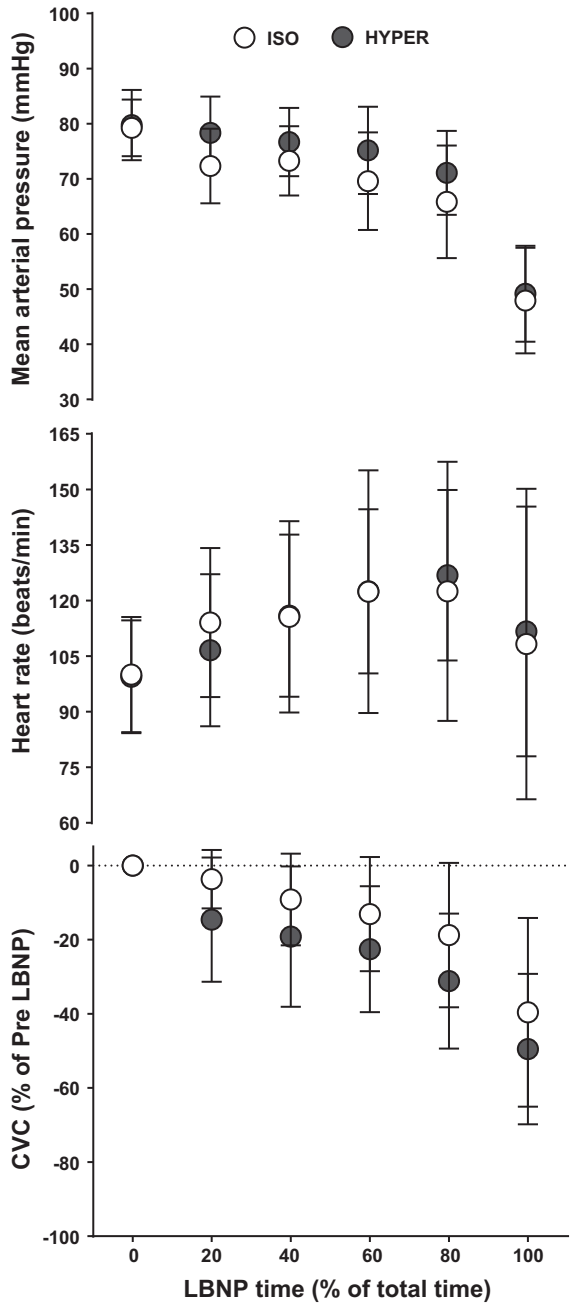

Fig. 2. Mean arterial pressure (*top*), heart rate (*middle*), and cutaneous vascular conductance (CVC, *bottom*) during progressive LBNP to presyncope performed under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. The data are plotted as a percentage of total LBNP time for 12 subjects. CVC data are plotted as a percentage change from pre-LBNP values. Values are means ± SD. Data were analyzed with a two-way repeated measures ANOVA.

DISCUSSION

In the current study, we examined the effect of plasma hyperosmolality on LBNP tolerance in heat-stressed humans. Infusion of hyperosmotic saline improved overall LBNP tolerance, relative to the infusion of ISO saline that minimized differences in plasma volume expansion between conditions. However, an improvement in LBNP tolerance with plasma hyperosmolality was not observed in all subjects. Some subjects demonstrated large improvements in LBNP tolerance (≥25%, responders), whereas little to no improvement was observed in others (≤5%, nonresponders). In the group of responders, plasma hyperosmolality was accompanied by a greater blood pressure and a greater increase in heart rate up to the point of presyncope. These results suggest that plasma hyperosmolality improves tolerance to central hypovolemia during heat stress in most, but not all individuals.

Heat stress profoundly impairs LBNP tolerance in humans (28). A primary factor contributing to this effect is central hypovolemia, as heat stress reduces central blood volume even in the absence of additional reductions in central blood volume (6). Restoration of blood volume, either through rapid bolus or continuous ISO saline infusion, improves LBNP tolerance in heat-stressed humans (18, 22). However, heat stress also increases plasma osmolality, which could have beneficial effects for tolerance during a central hypovolemic challenge. In animal models, hyperosmolality can be sympathoexcitatory through osmosensitive neurons located in the forebrain circumventricular organs (38, 39). In sheep, hyperosmotic saline infusion results in greater cardiac sympathetic nerve activity and a greater heart rate response during resuscitation from hemorrhage (15). In normothermic humans, oral salt loading results in greater forearm vasoconstriction during orthostatic stress and it improves orthostatic tolerance (4, 13). Infusion of hyperosmotic saline also increases resting MSNA and improves the baroreflex control of MSNA (3, 14, 41). The current study extends these observations by demonstrating that physiological responses mediated by hyperosmolality translate into improved LBNP tolerance during heat stress in humans. However, it should be noted that this observation was not consistent in all subjects.

During the hyperosmotic condition, a group of responders had relatively large improvements in LBNP tolerance (+106%), whereas little to no change was observed in a group of nonresponders (−8%). The most obvious difference between these groups is the markedly lower LBNP tolerance of responders, relative to nonresponders, during the iso-osmotic condition (~threefold). In fact, the four nonresponders had the four highest LBNP tolerances during the iso-osmotic condition. Thus hyperosmolality may only improve LBNP tolerance during heat stress in otherwise low-tolerant individuals. Alternatively, we cannot rule out the possibility of interindividual sensitivity to plasma hyperosmolality, analogous to the effects of salt consumption on blood pressure where individuals are often defined as "salt-sensitive" or "salt-insensitive" (40). For example, plasma hyperosmolality resulted in greater blood pressure and greater increases in heart rate up to the point of presyncope in responders. These effects were not observed in nonresponders, despite similar plasma osmolality before and immediately after the LBNP protocol. It is therefore possible that plasma hyperosmolality only improves tolerance to central hypovolemia in individuals who are physiologically sensitive to its effects.

The precise physiological mechanism(s) by which plasma hyperosmolality improves LBNP tolerance remains to be determined. As discussed, greater blood pressure and a greater increase in heart rate were observed up to the point of presyncope (Fig. 4). Preliminary observations suggest these responses could be driven by greater sympathetic activation, evidenced by elevated MSNA (Table 1). Such results are consistent with

Table 1. *Muscle sympathetic nerve activity during progressive lower-body negative pressure to presyncope performed under hyperthermic conditions after intravenous infusion of either iso-osmotic or hyperosmotic saline*

	Pre-LBNP	20%	40%	60%	80%	100%
			Burst frequency, bursts/min			
All subjects (n = 6)						
ISO	50 ± 23	71 ± 12	77 ± 19	75 ± 22	71 ± 19	56 ± 24
HYPER	61 ± 15	76 ± 16	83 ± 11	87 ± 11	84 ± 18	64 ± 10
Responders (n = 3)						
ISO	44 ± 12	69 ± 11	67 ± 9	60 ± 6	60 ± 12	35 ± 12
HYPER	65 ± 11	78 ± 19	83 ± 12	87 ± 12	78 ± 6	59 ± 7
Nonresponders (n = 3)						
ISO	56 ± 32	73 ± 14	86 ± 23	91 ± 22	81 ± 20	76 ± 7
HYPER	56 ± 20	73 ± 16	82 ± 12	87 ± 14	90 ± 26	69 ± 10
			Burst incidence, bursts/100 heartbeats			
All subjects (n = 6)						
ISO	50 ± 21	63 ± 14	68 ± 17	65 ± 19	60 ± 17	53 ± 12
HYPER	61 ± 19	68 ± 17	70 ± 13	71 ± 15	69 ± 16	61 ± 16
Responders (n = 3)						
ISO	49 ± 13	68 ± 11	72 ± 11	68 ± 16	67 ± 8	54 ± 14
HYPER	71 ± 12	77 ± 13	77 ± 12	80 ± 8	76 ± 0	72 ± 4
Nonresponders (n = 3)						
ISO	51 ± 31	58 ± 16	63 ± 23	62 ± 25	54 ± 23	52 ± 14
HYPER	50 ± 20	59 ± 16	62 ± 11	62 ± 15	63 ± 22	50 ± 15

Values are means ± SD and presented as a percentage of total lower-body negative pressure (LBNP) time for three subgroups: *1*) all subjects from whom appropriate muscle sympathetic nerve activity (MSNA) signals were obtained (n = 6); *2*) subjects in whom LBNP tolerance improved during hyperosmotic (HYPER) (responders, n = 3), and; *3*) subjects in whom LBNP tolerance did not improve during HYPER (nonresponders, n = 3). Pre-LBNP: values immediately before the LBNP protocol. ISO, iso-osmotic.

the observation that the increase in MSNA is an important determinant of LBNP tolerance in normothermic (5, 32) and heat stress (7) conditions and that hyperosmolality improves the baroreflex control of MSNA at rest under normothermic conditions (41). However, future studies are needed to test this hypothesis. In contrast, plasma hyperosmolality did not affect the reduction in cutaneous vascular conductance during LBNP (Fig. 4). The neural control of the cutaneous circulation is achieved by vasoconstrictor and active vasodilator systems (12, 27). Reductions in cutaneous vasodilation during combined heat stress and LBNP have been ascribed to both increased vasoconstrictor activity (31) and withdrawal of active vasodilation (19). Therefore, plasma hyperosmolality does not appear to result in greater cutaneous vasoconstrictor activity and/or greater withdrawal of active cutaneous vasodilation during combined heat stress and LBNP.

Perspectives and Significance

The infusion of hyperosmotic/oncotic solutions is favorable for the early resuscitation from severe hypovolemia (20) and oral salt loading improves orthostatic tolerance (4, 13). However, the general premise for using hyperosmotic solutions/oral salt loading is to create an osmotic gradient that draws fluid from the intracellular/interstitial compartments into the vascular space. It is therefore challenging to differentiate the effect of plasma hyperosmolality from that of hypervolemia on orthostatic tolerance. In the current study, we employed different infusion rates for ISO and HYPER saline to minimize differences in plasma volume expansion relative to when the same rate is used for both solutions (34, 35). Thus the current study extends previous findings by demonstrating that plasma hyperosmolality has a favorable effect upon LBNP tolerance during heat stress conditions, even when changes in intravascular volume are minimized. Given that hypovolemia and hyperosmolality often occur concurrently during heat stress (1, 17), these results suggest that the physiological effects of hyperosmolality may counter the detrimental effects of hypovolemia on LBNP tolerance in heat stressed humans.

Considerations. It is recognized that the expansion of plasma volume was not perfectly matched between trials before the LBNP protocol. Previously, we found that heat stress-induced reductions in LBNP tolerance were mitigated by ~36%

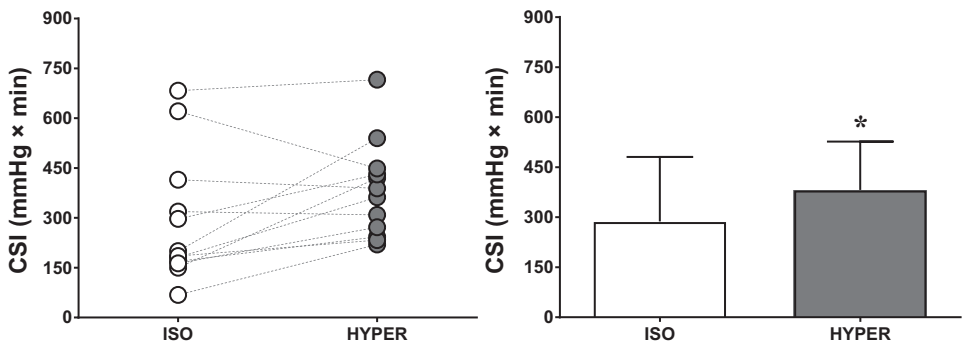

Fig. 3. Individual (*left*) and mean (*right*) values of tolerance to central hypovolemia under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. Tolerance to central hypovolemia was quantified using the cumulative stress index (CSI). Values are means ± SD for 12 subjects. Data were analyzed with a paired samples t-test. *$P \leq 0.05$ between conditions.

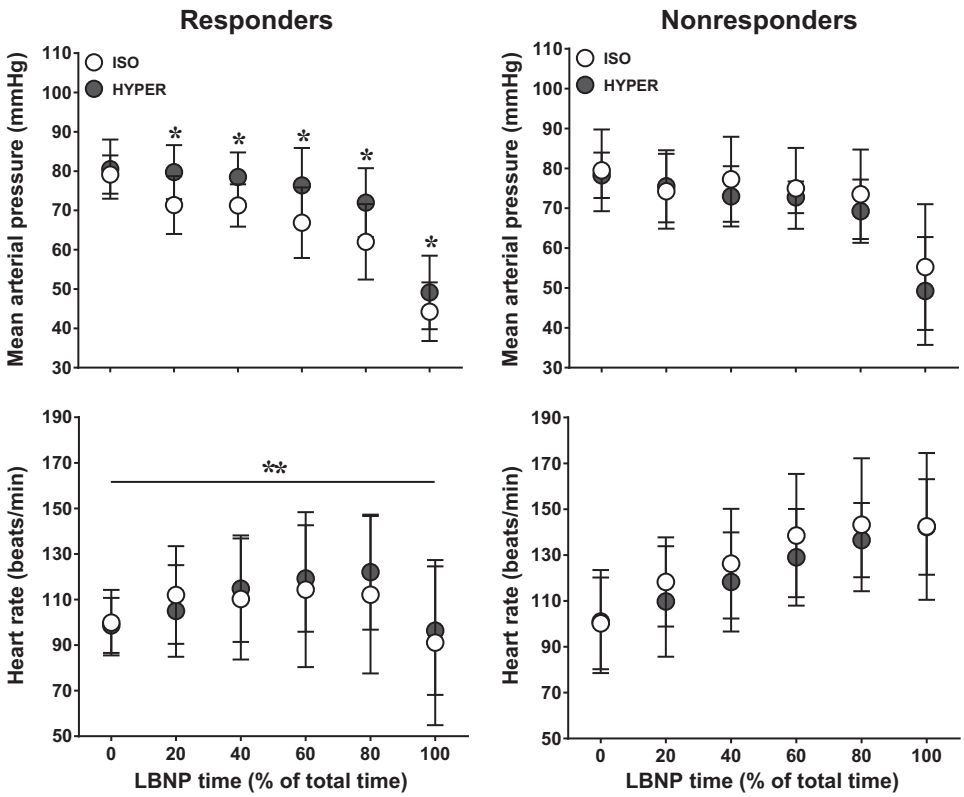

Fig. 4. Mean arterial pressure (*top*) and heart rate (*bottom*) during progressive LBNP to pre-syncope performed under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. The data are plotted as a percentage of total LBNP time for 8 subjects in whom LBNP tolerance improved during HYPER (responders, *left*) and for 4 subjects in whom LBNP tolerance did not improve during HYPER (nonresponders, *right*). Values are means ± SD. Data were analyzed with a two-way repeated measures ANOVA. $*P \leq 0.05$ between conditions at the indicated time point. $**P \leq 0.05$ condition × time interaction.

when saline was infused to offset sweat losses (22). In that study, heat stress alone decreased plasma volume by ~6% from baseline, whereas saline infusion maintained plasma volume at baseline levels. In contrast, plasma volume in the current study was ~5% greater than baseline before the onset of LBNP during the hyperosmotic condition, whereas it remained at baseline values during the iso-osmotic condition. During the iso-osmotic condition, LBNP tolerance was 27% lower overall (46% in responders) relative to the hyperosmotic condition. An important distinction between our previous (22) and current studies is the fact that the differences in plasma volume were due to a relatively greater plasma volume in the current study versus a relatively lower plasma volume in our previous study (22). Although a reduction in plasma volume from baseline can compromise LBNP tolerance (22, 26), it is unclear if and/or to what extent the 5% plasma volume expansion observed in the current study affects LBNP tolerance during heat stress relative to a condition in which plasma volume is maintained.

Despite the potential modulating effect of differences in plasma volume expansion, we propose that hyperosmolality was the primary factor responsible for the observed differences in LBNP tolerance for the following reasons. A greater increase in heart rate was observed during LBNP in the group of responders. Similarly, preliminary observations suggest that plasma hyperosmolality also led to greater MSNA in the group of responders, although we recognize that the interpretation of these data is limited by the low number of subjects from which appropriate signals could be obtained. Nonetheless, these responses occurred despite subjects beginning the LBNP protocol with a greater relative plasma volume expansion. Such observations support a direct effect of hyperosmolality on heart rate and MSNA, as these responses would be expected to be

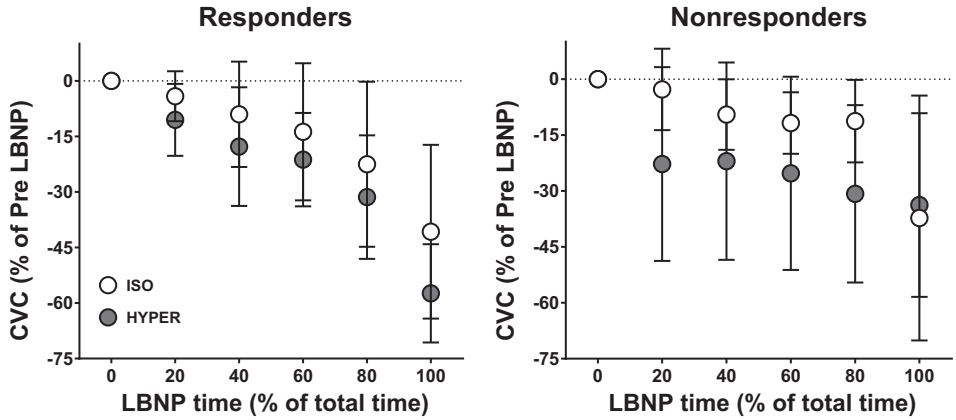

Fig. 5. Changes in cutaneous vascular conductance (CVC) during progressive LBNP to pre-syncope performed under hyperthermic conditions after intravenous infusion of either ISO or HYPER saline. The data are changes from pre-LBNP values and are plotted as a percentage of total LBNP time for 8 subjects in whom LBNP tolerance improved during HYPER saline (responders, *left*) and for 4 subjects in whom LBNP tolerance did not improve during HYPER saline (nonresponders, *right*). Values are means ± SD. Data were analyzed with a two-way repeated measures ANOVA.

accompanied by relative hypovolemia as opposed to the observed hypervolemia. It should also be considered that relative changes in plasma volume were similar between conditions immediately following LBNP. This observation suggests that differences in plasma volume expansion evident before LBNP did not persist throughout the LBNP protocol. However, the greater LBNP levels reached during the hyperosmotic condition could contribute to this observation.

In conclusion, the current study evaluated the effect of plasma hyperosmolality on LBNP tolerance in heat-stressed humans. The main findings show that plasma hyperosmolality improves LBNP tolerance during heat stress in most, but not all, individuals. In individuals who improved LBNP tolerance, plasma hyperosmolality resulted in greater blood pressure and a greater increase in heart rate up to the point of presyncope. Preliminary observations suggest that these responses could be mediated by greater sympathetic activation, although the precise physiological mechanism(s) remain to be determined.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.G., S.A.R., and C.G.C. conceived and designed research; D.G., S.A.R., H.N., and P.Y.P. performed experiments; D.G. analyzed data; D.G., S.A.R., H.N., P.Y.P., and C.G.C. interpreted results of experiments; D.G. prepared figures; D.G. drafted manuscript; D.G., S.A.R., H.N., P.Y.P., and C.G.C. edited and revised manuscript; D.G., S.A.R., H.N., P.Y.P., and C.G.C. approved final version of manuscript.

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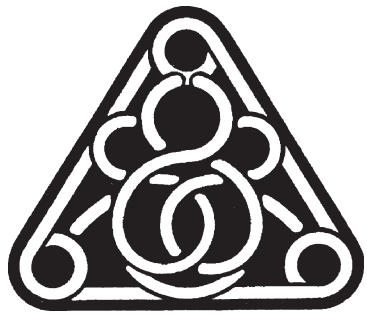

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Forehead versus forearm skin vascular responses at presyncope in humans

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Gagnon D, Brothers RM, Ganio MS, Hastings JL, Crandall CG. Forehead versus forearm skin vascular responses at presyncope in humans. *Am J Physiol Regul Integr Comp Physiol* 307: R908–R913, 2014. First published August 6, 2014; doi:10.1152/ajpregu.00204.2014.—Facial pallor is commonly observed at presyncope in humans, suggestive of reductions in facial skin blood flow (SkBF). Yet, cutaneous vasoconstriction is usually minimal at presyncope when measured at the forearm. We tested the hypothesis that reductions in forehead SkBF at presyncope are greater than in the forearm. Forehead and forearm SkBF (laser-Doppler) and blood pressure (Finometer or radial artery catheterization) were measured during lower body negative pressure (LBNP) to presyncope in 11 normothermic and 13 heat-stressed subjects (intestinal temperature increased ~1.4°C). LBNP reduced mean arterial pressure from 91 ± 5 to 57 ± 7 mmHg during normothermia ($P \leq 0.001$) and from 82 ± 5 to 57 ± 7 mmHg during heat stress ($P \leq 0.001$). During normothermia, LBNP decreased forehead SkBF 55 ± 14% compared with 24 ± 11% at the forearm ($P = 0.002$), while during heat stress LBNP decreased forehead SkBF 39 ± 11% compared with 28 ± 8% in the forearm ($P = 0.007$). In both conditions, most (≥68%) of the decreases in SkBF were due to decreases in blood pressure. However, a greater contribution of actively mediated reductions in SkBF was observed at the forehead, relative to the forearm during normothermia (32 ± 13% vs. 11 ± 11%, $P = 0.031$) and heat stress (30 ± 13% vs. 10 ± 13%, $P = 0.004$). These data suggest that facial pallor at presyncope is due to a combination of passive decreases in forehead SkBF secondary to reductions in blood pressure and to active decreases in SkBF, the latter of which are relatively greater than in the forearm.

heat; lower body negative pressure; skin blood flow; syncope; vasoconstriction

IT IS WELL ESTABLISHED THAT the control of human skin blood flow (SkBF) differs between glabrous and nonglabrous regions. Although SkBF within these regions generally responds similarly to many stimuli, discrepancies in responses can occur within a given skin type. In particular, cutaneous vessels of forehead skin vasodilate similarly relative to other nonglabrous areas during local (11) and whole body (3, 6, 7) heating, yet forehead cutaneous vasoconstriction has been reported to be both attenuated (4) and greater (15) in response to hypocapnia, as well as nonexistent during cold stress (5, 17). Microneurographic recordings of the supraorbital nerve, which serves forehead skin, have also demonstrated an absence of sympathetic vasoconstrictor activity during cold stress (12). Thus, despite forehead skin being considered nonglabrous, it may respond differently relative to other nonglabrous regions.

Facial pallor is a typical observation in humans during profound hypotension sufficient to cause presyncope. When the hypotensive challenge is accompanied by heat stress, this occurrence is particularly noticeable. The pallor observed in the face is in contrast to what is observed in other nonglabrous areas, such as the forearm. As forehead SkBF is generally greater compared with the forearm, particularly during heat stress (6, 14, 15), facial pallor may be more noticeable due to a greater decrease in facial SkBF relative to the forearm, although the mechanisms for this presumed response have not been studied. Facial pallor could result from passive reductions in SkBF secondary to the rapid and pronounced reductions in blood pressure at the point of presyncope (1, 21), from neurally mediated vasoconstriction (should this exist in forehead skin) and/or withdrawal of active vasodilation, or a combination of these mechanisms. Although the neural control of SkBF may differ between the forehead and the forearm, the passive effect of reductions in blood pressure would be expected to similarly affect the skin circulation of both regions. Therefore, it can be hypothesized that facial pallor at presyncope is associated with a greater neurally mediated reduction in facial SkBF relative to the forearm.

The purpose of this study was to compare forehead and forearm SkBF responses during a central hypovolemic challenge performed to presyncope under normothermic, as well as heat stress conditions. Furthermore, we determined the fractional contributions by which decreases in forehead and forearm SkBF were "passive" i.e., as a result of decreases in blood pressure, or "active" i.e., due to influences on the skin vasculature, which were presumed to be of neural origin. We tested the hypotheses that *1*) the magnitude of the reduction in SkBF would be greater in the forehead compared with the forearm at presyncope, while normothermic or heat stressed, which may explain facial pallor subjectively observed under such conditions, and *2*) greater reductions in forehead SkBF relative to the forearm at presyncope would be due to a greater "active" component.

METHODS

Subjects. Simultaneous measurements of forehead and forearm SkBF were retrospectively analyzed from 24 subjects who underwent incremental lower body negative pressure (LBNP) to presyncope. Eleven of these subjects (three females) underwent the protocol while normothermic, and 13 subjects (six females) performed the protocol while heat-stressed. Of the 13 subjects that completed the heat stress trial, 2 of them performed the protocol twice, on separate days. The averaged data from both trials were analyzed for these two subjects.

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Five of the subjects completed the protocol under both thermal conditions, although on different days. Subject characteristics are as follows: age, 37 ± 11 years; height, 172 ± 6 cm; weight, 71.1 ± 12.0 kg. All subjects were free of any known cardiovascular, respiratory, neurological or metabolic diseases. Phase of menstrual cycle was recorded, but not controlled for in female subjects. All procedures were approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and at Texas Health Presbyterian Hospital Dallas. Written informed consent was obtained from all subjects prior to their participation in the study.

Measurements. SkBF, in arbitrary units (au), was measured by laser-Doppler flowmetry using integrated probes (Moor Instruments, Devon, United Kingdom; or Perimed, North Royalton, OHA) placed on forearm and forehead skin. The forearm SkBF probe was placed on the dorsal side of the forearm, in a region that visually was devoid of large veins. The forehead SkBF probe was placed at the center of the forehead. Local skin temperature at each site was not controlled and was, therefore, allowed to fluctuate freely. This approach was selected to maximize the practical validity of the obtained SkBF values. Blood pressure was continuously measured noninvasively using photoplethysmography (Finometer Pro, FMS, Amsterdam, Netherlands) and corrected to pressures obtained by auscultation of the brachial artery (Tango+; SunTech Medical, Morrisville, NC). In seven of the subjects who performed the heat stress trial, blood pressure was measured by direct cannulation of the radial artery (Baxter Healthcare, Irving, CA). Cutaneous vascular conductance (CVC) was calculated as SkBF divided by mean arterial pressure. Heart rate was obtained from an electrocardiogram (Agilent, Munich, Germany) that was interfaced with a cardiotachometer (CWE, Ardmore, PA). Internal body temperature was measured by an ingestible telemetric pill (HQ Palmetto, FL) that was swallowed by the subject upon arrival, ~2 h prior to data collection. Mean skin temperature was measured as the weighted average of six thermocouples attached to the skin surface on the abdomen, calf, chest, lower back, shoulder, and quadriceps (22).

Experimental protocol. Upon arrival to the laboratory, the subjects swallowed the telemetric pill for the measurement of internal body temperature before being dressed in a two-piece tube-lined suit that covered the entire body except for the head, hands, feet, and one forearm. While supine, the subjects were sealed at the waist within a custom-made LBNP chamber. The subjects were then instrumented for the measurement of heart rate, blood pressure, forehead and forearm SkBF, and mean skin temperature. Following a baseline rest period, incremental LBNP to presyncope was performed for the normothermic trial, while the subjects in the heat stress trial underwent incremental LBNP to presyncope after internal body temperature had increased by ~1.4°C. The LBNP protocol began at 20 mmHg, with an increase in LBNP of 10 mmHg every 3 min until presyncope. During the normothermic condition, mean skin temperature was clamped by circulating water maintained at ~34°C through the tube-lined suit, while whole-body heat stress was achieved by circulating water at 48°C through the tube-lined suit. Once the desired increase in internal body temperature was achieved, the temperature of the water perfusing the suit was slightly reduced to 46°C to maintain internal body temperature relatively constant during the subsequent LBNP period. Criteria for the termination of the LBNP protocol included continued self-reporting by the subject of feeling faint, sustained nausea, rapid and progressive decrease in blood pressure resulting in a sustained systolic blood pressure being <80 mmHg, and/or relative bradycardia accompanied with a narrowing of pulse pressure.

Data analysis. Data were collected with data acquisition software (Biopac MP150, Santa Barbara, CA) at a minimum sampling frequency of 50 Hz. The data were analyzed at baseline rest, during heat stress prior to the initiation of LBNP, and during the final 100 s prior to the cessation of LBNP. Minute averages were performed for the baseline normothermic and heat stress data, while the data during the final 100 s of LBNP were averaged into 5-s segments. To account for differences in absolute values of SkBF and CVC between the forehead and forearm (see RESULTS section), the percent reduction in SkBF and CVC from pre-LBNP was calculated for each site. To determine the contribution by which decreases in SkBF occurred due to the decrease in blood pressure (i.e., passive), the ratio of the percent reduction in blood pressure to the percent reduction in SkBF during LBNP was calculated using the following formula: passive contribution (%) = [percent reduction in blood pressure from pre-LBNP ÷ percent reduction in SkBF from pre-LBNP] × 100. The remaining contribution (i.e., 100% − passive contribution) was considered to be due to active, presumably neurally mediated decreases in SkBF. In some instances, the percent reduction in mean arterial pressure was greater than the percent reduction in SkBF. In these cases, a value of 100% was attributed to the passive contribution, while a value of 0% was attributed to the active contribution, given that under such conditions, there was no evidence of a neurally mediated (i.e., active) vasoconstrictor response.

Statistical analysis. Within each thermal condition, the percent reduction in SkBF was analyzed using a two-way repeated-measures ANOVA using the repeated factors of skin site (levels: forehead and forearm) and time (levels: pre-LBNP and every 5 s during the final 100 s of LBNP). Mean arterial pressure was analyzed within each thermal condition using a one-way repeated-measures ANOVA, using the repeated factor of time (pre-LBNP and every 5 s during the final 100 s of LBNP). Differences between sites in absolute values of SkBF at baseline and heat stress, in the overall reduction in SkBF and CVC from pre-LBNP to presyncope, as well as in the relative contributions of passive vs. active reductions in SkBF, were analyzed using paired-samples Student's *t*-test. The level of significance was set at an alpha of $P \leq 0.05$, and a Holm-Bonferroni correction was applied when multiple comparisons were made. Statistical analyses were performed using commercially available statistical software (SPSS 20.0 for Windows, SPSS, Chicago, IL). All variables are reported as means ± 95% confidence intervals. Confidence intervals were calculated as 1.96 × standard error of the mean.

RESULTS

Normothermic condition. Baseline SkBF was greater at the forehead (96.3 ± 19.7 au) compared with the forearm (16.1 ± 4.7 au, $P \leq 0.001$). LBNP time and level at presyncope averaged 1,251 ± 173 s and 75 ± 9 mmHg, respectively, while in this thermal condition. Mean arterial pressure decreased during the final 100 s of LBNP ($P \leq 0.001$), averaging 57 ± 7 mmHg at presyncope compared with 91 ± 5 mmHg at baseline rest ($P \leq 0.001$). Relative to pre-LBNP, forehead, and forearm SkBF decreased over the final 100 s of LBNP ($P \leq 0.001$, Fig. 1), with the magnitude of the reduction in SkBF over time being different between skin sites (site × time interaction; $P = 0.009$). At presyncope, forehead SkBF was reduced 55 ± 14% compared with 24 ± 11% at the forearm ($P \leq 0.001$; Fig. 3). At the forehead, 68 ± 13% of that decrease in SkBF was attributed to decreases in blood pressure, compared with 89 ± 11% at the forearm ($P = 0.031$ between sites; Fig. 4). As such, 32 ± 13% and 11 ± 11% of the decrease in forehead and forearm SkBF, respectively, were active in origin ($P = 0.031$ between sites, Fig. 4). At presyncope, forehead CVC decreased by 31 ± 15% ($P = 0.003$), while forearm CVC increased by 27 ± 25% although this increase was not statistically significant ($P = 0.061$). The difference in CVC between the forehead and forearm at presyncope was significant ($P = 0.002$).

Heat stress condition. Prior to heat stress, SkBF was greater at the forehead (121.5 ± 37.5 au) compared with the forearm

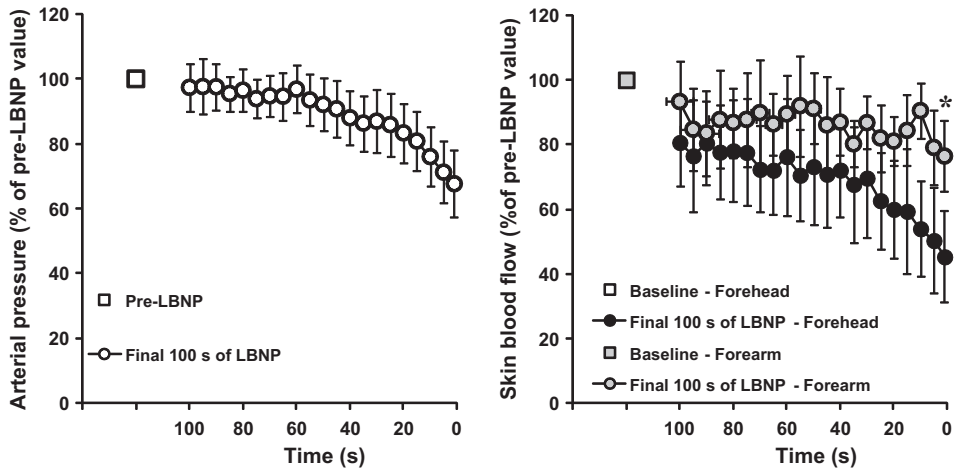

Fig. 1. Relative decreases in mean arterial pressure (*left*), as well as forehead and forearm skin blood flows (*right*) during the last 100 s of incremental lower body negative pressure (LBNP) performed to presyncope in a normothermic condition. The values are presented as a percentage of the values measured prior to LBNP (i.e., pre-LBNP). Values are expressed as means ± confidence intervals. *Significantly different from the forehead.

(20.6 ± 3.7 au; $P \leq 0.001$). Whole body heat stress increased mean skin temperature from 34.5 ± 0.4°C to 38.9 ± 0.5°C ($P \leq 0.001$) and internal body temperature by 1.42 ± 0.12°C. Forehead SkBF (311.1 ± 36.5 au) remained greater compared with the forearm (149.8 ± 33.0 au; $P \leq 0.001$) with heat stress. Heat stress also increased heart rate from 63 ± 4 bpm to 110 ± 9 bpm ($P \leq 0.001$) and reduced mean arterial pressure from 90 ± 5 mmHg to 82 ± 5 mmHg ($P \leq 0.001$). LBNP time and LBNP level at presyncope averaged 682 ± 159 s and 47 ± 8 mmHg, respectively. Mean arterial pressure decreased over the last 100 s of LBNP during heat stress ($P \leq 0.001$), averaging 57 ± 7 mmHg at presyncope ($P \leq 0.001$ relative to baseline). Relative to pre-LBNP, forehead and forearm SkBF decreased over the last 100 s of LBNP ($P \leq 0.001$, Fig. 2), with the pattern of response differing between sites (site × time interaction; $P = 0.003$). Overall, forehead SkBF decreased by 39 ± 11% during LBNP compared with a reduction in forearm SkBF of 28 ± 8% ($P = 0.007$, Fig. 3). At the forehead, 70 ± 13% of that decrease in SkBF was due to the decrease in mean arterial pressure, compared with 90 ± 13% at the forearm ($P = 0.004$ between sites, Fig. 4). As such, 30 ± 13% of the decrease in forehead SkBF and 10 ± 13% of the decrease in forearm SkBF was active in nature ($P = 0.004$ between sites, Fig. 4). The relative contribution of passive and active decreases in SkBF did not differ between thermal conditions at both sites (all $P > 0.05$). Forehead CVC decreased by 15 ± 13% ($P = 0.047$) during LBNP, while it increased 5 ± 9% at the forearm, although this change was not statistically significant ($P = 0.334$). The difference in CVC between the forehead and forearm at presyncope was significant ($P = 0.018$).

DISCUSSION

This study examined forehead and forearm SkBF responses during incremental LBNP to presyncope in both normothermic and heat-stressed humans. Regardless of the thermal condition, the relative decrease in forehead SkBF at presyncope was greater compared with that in the forearm. While the primary mechanism for these decreases in SkBF is accounted for by decreases in blood pressure, a greater proportion of actively mediated decreases in forehead SkBF (relative to forearm) was observed in both conditions. These findings provide a potential mechanism for the facial pallor commonly observed at presyncope in humans.

Previous studies have noted a lack of decrease in forehead SkBF during the local application of cold to the forehead (5), as well as during whole body cold stress (17). Furthermore, attenuated decreases in forehead SkBF relative to the forearm have been observed during hyperventilation-induced hypocapnia (4). Together, these studies suggest that the forehead skin

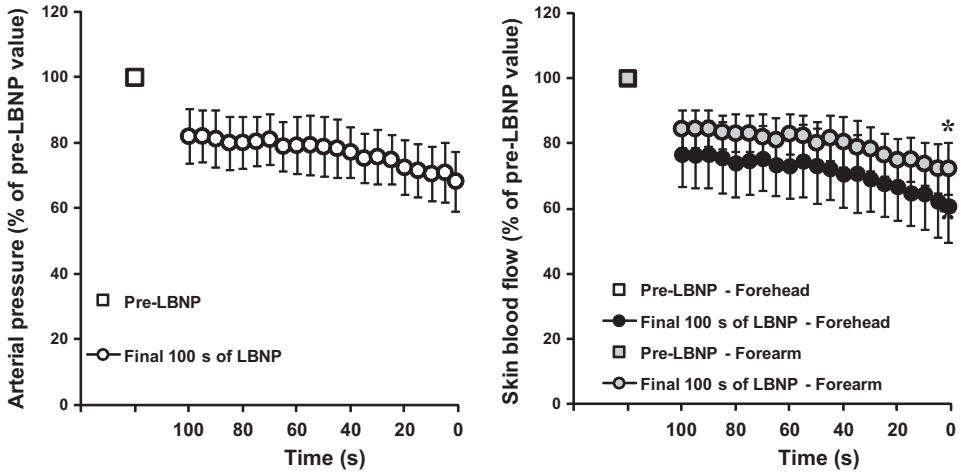

Fig. 2. Relative decreases in mean arterial pressure (*left*), as well as forehead and forearm skin blood flows (*right*) during the last 100 s of incremental LBNP performed to presyncope during a heat stress condition. The values are presented as a percentage of the values measured prior to LBNP (i.e., pre-LBNP). Values are expressed as means ± confidence intervals. *Significantly different from the forehead.

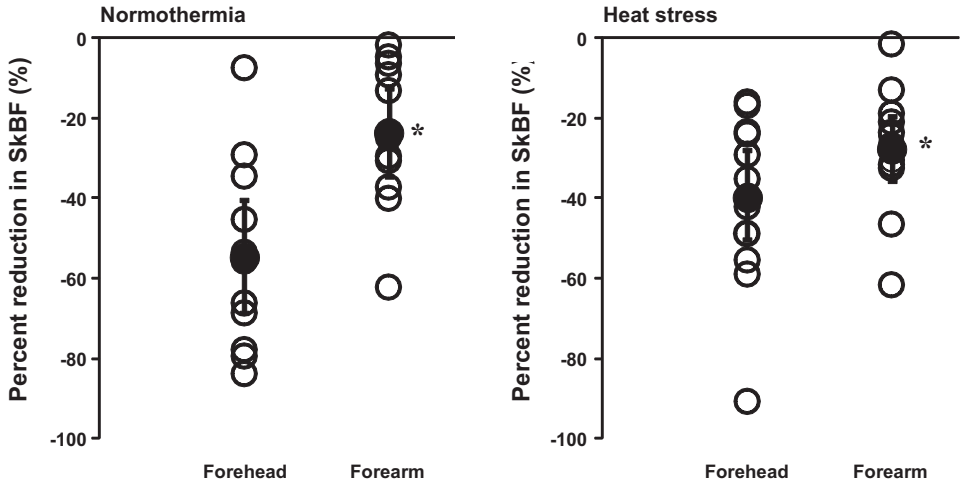

Fig. 3. The percent reduction in forehead and forearm skin blood flow (SkBF) at presyncope, relative to the period just prior to LBNP, under normothermic and heat stress conditions. The open circles represent individual data points, while the solid circles represent the means ± confidence intervals. *Significantly different from the forehead.

circulation may not be responsive to sympathetic vasoconstriction stimuli. In fact, Nordin (12) performed direct microneurographic recordings of cutaneous sympathetic activity in the supraorbital nerve and observed little background activity when individuals rested at normal ambient temperatures and no increase in nerve activity during body cooling. Although the present results do not support or refute sympathetic vasoconstrictor control of forehead SkBF, they clearly demonstrate that forehead SkBF is capable of decreasing during a sympathoexcitatory stimulus, regardless of whether it is performed under normothermic or heat stress conditions. Furthermore, the relative decreases in forehead SkBF were greater compared with those observed in the forearm. It should be noted that the stimulus employed in the current study (i.e., LBNP) elicited substantial reductions in blood pressure, whereas most of the previous stimuli used to examine the control of forehead SkBF (e.g., local application of cold, cold stress) likely resulted in either no change or an increase in blood pressure. Given that decreases in blood pressure were the main determinant for the reduction in forehead SkBF under both thermal conditions, the lack of change in forehead SkBF in previous studies could have been due to a lack of decrease in blood pressure. To this effect, a more recent study has reported decreases in forehead SkBF during acute and pronounced reductions in blood pressure under normothermic conditions (13).

Overall, reductions in blood pressure during LBNP explained the majority of the reductions in forehead and forearm SkBF under both thermal conditions. However, a novel finding of the current study is the greater contribution of active influences upon the reduction in forehead SkBF relative to the forearm. The mechanisms by which a larger fraction of forehead SkBF is "actively" decreased during presyncopal LBNP include greater cutaneous vasoconstriction and/or greater withdrawal of active vasodilation. Since active vasodilation occurs only after core and skin temperatures have increased beyond an onset threshold (10, 18), it is unlikely that a withdrawal of active vasodilation can explain the greater proportion of active influences upon the reduction in forehead SkBF during the normothermic condition. As such, the greater actively mediated reduction in forehead SkBF during the normothermic condition was most likely driven by greater vasoconstrictor activity, should it exist in forehead skin. Although Nordin (12) did not report any increase in nerve activity from the supraorbital nerve during body cooling, it was acknowledged that vasoconstrictor fibers supplying forehead skin may run through other nerves than the one measured. It should also be considered that absolute values of SkBF were greater at the forehead compared with the forearm, which could contribute to a greater relative decrease in blood flow, since there was more "reserve" for SkBF to decrease. This could potentially account for a greater relative decrease in mean arterial pressure, which exceeded that for SkBF in a few subjects. This was particularly noticeable in the CVC values, as attenuated decreases in forearm SkBF combined with a continued decrease in mean arterial pressure led to CVC values that indicated a vasodilation (i.e., increase in conductance) in the forearm as opposed to

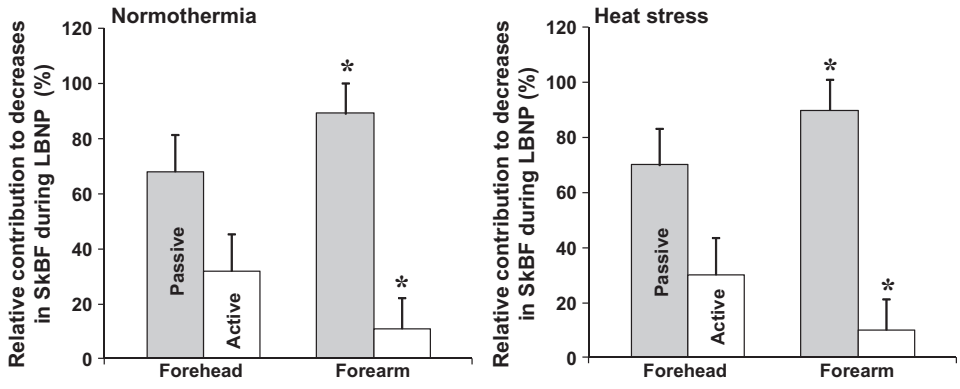

Fig. 4. The relative contribution of passive (i.e., due to reductions in blood pressure, gray bars) and active (i.e., presumably neurally mediated, open bars) reductions in forehead and forearm SkBF at presyncope in normothermic and heat stressed subjects. Values are expressed as means ± confidence intervals. *Significantly different from the forehead for the indicated contribution.

vasoconstriction (i.e., a decrease in conductance) in the forehead at presyncope. Although this possibility may especially be true for the normothermic condition, nonetheless, we observed a greater relative decrease in forehead SkBF during the heat stress condition when forearm SkBF was substantially elevated, thereby minimizing any potential for its decrease to be limited.

Minor reductions in forearm CVC at presyncope have been reported previously during heat stress (1). The cutaneous vasculature represents the greatest reservoir from which blood volume, as well as vascular conductance, can be drawn upon to maintain blood pressure during a central hypovolemic challenge under heat stress conditions. However, heat stress itself adversely affects the responsiveness of the forearm cutaneous vasculature to constrict to adrenergic agonists (23), in part, because of an inhibitory effect of nitric oxide (2, 19, 20). As such, the current results in the forearm support previous observations of minimal reductions in forearm CVC at the point of presyncope in heat-stressed humans and suggest that the reductions in SkBF that do occur are primarily passive in nature due to the decrease in blood pressure. In contrast, the current results suggest that active influences contribute to the relatively greater decrease in forehead SkBF during LBNP to presyncope in heat-stressed humans. Since the forehead cutaneous vasculature is under the control of the active vasodilator system (3), the greater actively mediated reduction in forehead SkBF during the heat stress condition could be due to a greater cutaneous vasoconstriction and/or a greater withdrawal of active vasodilation. Regardless of the potential mechanism(s), the results of the current study suggest that facial pallor observed at presyncope is associated with relatively greater decreases in forehead SkBF (compared to the forearm), which are primarily related to the decreases in blood pressure, as well as with an added active influence upon the cutaneous vasculature.

Limitations. Hyperventilation-induced hypocapnia commonly occurs during LBNP performed to presyncope under both normothermic and heat stress conditions (16). Although hypocapnia itself has been shown to reduce forehead but not forearm SkBF during heat stress (15), these reductions were small (~5%) relative to the decreases in SkBF observed at presyncope in the current study (~39%). Furthermore, Fujii et al. (4) reported that hypocapnia (~20 mmHg reduction in end-tidal CO_2) induced by voluntary hyperventilation similarly affected the forehead and forearm cutaneous circulations during passive heat stress sufficient to elevate internal body temperature by 1°C. Therefore, it is unlikely that the greater decreases in forehead SkBF observed in the current study can be attributed to regional differences in the sensitivity of the cutaneous vasculature to hypocapnia. Further work is needed to determine the exact mechanism by which forehead SkBF decreases to a greater extent compared with forearm SkBF during LBNP performed to presyncope. Potential mechanisms could be addressed by the application of drugs (e.g., via microdialysis, intradermal injection, iontophoresis, etc.) that block the sympathetic vasoconstrictor (8) and active vasodilator (9, 19) systems. For cosmetic reasons, we chose to refrain from using such techniques and rather sought to first identify whether differences in forehead and forearm SkBF responses exist, prior to seeking cosmetically favorable approaches to investigate more mechanistic answers. It should also be noted that the current study only examined forehead SkBF, and therefore, the results might not be applicable to SkBF in other areas of the face, particularly those that are considered glabrous in nature (e.g., ears, nose, lips, etc.).

In conclusion, the results of the current study show that relative decreases in SkBF are greater in the forehead compared with the forearm during incremental LBNP to presyncope in normothermic and heat-stressed humans. Although reductions in blood pressure explain the majority of the decrease in SkBF at both skin sites, a significantly greater proportion of actively mediated decreases in forehead SkBF was observed under both thermal conditions. Overall, these results suggest that the forehead cutaneous vasculature is more responsive relative to that of the forearm during incremental LBNP, which could explain the commonly observed facial pallor in individuals at the point of presyncope.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.G. and C.G.C. analyzed data; D.G., R.M.B., M.S.G., J.L.H., and C.G.C. interpreted results of experiments; D.G. prepared figures; D.G. drafted manuscript; D.G., R.M.B., M.S.G., J.L.H., and C.G.C. edited and revised manuscript; D.G., R.M.B., M.S.G., J.L.H., and C.G.C. approved final version of manuscript; R.M.B., M.S.G., J.L.H., and C.G.C. conception and design of research; R.M.B., M.S.G., J.L.H., and C.G.C. performed experiments.

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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

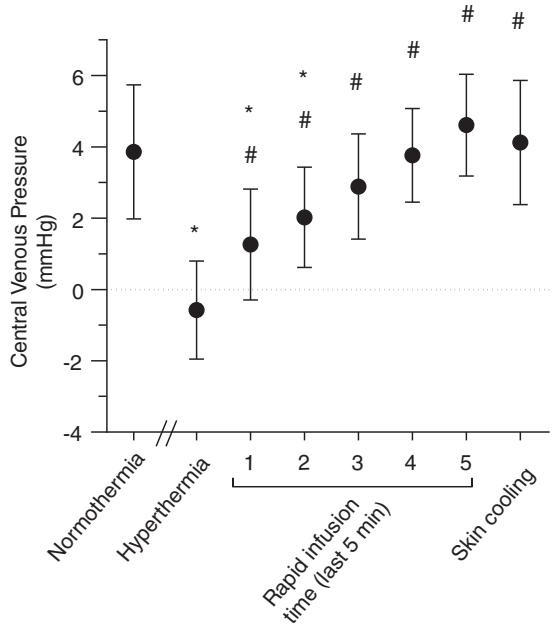

Figure 1. Central venous pressure ($n = 8$) immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the final 5 min of rapid saline infusion while hyperthermic
*Significantly different from normothermia, $P < 0.05$; and #significantly different from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

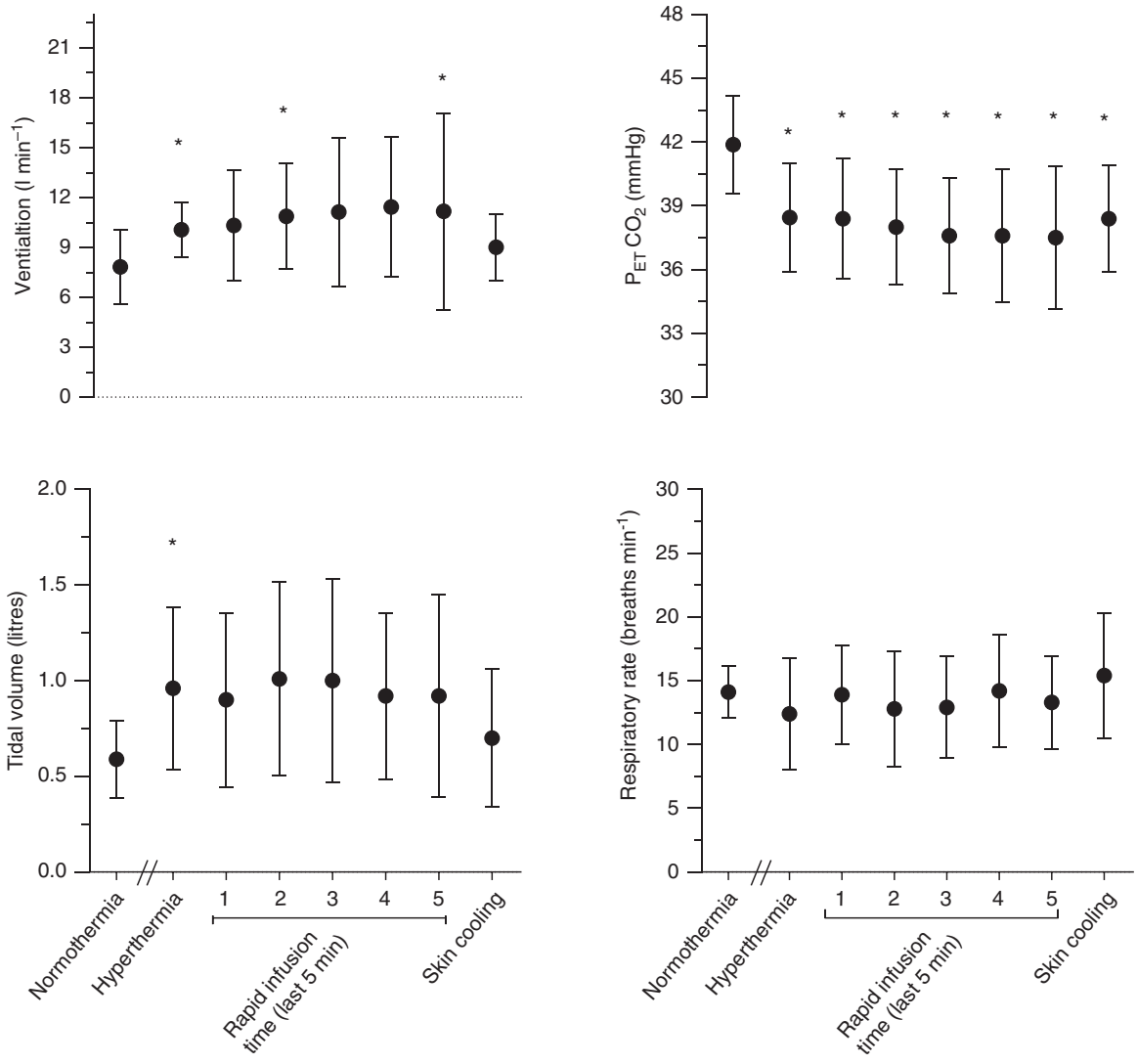

Figure 2. Respiratory responses immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the final 5 min of rapid saline infusion while hyperthermic
*Significantly different from normothermia, $P < 0.05$.

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

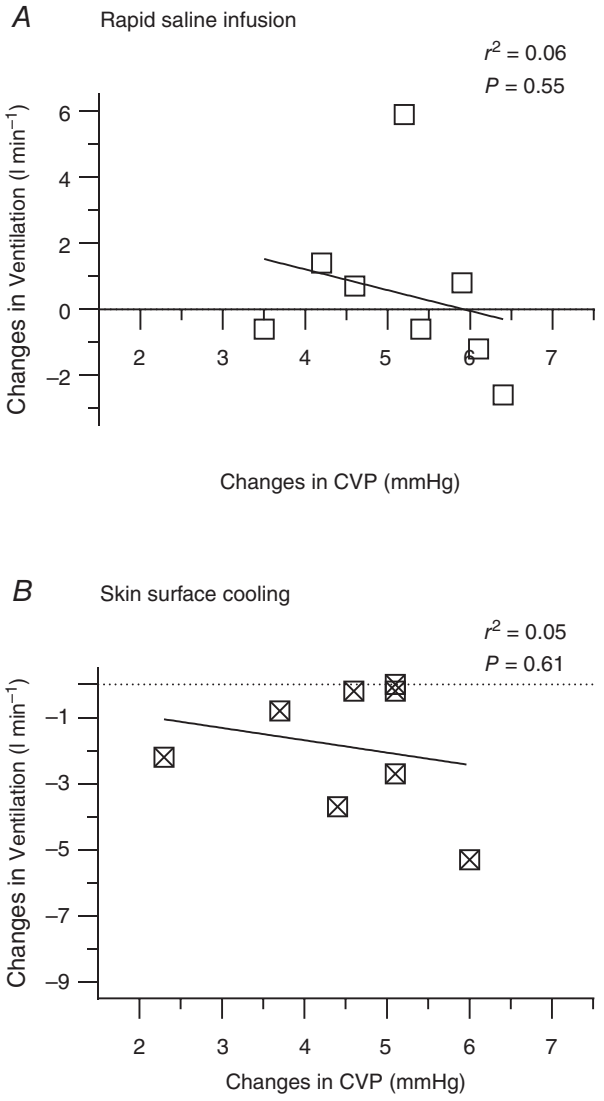

Figure 3. The relationships between changes in ventilation and central venous pressure (CVP) following rapid saline infusion (relative to hyperthermia; *A*) and following skin surface cooling (relative to hyperthermia; *B*)
Data are individual responses to rapid saline infusion ($n = 8$).

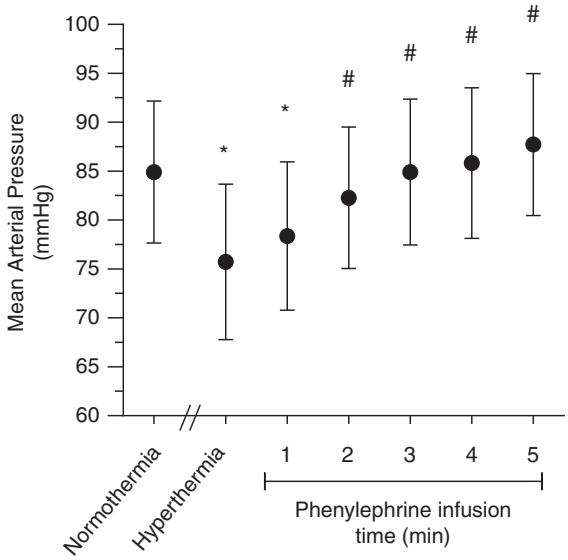

Figure 4. Mean arterial pressure immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the first 5 min of phenylephrine infusion
*Significantly different from normothermia, $P < 0.05$; and #significantly different from hyperthermia, $P < 0.05$.

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

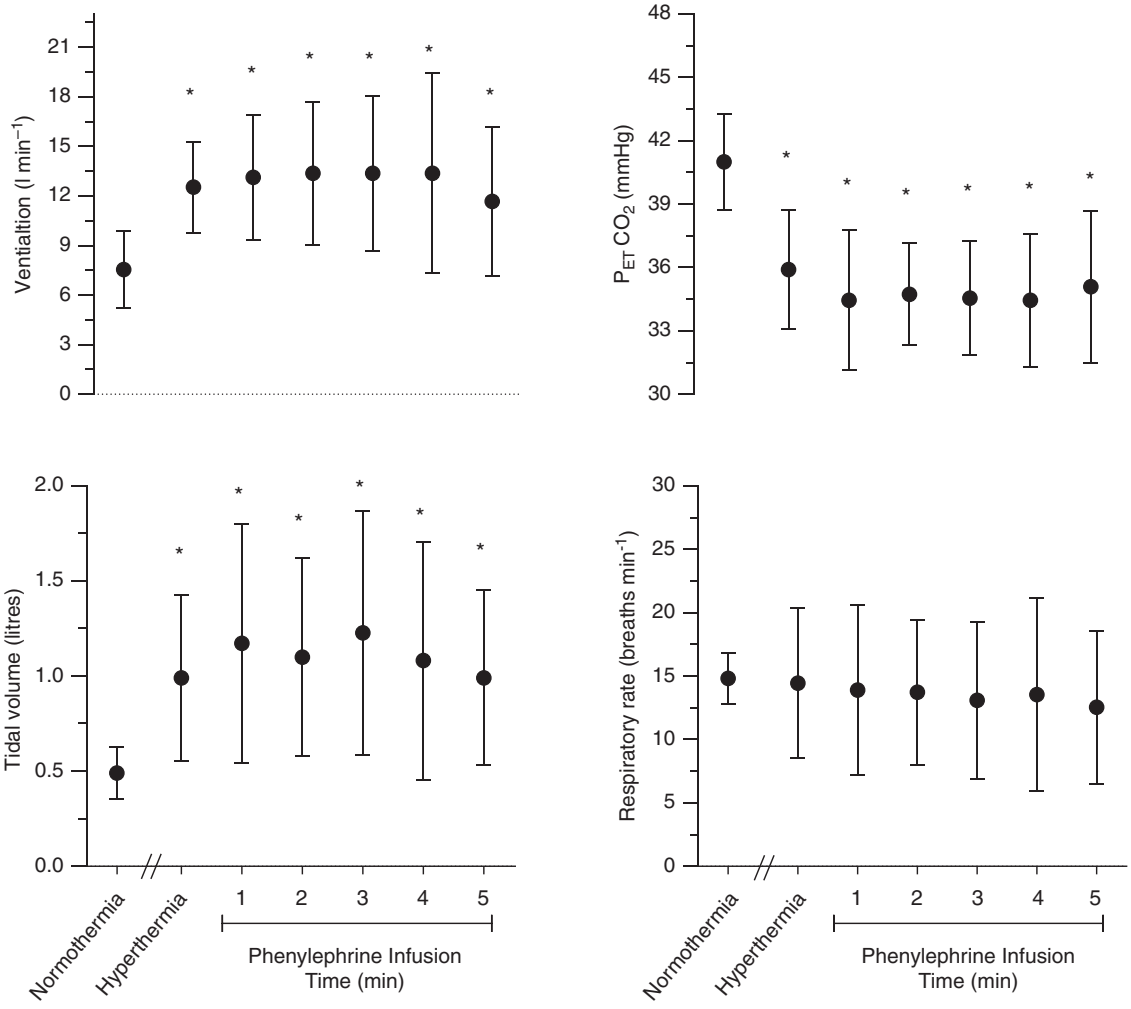

Figure 5. Respiratory responses immediately prior to whole-body passive hyperthermia (normothermia), during hyperthermia alone and throughout the first 5 min of phenylephrine infusion
*Significantly different from normothermia, $P < 0.05$.

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

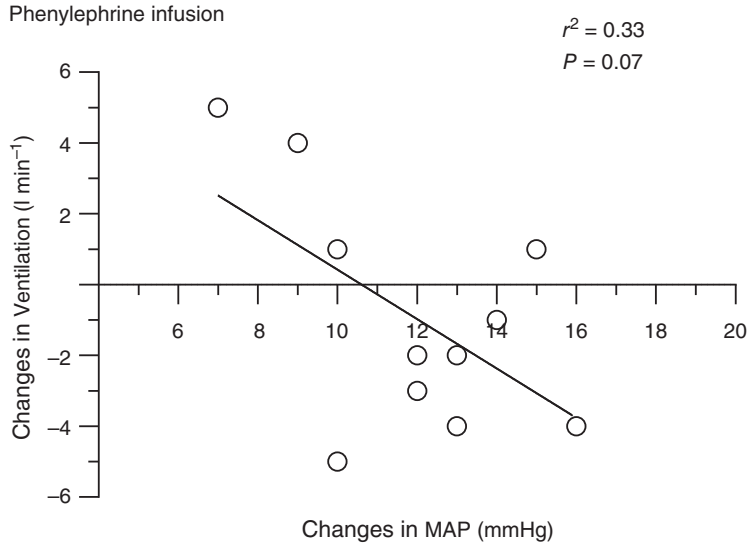

Figure 6. The relationships between changes in ventilation and Mean arterial pressure (MAP) following phenylephrine infusion (relative to hyperthermia)
Data are individual responses to PE infusion (n = 11).

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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Research Paper

Elevated skin and core temperatures both contribute to reductions in tolerance to a simulated haemorrhagic challenge

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New Findings

- **What is the central question of this study?**
  Combined increases in skin and core temperatures reduce tolerance to a simulated haemorrhagic challenge. The aim of this study was to examine the separate and combined influences of increased skin and core temperatures upon tolerance to a simulated haemorrhagic challenge.
- **What is the main finding and its importance?**
  Skin and core temperatures increase during many occupational settings, including military procedures, in hot environments. The study findings demonstrate that both increased skin temperature and increased core temperature can impair tolerance to a simulated haemorrhagic challenge; therefore, a soldier's tolerance to haemorrhagic injury is likely to be impaired during any military activity that results in increased skin and/or core temperatures.

Tolerance to a simulated haemorrhagic insult, such as lower-body negative pressure (LBNP), is profoundly reduced when accompanied by whole-body heat stress. The aim of this study was to investigate the separate and combined influence of elevated skin (T_{skin}) and core temperatures (T_{core}) on LBNP tolerance. We hypothesized that elevations in T_{skin} as well as T_{core} would both contribute to reductions in LBNP tolerance and that the reduction in LBNP tolerance would be greatest when both T_{skin} and T_{core} were elevated. Nine participants underwent progressive LBNP to presyncope on four occasions, as follows: (i) control, with neutral T_{skin} (34.3 ± 0.5°C) and T_{core} (36.8 ± 0.2°C); (ii) primarily skin hyperthermia, with high T_{skin} (37.6 ± 0.2°C) and neutral T_{core} (37.1 ± 0.2°C); (iii) primarily core hyperthermia, with neutral T_{skin} (35.0 ± 0.5°C) and high T_{core} (38.3 ± 0.2°C); and (iv) combined skin and core hyperthermia, with high T_{skin} (38.8 ± 0.6°C) and high T_{core} (38.1 ± 0.2°C). The LBNP tolerance was quantified via the cumulative stress index (in millimetres of mercury × minutes). The LBNP tolerance was reduced during the skin hyperthermia (569 ± 151 mmHg min) and core hyperthermia trials (563 ± 194 mmHg min) relative to control conditions (1010 ± 246 mmHg min; both $P < 0.05$). However, LBNP tolerance did not differ between skin hyperthermia and core hyperthermia trials ($P = 0.92$). The lowest LBNP tolerance was observed during combined skin and core

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hyperthermia (257 ± 106 mmHg min; $P < 0.05$ relative to all other trials). These data indicate that elevated skin temperature, as well as elevated core temperature, can both contribute to reductions in LBNP tolerance in heat-stressed individuals. However, heat stress-induced reductions in LBNP tolerance are greatest in conditions when both skin and core temperatures are elevated.

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Introduction

Whole-body heat stress increases skin and body core temperatures, resulting in elevations in skin blood flow, via both neurally mediated (i.e. reflex; Roddie *et al.* 1957; Johnson *et al.* 1976) and locally mediated cutaneous vasodilatation (i.e. direct; Kellogg *et al.* 1999), along with parallel reductions in central blood volume (Rowell *et al.* 1969; Minson *et al.* 1998; Crandall *et al.* 2008). Such increases in skin blood flow, coupled with insufficient cutaneous vasoconstriction during progressive central hypovolaemia (Crandall *et al.* 2010; Pearson *et al.* 2013), are problematic for the maintenance of arterial blood pressure. The resultant effect is a profound reduction in tolerance to a simulated haemorrhagic challenge, such as progressive lower-body negative pressure (LBNP) to presyncope (Lind *et al.* 1968; Allan & Crossley, 1972; Johnson *et al.* 1973; Wilson *et al.* 2006; Keller *et al.* 2009).

When heat stress-induced reductions in central blood volume are countered via rapid saline/dextran infusion during the heat stress, LBNP tolerance is returned to non-heat stress levels (Keller *et al.* 2009). Likewise, the cardiovascular responses to a mild subpresyncopal orthostatic challenge are improved when skin temperature is returned to normothermic values (Lucas *et al.* 2010) or reduced below normothermic values (Wilson *et al.* 2002). These improvements with skin-surface cooling were associated with increased arterial blood pressure, perhaps owing in part to a transfer of blood volume from the skin to the central vasculature. Furthermore, a slightly lower skin temperature modestly improved LBNP tolerance in hyperthermic individuals following exercise (Pearson *et al.* 2014), although in that protocol skin temperatures were still elevated above normothermic values, and a normothermic reference LBNP challenge was not imposed. It is unknown whether returning skin surface temperatures to normothermic values, without actively lowering skin temperature below normothermic values, would return LBNP tolerance to that observed with a normothermic LBNP challenge, despite core temperature remaining elevated. That is, given the parallel effects of skin temperature on skin blood flow, and the presumed reciprocal effect on central blood volume, reducing skin surface temperature in heat-stressed individuals may therefore normalize LBNP tolerance, despite core temperature remaining elevated. However, little is known regarding the role of primarily skin hyperthermia *versus* primarily core hyperthermia on LBNP tolerance. The latter conditions may occur during exercise with adequate evaporative cooling of the skin surface, resulting in elevated core temperatures with relatively normothermic skin temperatures, whereas the former conditions may be experienced during brief passive exposure to high environmental temperatures. Investigating the effect of separate and combined increases in skin and core temperature upon LBNP tolerance may provide insight towards the treatment of heat-stressed individuals who are experiencing a haemorrhagic challenge. Therefore, the aim of this study was to examine the separate and combined influences of increased skin and body core temperatures upon tolerance to a simulated haemorrhagic challenge. Specifically, we hypothesized that LBNP tolerance would be reduced when skin temperature is primarily elevated (with minimal accompanying increases in core temperature), as well as when core temperature is primarily elevated (with minimal accompanying increases in skin temperature). Furthermore, we hypothesized that the greatest reduction in LBNP tolerance would occur with combined increases in skin and core temperatures.

Methods

Ethical approval

Nine subjects (eight men) participated in this study. Subject characteristics (mean ± SD) were as follows: age, 29 ± 5 years; height, 184 ± 12 cm; and weight, 82.3 ± 13.2 kg. The one female participant was tested in the follicular phase of the menstrual cycle for all trials. Subjects were not taking medications, were non-smokers, were free of any known cardiovascular, metabolic or neurological diseases and refrained from alcohol, caffeine and exercise for 24 h before the study. Subjects were informed of the purpose, procedures and risks of the study before providing their 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 Dallas (reference number: STU 0602011-099). The study

conformed to the standards set by the *Declaration of Helsinki*.

Instrumentation and experimental protocol

On experimental days, adequate hydration was confirmed via urine specific gravity (<1.020), measured using a digital refractometer. Approximately 2 h before the onset of data collection, subjects swallowed an ingestible telemetry pill for the measurement of core (intestinal) temperature (HQ, Palmetto, FL, USA). Subjects voided their bladder before nude body mass was recorded, which was also obtained after each trial to provide an indication of fluid loss. Height was measured using a stadiometer. Mean skin temperature was measured from the weighted average temperature across six sites (Taylor *et al.* 1989) using thermocouples fixed to the skin with porous adhesive tape. Arterial blood pressure was continuously measured non-invasively using photoplethysmography (Finometer Pro; FMS, Amsterdam, The Netherlands), while intermittent blood pressure was obtained via auscultation of the brachial artery (Tango; Suntech Medical Instruments, Raleigh, NC, USA). Heart rate was obtained from an ECG (Agilent, Munich, Germany) that was interfaced with a cardiotachometer (1000 Hz sampling rate; CWE, Ardmore, PA, USA). Cardiac output and stroke volume were measured using a foreign gas rebreathing system (Innovision A/S, Odense, Denmark). Mean blood flow velocity in the right middle cerebral artery served as an index of cerebral perfusion, which was measured using 2 MHz pulsed Doppler ultrasound (Multiflow; DWL Elektronische Systeme, Singen, Germany). The Doppler probe was maintained in position throughout the protocol using a commercially available headpiece. The partial pressure of end-tidal carbon dioxide (P_{ET,CO_2}) was sampled from a nasal cannula connected to a capnograph (9004 Capnocheck® Plus; Smiths Medical International Ltd, Watford, UK).

Skin blood flow was measured from the dorsal forearm via laser Doppler probes (Periflux413; Perimed, North Royalton, OH, USA) connected to a laser-Doppler flowmeter (Periflux5010; Perimed). Probes were fitted inside a local heating device (Peritemp 4005; Perimed) capable of controlling local skin temperature at that site. The local heater and laser Doppler probe assembly was placed over a fine-wire skin thermocouple (RET-4, Type T thermocouple; Physitemp Instruments Inc., Clifton, NJ, USA). Local heaters were continually adjusted to match mean skin temperatures under the water-perfused suit. At the end of each trial, local heat-induced maximal skin blood flow was assessed via 30 min of local heating at 42°C measured at the skin and local heater interface.

Following instrumentation, subjects rested in the supine position for 30 min to allow for the stabilization of fluid shifts. Baseline data were subsequently obtained. Subjects were then exposed to one of four trials, on separate days and in a randomized order. Of these four trials, one was a control trial, in which skin and core temperatures remained at neutral levels throughout the protocol. The three remaining trials were designed to elicit either an increase in primarily skin temperature (skin hyperthermia), primarily core temperature (core hyperthermia) or both skin and core temperatures (combined skin and core hyperthermia). Each trial was separated by at least 3 days. To obtain the desired skin and core temperatures, subjects donned a water-perfused tube-lined suit (Med-Eng, Ottawa, ON, Canada) that covered their entire body except for the head, hands, feet and the left forearm, on which skin blood flow was measured. The suit permitted the control of whole-body skin and core temperatures by adjusting the temperature of the water perfusing the suit.

During the control trial, 34°C water was perfused through the suit. During the three trials where either skin or core temperatures were increased, subjects were exposed to slightly different heating protocols before the onset of a simulated haemorrhagic challenge (LBNP). During the skin hyperthermia trial, water at 48–50°C was perfused through the suit to elevate skin temperatures quickly to ∼38°C while limiting increases in core temperature. This period of heating, lasting between 20 and 25 min, was immediately followed by the LBNP challenge. During the core hyperthermia trial, water at 48–50°C was perfused through the suit, elevating skin temperatures to ∼38°C. This period of heating was sustained for ∼50 min, resulting in an increase in core temperature of ∼1.2°C. Upon reaching this increase in core temperature, the temperature of the water perfusing the suit was reduced to ∼10°C for ∼6 min and thereafter held at ∼37°C to return skin temperatures to baseline values (∼34–35°C), while minimizing decreases in core temperature. The LBNP challenge then ensued. During the skin and core hyperthermia trial, water at 48–50°C was perfused through the suit, elevating skin temperatures to ∼38°C. This period of heating was sustained for ∼50 min, resulting in an increase in core temperature of ∼1.2°C, after which subjects underwent the LBNP challenge. In each trial, all measurements were obtained at the end of the heating protocol specific to that trial, immediately before the onset of LBNP.

For all trials, LBNP began at 20 mmHg for 3 min, followed by increasing negative pressure by 10 mmHg in 3 min stages until presyncope. The termination of LBNP was based upon the subject self-reporting of feeling faint and/or nauseous, a rapid and progressive decrease in blood pressure resulting in sustained systolic blood pressure of <80 mmHg and/or a relative and pronounced bradycardia. Throughout LBNP, arterial blood pressures were also measured at the brachial artery by automated auscultation (Tango; Suntech Medical

Instruments). Tolerance to LBNP was quantified using the cumulative stress index (CSI; Luft *et al.* 1976), calculated by summing the time at each level of LBNP multiplied by LBNP level (i.e. 20 mmHg × 3 min + 30 mmHg × 3 min + 40 mmHg × 3 min, etc.) until presyncope.

Data analysis

Temperature and haemodynamic data were collected via a data-acquisition system (Biopac Systems Inc., Santa Barbara, CA, USA). Data were averaged across 60 s at baseline and after the desired increase in core and mean skin temperatures prior to LBNP. Cardiac output and stroke volume were measured at normothermic baseline and before the onset of LBNP in each trial. Skin blood flow is reported as cutaneous vascular conductance [in arbitrary units (a.u.) per millimetre of mercury], calculated as skin blood flow units (in arbitrary units) divided by mean arterial pressure (in millimetres of mercury). Changes in cutaneous vascular conductance during LBNP are expressed as a percentage of the maximal cutaneous vascular conductance values obtained after 30 min of local heating. To express data throughout LBNP of varying durations between trials, thermal data during LBNP were averaged over a 30 s period immediately preceding 20, 40, 60 and 80% of maximal CSI, as well as during a 15 s period immediately preceding the termination of LBNP (i.e. presyncope).

With the exception of CSI, data were statistically analysed using a two-way repeated-measures ANOVA with main factors of thermal condition (control, skin hyperthermia, core hyperthermia and combined skin and core hyperthermia) and time. Analysis of body temperatures was completed with levels for time of baseline, pre-LBNP, 20, 40, 60 and 80% CSI, and presyncope. Analysis of blood pressure and cutaneous vascular conductance responses was completed with levels of time of baseline, pre-LBNP, 90, 80, 70, 60, 50, 40, 30, 20 and 10 s prior to presyncope, and presyncope). These data were analysed during the final 90 s prior to presyncope and cessation of LBNP in order to examine the cutaneous vascular responses to arterial baroreceptor unloading in all trials. The CSI data were analysed via one-way repeated-measures ANOVA. *Post hoc* analyses were performed using repeated-sampling corrected paired *t* tests (Bonferroni). Data were analysed using GraphPad Prism (version 6; GraphPad Software, Inc., La Jolla, CA, USA) and SPSS v20 (IBM, Armonk, NY, USA), with *a priori* statistical significance set at $P < 0.05$. Data are reported as mean values ± SD.

Results

Skin and core temperature responses for each trial are depicted in Fig. 1. At baseline, mean skin and

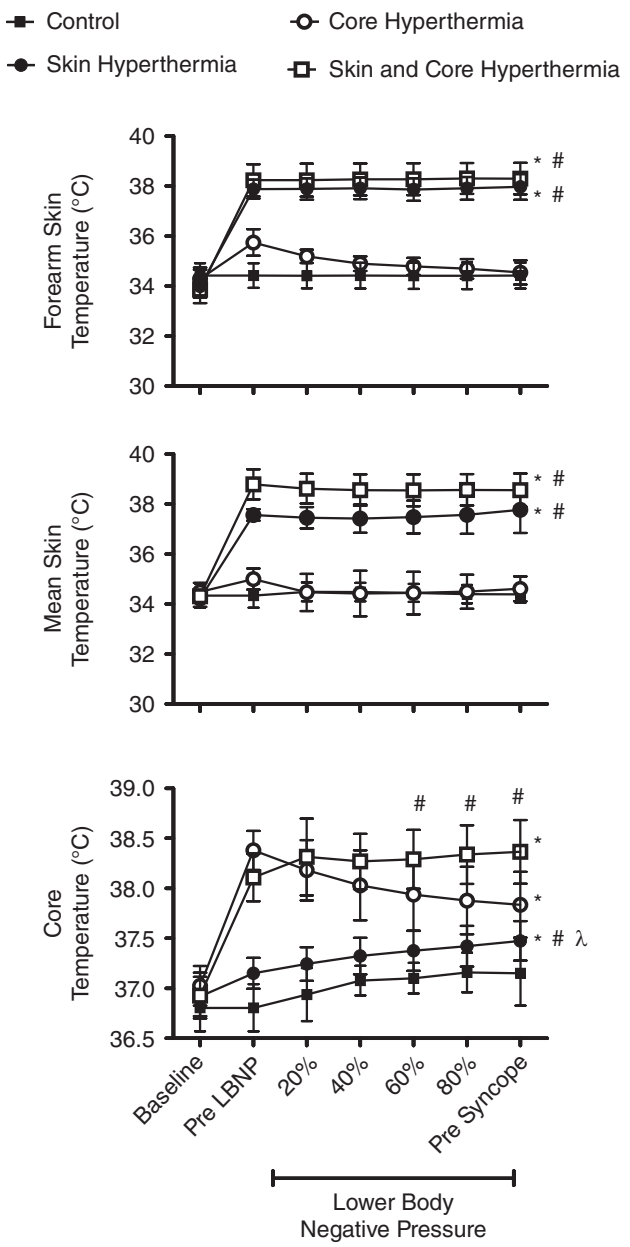

Figure 1. Internal core and mean skin temperatures in each trial prior to and throughout lower-body negative pressure (LBNP) to presyncope
Skin and core temperature responses to the applied thermal conditions and subsequent LBNP to presyncope. Forearm skin temperature was obtained from the site where skin blood flow was assessed. Mean skin temperature is the weighted average of temperatures at six locations. Core temperature was from the ingestible temperature pill. *Different from the control trial (main effect, $P < 0.05$). #Different from the core hyperthermia trial at that respective time point when symbols are placed within the figure or a main effect when the symbol is presented at the side of the figure ($P < 0.05$). λDifferent from the combined skin and core hyperthermia trial (main effect, $P < 0.05$).

core temperatures were not different between trials (all $P > 0.05$). During the control trial, mean skin and core temperatures did not change from baseline throughout LBNP (both $P > 0.05$). During the core hyperthermia trial, mean skin temperatures were similar to baseline values prior to and throughout LBNP (all $P > 0.05$). Mean skin temperatures were increased at pre-LBNP, relative to baseline, during both the skin hyperthermia and the combined skin and core hyperthermia trials (both $P < 0.05$) and remained greater throughout LBNP relative to control and core hyperthermia trials (all $P < 0.05$). By experimental design, forearm skin temperatures at the location of the laser-Doppler probe followed the changes in mean skin temperature during each trial.

During the skin hyperthermia trial, core temperature increased slightly throughout LBNP such that it was greater relative to the control trial ($P < 0.001$). By design, core temperature was elevated during the core hyperthermia and combined skin and core hyperthermia trials, and remained greater throughout LBNP during these trials relative to the control and skin hyperthermia trials (all $P < 0.05$). In the latter stages of LBNP, core temperature declined slightly during the core hyperthermia trial and was lower relative to the combined skin and core hyperthermia trial ($P < 0.05$).

Tolerance to the LBNP challenge during each trial is depicted in Fig. 2. The LBNP tolerance was greatest during the control trial (1010 ± 246 mmHg min, $P < 0.001$) relative to both the skin hyperthermia (569 ± 151 mmHg min) and core hyperthermia trials (563 ± 194 mmHg min; both $P < 0.05$). However, despite large differences in skin and core temperatures, LBNP tolerance was not different between skin hyperthermia and core hyperthermia trials ($P = 0.92$). The lowest LBNP tolerance occurred during the combined skin and core hyperthermia trial (257 ± 106 mmHg min; $P < 0.001$ relative to all other trials).

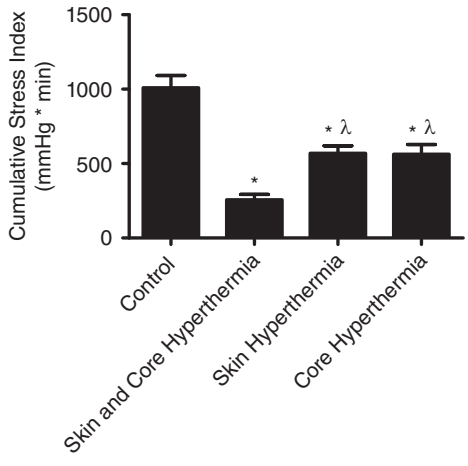

Figure 2. Lower-body negative pressure tolerance expressed as cumulative stress index for each trial
The LBNP tolerance was highest in the control trial. The LBNP tolerance was not different between core hyperthermia and skin hyperthermia trials, but both were higher relative to the combined skin and core hyperthermia trial. *Different from the control trial ($P < 0.05$). λDifferent from the combined skin and core hyperthermia trial ($P < 0.05$).

Cutaneous vascular conductance responses are shown in Fig. 3. Prior to LBNP, cutaneous vascular conductance was not different between the combined skin and core hyperthermia (89 ± 14% max), skin hyperthermia (77 ± 14% max) and core hyperthermia trials (76 ± 11% max, all $P > 0.05$), while each was greater than cutaneous vascular conductance in the control trial (23 ± 14% max; all $P < 0.01$). At presyncope, cutaneous vascular conductance was reduced slightly relative to pre-LBNP during the control trial (−8 ± 46% max), although the variability of this response was fairly large. The magnitude of reduction in cutaneous vascular conductance, from pre-LBNP to presyncope, did not differ between the combined skin and core hyperthermia (−12 ± 14% max) and skin hyperthermia trials (−6 ± 34% max, $P = 0.73$). During the core hyperthermia trial, cutaneous vascular conductance decreased by 65 ± 8% max from pre-LBNP to presyncope ($P < 0.05$ relative to all other trials) as a result of the combined effect of reduced skin temperature and LBNP.

Haemodynamic responses are shown in Table 1. Cardiac output was not different between trials at baseline (all $P > 0.05$) but increased in all thermal trials at pre-LBNP (all $P < 0.05$). Changes in heart rate, middle cerebral blood velocity and P_{ET,CO_2} in response to LBNP were not different between trials (all $P > 0.05$). Mean arterial pressure decreased during LBNP in all trials (all $P < 0.05$; Fig. 4), was slightly lower during LBNP in the combined skin and core hyperthermia trial relative to all other trials, yet was not different between trials at baseline, pre-LBNP and presyncope (all $P > 0.05$).

Body weight was reduced following the skin hyperthermia, core hyperthermia and combined skin and core hyperthermia trials (−0.9 ± 0.3, −1.9 ± 0.7 and −2.1 ± 0.7% body mass, respectively) relative to the control trial, where body weight was unchanged (all $P < 0.05$). The magnitude of body weight loss was greatest during the core hyperthermia and combined skin and core hyperthermia trials relative to the skin hyperthermia trial (both $P < 0.05$), while there was no difference in body weight loss between core hyperthermia and combined skin and core hyperthermia trials ($P = 0.50$).

Discussion

The aim of this study was to investigate the separate and combined contributions of elevated skin and core temperatures on LBNP tolerance during heat stress.

As expected, combined elevations in skin and core temperature with passive heat stress greatly reduced LBNP tolerance relative to the control conditions. Although primarily skin hyperthermia and primarily core hyperthermia both reduced LBNP tolerance relative to the control trial, LBNP tolerance was not different between these separate hyperthermic conditions. These data demonstrate that elevations in skin and core temperatures that are primarily separate from one another can both contribute to impaired LBNP tolerance during passive heat stress. Interestingly, the similar reduction in LBNP tolerance with increases in either primarily skin or primarily core temperature occurred despite a markedly different cutaneous vascular conductance during the LBNP challenge between these trials.

Hyperthermia and LBNP tolerance

Whole-body heat stress increases both skin and core body temperatures, which is accompanied by cutaneous vasodilatation that, in extreme conditions, can increase skin blood flow upwards of 7 l min^{-1} (Rowell *et al.* 1969). It is well established that whole-body passive heat stress severely compromises LBNP tolerance (Lind *et al.* 1968; Allan & Crossley, 1972; Johnson *et al.* 1973; Wilson *et al.* 2006; Keller *et al.* 2009). Prior to the present study, however, the primarily separate contribution of elevated skin and core temperatures to this reduced LBNP tolerance was unknown. As expected, we observed substantially lower LBNP tolerance when skin and core temperatures were simultaneously elevated by whole-body passive heat stress (Fig. 2). The LBNP tolerance was also reduced by increases in primarily skin temperature, as well as increases in primarily core temperature (Fig. 2). These data indicate that LBNP tolerance is reduced to a similar extent regardless of whether heat stress results primarily in elevated skin or core temperature, although a further reduction in LBNP tolerance occurred when both were elevated simultaneously. Taken together, these data suggest that elevated skin and core temperatures can both contribute to a compromised LBNP tolerance during heat stress, but when elevations in skin and core temperature are combined the reduction in LBNP tolerance is even further compromised.

Compromised LBNP tolerance in heat-stressed individuals has been attributed, in part, to reduced central blood volume (Keller *et al.* 2009) and an increased skin blood flow (Rowell *et al.* 1969; Minson *et al.*

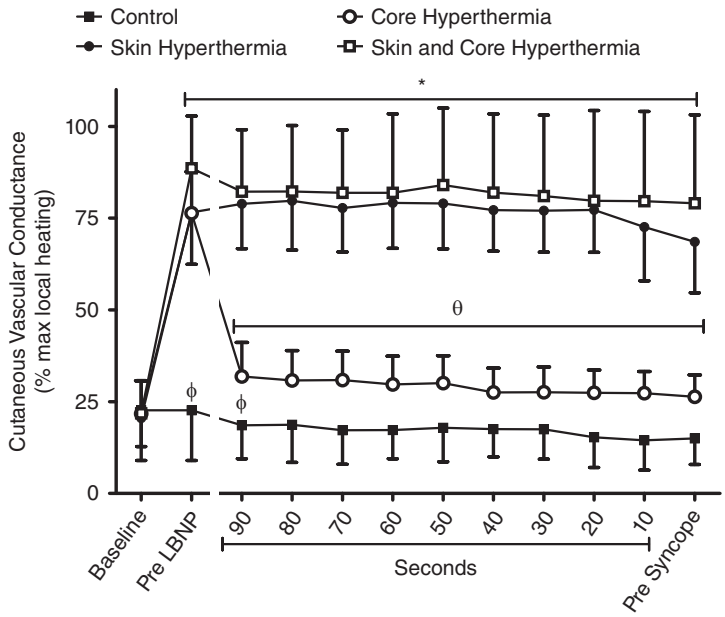

Figure 3. Cutaneous vascular conductance expressed as a percentage of maximum in each trial prior to and throughout the final 90 s of LBNP to presyncope
Cutaneous vascular conductance was elevated to a similar exent relative to baseline in all three heat stress trials compared with the control conditions. During the final 90 s of LBNP, cutaneous vascular conductance remained higher than control values in the skin hyperthermia and combined skin and core hyperthermia trials, but was reduced to control trial values in the core hyperthermia trial. ∗Different from the control trial ($P < 0.05$). $^{\theta}$Cutaneous vascular conductance in the core hyperthermia trial was different from that in the skin hyperthermia and combined hyperthermia trials ($P < 0.05$). $^{\varphi}$Cutaneous vascular conductance was different between control and core hyperthermia trials at that respective time point ($P < 0.05$).

Table 1. Haemodynamic and cerebrovascular measurements at baseline, during the applied thermal conditions and at presyncope for each trial

	Control		Skin and core hyperthermia			Skin hyperthermia			Core hyperthermia		
Parameter	Baseline/ pre-LBNP	Presyncope	Baseline	Pre-LBNP	Presyncope	Baseline	Pre-LBNP	Presyncope	Baseline	Pre-LBNP	Presyncope
Mean arterial pressure (mmHg)	83 ± 12	58 ± 8†	78 ± 11	75 ± 7	54 ± 5*†	81 ± 6	80 ± 5	57 ± 7*†	81 ± 12	80 ± 8	60 ± 17*†
Cardiac output (l min^{-1})	6.7 ± 1.3	—	6.5 ± 1.0	9.7 ± 2.0*‡	—	6.5 ± 0.7	8.2 ± 0.6*‡¶	—	6.8 ± 1.3	8.9 ± 1.6*‡	—
Heart rate (beats min^{-1})	57 ± 10	101 ± 29*	59 ± 13	96 ± 16*	121 ± 30*	58 ± 14	71 ± 7	117 ± 26*†	59 ± 10	77 ± 12	116 ± 20*†
Stroke volume (ml)	106 ± 17	—	111 ± 22	105 ± 28	—	113 ± 14	116 ± 11	—	116 ± 19	107 ± 21*	—
CVC (a.u. mmHg^{-1})	0.6 ± 0.4	0.4 ± 0.2	0.5 ± 0.2	2.3 ± 0.6*‡	2.1 ± 0.8*‡	0.5 ± 0.2	2.1 ± 0.6*‡	1.9 ± 0.6*‡	0.5 ± 0.3	1.9 ± 0.8*‡¶	0.7 ± 0.3 †§
CVC (% max)	23 ± 14	15.0 ± 7.1	22 ± 9	89 ± 14*‡	79 ± 24*‡	21 ± 8	77 ± 14*‡	69 ± 14*‡	22 ± 9	76 ± 11*‡¶	26 ± 6†§
MCAv (cm s^{-1})	68.4 ± 6.4	41.9 ± 8.7*	65.9 ± 8.0	58.4 ± 9.2	36.7 ± 8.9*†	71.2 ± 8.6	63.8 ± 10.7	41.3 ± 12.3*†	67.1 ± 11.6	61.6 ± 14.8	41.00 ± 10.5*†
P_{ET,CO_2} (mmHg)	43 ± 2	30 ± 8*	44 ± 3	38 ± 6	26 ± 9*†	43 ± 4	40 ± 4	29 ± 5*†	42 ± 4	38 ± 6	29 ± 6*†

Abbreviations: CVC, cutaneous vascular conductance; LBNP, lower-body negative pressure; MCAv, middle cerebral artery blood velocity; and P_{ET,CO_2}, partial pressure of end-tidal carbon dioxide. Values are means ± SD for nine participants, except cardiac output, which is from eight individuals. Mean arterial pressure and CVC data are from nine participants, except at presyncope in both the combined skin and core hyperthermia trial and the core hyperthermia trial, where data are from eight participants. *Different from baseline within the trial ($P < 0.05$). †Different from Pre-LBNP within trial ($P < 0.05$). ‡Different from the control trial at that respective time point ($P < 0.05$). ¶Different from the combined skin and core hyperthermia trial at that respective time point ($P < 0.05$). §Different from both skin hyperthermia and combined skin and core hyperthermia trials within that respective time point ($P < 0.05$).

1998; Crandall *et al.* 2008) coupled with insufficient cutaneous vasoconstriction during LBNP (Crandall *et al.* 2010; Pearson *et al.* 2013). Prior to LBNP, mean skin temperatures and cutaneous vascular conductance were elevated to a similar extent between the skin hyperthermia and combined skin and core hyperthermia trials. Though speculative, these observations suggest that reductions in central blood volume that accompany cutaneous vasodilatation (Rowell *et al.* 1969; Minson *et al.* 1998; Crandall *et al.* 2008) were also likely to be similar between these two trials. However, central blood volume was not measured in the present study to confirm this speculation. Furthermore, the reduction in cutaneous vascular conductance at presyncope was rather minimal (~6–12%) and not different between these two trials. Despite these important similarities, the reduction in LBNP tolerance was greater in the combined skin and core hyperthermia trial relative to the skin hyperthermia trial (Fig. 3). The LBNP tolerance was not different between the core hyperthermia and skin hyperthermia trials, despite a far greater reduction in cutaneous vascular conductance during the LBNP challenge in the core hyperthermia trial (45 *versus* 8%, respectively; Fig. 3). These data suggest that the level of cutaneous vasodilatation prior to LBNP and/or the reductions in cutaneous vascular conductance during LBNP may have only a minimal influence on LBNP tolerance in the thermal conditions investigated herein, and/or different mechanisms may be responsible for the reduction in LBNP tolerance between these thermal trials. This suggestion is in contrast to previous data suggesting that an insufficient cutaneous vasoconstriction whilst arterial blood pressure declines may be a leading factor contributing to impaired LBNP tolerance in heat-stressed individuals (Crandall *et al.* 2010; Pearson *et al.* 2013). Although speculative, this apparent contrast suggests that the changes in central blood volume and cutaneous vascular conductance during heat stress may not share a linear relationship. That is, during LBNP the central blood volume declines owing to blood pooling in the lower limbs; however, if central blood volume was different before the onset of LBNP between the different thermal conditions, this may help to explain differences in tolerance between conditions despite similarities or differences in cutaneous vascular conductance responses between these trials. Central blood volume was not measured in this study and therefore we do not know how it was influenced prior to and during LBNP between the different thermal trials. In order to understand more fully the influence of cutaneous vascular conductance upon arterial blood pressure regulation during a combined heat stress and haemorrhagic challenge and to reconcile the present data with the aforementioned proposed hypotheses (Crandall *et al.* 2010; Pearson *et al.* 2013), it may be necessary to measure central blood volume during similar trials.

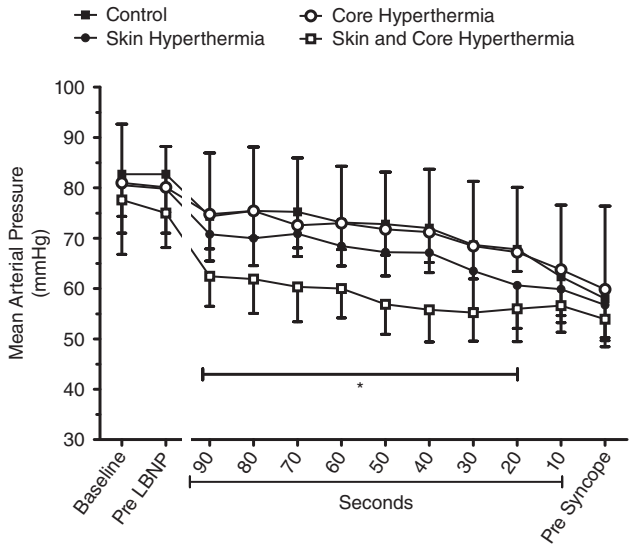

Figure 4. Blood pressure responses to each trial prior to and throughout the final 90 s of LBNP to presyncope
Mean arterial pressure was not different between trials at baseline, pre-LBNP or presyncope. However, mean arterial pressure was lower in the combined skin and core hyperthermia trials relative to both the control and core hyperthermia trials in the time period of 90–20 s prior to presyncope. There were no differences in mean arterial pressure between the skin hyperthermia and the combined skin and core hyperthermia trial at any time point. *Combined skin and core hyperthermia trial was different from both the control and the core hyperthermia trial ($P < 0.05$).

In heat-stressed individuals, cooling of the skin surface improves the cardiovascular and cerebrovascular responses during subpresyncopal upright tilt testing (Wilson *et al.* 2002) and LBNP (Lucas *et al.* 2010), relative to when skin surface temperature remains elevated. These improved haemodynamic responses are presumably attributable to decreases in skin temperature and accompanying cutaneous vasoconstriction, which return skin blood flow toward baseline values, thereby enabling a prolonged maintenance of central blood volume, arterial blood pressure and cerebral blood flow. Such responses would suggest a greater capacity to withstand a haemorrhagic insult when skin surface temperature is actively reduced below typical normothermic values (~34°C). Consistent with this suggestion, the present study showed that LBNP tolerance improved relative to the combined skin and core hyperthermia trial when skin temperature was returned to control values during the core hyperthermia trial. That said, despite a lowered cutaneous vascular conductance accompanying reduced skin temperature in the core hyperthermia trial, LBNP tolerance was similar to the skin hyperthermia trial where skin temperatures and cutaneous vascular conductance were higher throughout LBNP. Furthermore,

LBNP tolerance was reduced during the core hyperthermia trial relative to the control trial (Fig. 2), despite no difference in cutaneous vascular conductance across the final 80 s of LBNP between these two trials (Fig. 3). These findings suggest that if core temperature remains elevated, returning the skin to a neutral temperature may not be sufficient to preserve LBNP tolerance completely relative to thermoneutral conditions. Therefore, these observations suggest that in a heated individual the skin surface needs to be cooled perhaps to levels lower than normothermic skin temperatures to restore tolerance to a haemorrhagic challenge relative to when the individual is in a thermoneutral state.

Each thermal trial caused a greater reduction in body mass compared with the control trial. Furthermore, body mass loss was greatest during the core hyperthermia and combined skin and core hyperthermia trials relative to the skin hyperthermia trial (∼1.9%, 2.1% and 0.9% body mass, respectively). Differences in body mass loss were likely to be a result of differences in sweat loss owing to the different duration of passive heat stress necessary to create the applied thermal conditions, in combination with minimal increases in core temperature during the skin hyperthermia trial. Fluid loss associated with heat stress can influence tolerance to a simulated haemorrhagic challenge. We previously reported that passive heat stress-induced reductions in total body water of ∼1.6% body mass reduced LBNP tolerance by ∼225 CSI units relative to conditions in which fluid loss was prevented (Lucas *et al.* 2013). Given this observation, it is unlikely that the small reductions in total body water can account for the observed reductions in LBNP tolerance during the skin hyperthermia trial relative to the control trial. It is noteworthy that reductions in body mass between core hyperthermia and combined skin and core hyperthermia were similar despite a significant difference in LBNP tolerance between these trials. Therefore, although body mass loss primarily owing to sweat loss may explain some of the decrease in tolerance between thermal trials, its impact upon LBNP tolerance is likely to be secondary to that of thermal stress (Lucas *et al.* 2013).

Limitations

During all trials, core temperature changed slightly throughout LBNP (Fig. 1), although the magnitude of this change was minimal, except for the core hyperthermia trial when the skin was cooled. We cannot exclude the possibility that a decline in core temperature during the core hyperthermia trial may have improved LBNP tolerance relative to the combined skin and core hyperthermia trial. That said, despite this reduction in core temperature, LBNP tolerance to the core hyperthermia trial remained substantially reduced relative to the control trial.

Perspectives, significance and conclusions

Elevations in internal core temperature that are largely separate from skin temperatures allow a unique insight into the role of core hyperthermia upon LBNP tolerance. Such conditions occur during exercise with adequate evaporative cooling of the skin surface. Likewise, elevations in skin temperature that are largely separate from increased internal core temperatures would allow a unique insight into the role of skin hyperthermia upon LBNP tolerance. Such conditions may be found during a brief exposure to high environmental temperatures. Both skin and core temperatures can increase appreciably in various settings where blood pressure regulation can be compromised owing to the combined effects of increased thermal strain and the physical demands of the occupation, such as in military personnel, firefighters and construction workers, and where the risk of a haemorrhagic injury is also elevated. For example, during military procedures in hot environments the skin and internal core temperatures can increase to the levels achieved within the present study (Buller *et al.* 2008). As we observed that increases in primarily skin temperature, as well as increases in primarily core temperature, can both reduce tolerance to an LBNP challenge, an increase in both skin and core temperatures is not a prerequisite to reduce tolerance to a haemorrhagic injury. In other words, the ability of individuals to tolerate a haemorrhagic injury is likely to be reduced at any point during day-to-day activities that result in increased skin and/or core temperatures. These data also indicate that any form of body core or skin temperature reduction towards neutral/control temperatures can improve tolerance to a haemorrhagic injury and may therefore be beneficial in the treatment of a haemorrhaging hyperthermic individual in the prehospital setting.

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Additional information

Competing interests

None declared.

Author contributions

All experiments took place at the Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital, Dallas, TX, USA. J.P. and C.G.C. contributed to conception and design of the experiments. J.P., C.G.C., R.A.I.L., Z.J.S. and D.G. contributed to acquisition, analysis and/or interpretation of data and experimental results. J.P., R.A.I.L., Z.J.S., D.G. and C.G.C. contributed to drafting the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Active and passive heat stress similarly compromise tolerance to a simulated hemorrhagic challenge

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[1]*Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas and University of Texas Southwestern Medical Center, Dallas, Texas;* [2]*School of Health Sciences, Cardiff Metropolitan University, Cardiff, United Kingdom;* [3]*Center for Global Health Research, Umea University, Umea, Sweden; and* [4]*China Institute of Sport Science, Beijing, China*

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Pearson J, Lucas RA, Schlader ZJ, Zhao J, Gagnon D, Crandall CG. Active and passive heat stress similarly compromise tolerance to a simulated hemorrhagic challenge. *Am J Physiol Regul Integr Comp Physiol* 307: R822–R827, 2014. First published July 30, 2014; doi:10.1152/ajpregu.00199.2014.—Passive heat stress increases core and skin temperatures and reduces tolerance to simulated hemorrhage (lower body negative pressure; LBNP). We tested whether exercise-induced heat stress reduces LBNP tolerance to a greater extent relative to passive heat stress, when skin and core temperatures are similar. Eight participants (6 males, 32 ± 7 yr, 176 ± 8 cm, 77.0 ± 9.8 kg) underwent LBNP to presyncope on three separate and randomized occasions: *1)* passive heat stress, *2*) exercise in a hot environment (40°C) where skin temperature was moderate (36°C, *active 36*), and *3*) exercise in a hot environment (40°C) where skin temperature was matched relative to that achieved during passive heat stress (~38°C, *active 38*). LBNP tolerance was quantified using the cumulative stress index (CSI). Before LBNP, increases in core temperature from baseline were not different between trials (1.18 ± 0.20°C; $P > 0.05$). Also before LBNP, mean skin temperature was similar between passive heat stress (38.2 ± 0.5°C) and *active 38* (38.2 ± 0.8°C; $P = 0.90$) trials, whereas it was reduced in the *active 36* trial (36.6 ± 0.5°C; $P \leq 0.05$ compared with passive heat stress and *active 38*). LBNP tolerance was not different between passive heat stress and *active 38* trials (383 ± 223 and 322 ± 178 CSI, respectively; $P = 0.12$), but both were similarly reduced relative to *active 36* (516 ± 147 CSI, both $P \leq 0.05$). LBNP tolerance is not different between heat stresses induced either passively or by exercise in a hot environment when skin temperatures are similarly elevated. However, LBNP tolerance is influenced by the magnitude of the elevation in skin temperature following exercise induced heat stress.

exercise; heat stress; orthostatic tolerance

PASSIVE HEAT STRESS increases core and skin temperatures and is accompanied with profound reductions in tolerance to central hypovolemia [e.g., lower body negative pressure (LBNP)], which simulates a hemorrhagic state (2, 26, 29, 31, 48, 51). This is due, in part, to a large displacement of blood to the cutaneous circulation and associated reductions in systemic vascular resistance (42) and central blood volume (12, 13), coupled with inadequate cutaneous vasoconstriction during the hypotensive challenge (11, 38). Such tolerance is likewise reduced following short-term exercise in a thermoneutral environment that is not accompanied by profound increases in skin and core temperatures (6). This response may be due to postexercise reductions in baroreflex sensitivity (40, 49), lowered arterial blood pressure (9, 16, 27, 39), and an impaired transduction of sympathetic outflow into vasoconstriction (20), coupled with elevations in vascular conductance in the previously active limb (34).

Blood pressure and vascular alterations following exercise may be exacerbated if the exercise is performed under hot environmental conditions owing to heighted skin and core temperatures, as well as elevated limb muscle and skin vascular conductances (34, 36, 41, 44, 50). Such a response may reduce tolerance to a simulated hemorrhagic challenge to a greater extent relative to a passive heat stress, when increases in core and skin temperatures are similar. To that end, the first objective of this project was to test the hypothesis that tolerance to a simulated hemorrhagic challenge (via LBNP) is lower following exercise in a hot environment relative to a similar thermal provocation induced by passive heat stress.

Skin temperatures following passive heat stress can markedly affect tolerance to a subsequent hypotensive challenge, with cooler skin improving this tolerance (52). It remains unknown whether skin temperature following an exercise heat stress likewise affects tolerance to such a challenge. To this end, the second objective of this study was to test the hypothesis that tolerance to a simulated hemorrhagic challenge following exercise in a hot environment is influenced by skin temperature. The obtained information has direct implications for the understanding of blood pressure control in a soldier who may be heat stressed passively (e.g., turret gunner, sniper, etc.) or actively (e.g., foot patrol) and experiences a subsequent hemorrhagic injury.

METHODS

Subjects. Eight subjects (six males) participated in this study. Subject characteristics were the following: age 32 ± 7 years; height, 176 ± 8 cm; weight 77.0 ± 9.8 kg; peak oxygen uptake ($\dot{V}O_{2peak}$) 43.6 ± 8 ml·kg^{-1}·min^{-1}; and peak power output 262 ± 33 watts (means ± SD). Women were tested in the follicular phase of the menstrual cycle or the placebo phase if they were taking birth control pills. Subjects were not taking medications (aside from birth control pills); were nonsmokers; were free of any known cardiovascular, metabolic, or neurological diseases; and refrained from alcohol, caffeine, and exercise for 24 h before the study. Subjects were informed of the purpose, procedures, and risks of the study before providing their 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 Dallas.

Instrumentation and experimental protocol. In preparation for experimental days, subjects completed a graded exercise test on a cycle

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ergometer (Lode, Groningen, The Netherlands) in thermoneutral conditions. Power output was recorded from the cycle ergometer while oxygen uptake, including peak, was measured using standard indirect calorimetry procedures (Parvo Medics' TrueOne 2400, Sandy, UT).

On experimental days, ~2 h before the onset of data collection, subjects swallowed an ingestible telemetry pill for the measurement of core temperature from intestinal temperature (HQ, Palmetto, FL). Subjects voided their bladder before nude body mass was recorded. Adequate hydration was confirmed via urine specific gravity (<1.028), which was measured using a digital refractometer. Height was measured using a stadiometer.

Mean skin temperature was measured from the weighted average temperature across six sites (49a)using thermocouples fixed to the skin with porous adhesive tape. Arterial blood pressure was continuously measured noninvasively using photoplethysmography (Finometer Pro, FMS, Amsterdam, The Netherlands), which was used to calculate mean arterial pressure. Heart rate was obtained from an electrocardiogram (ECG, Agilent, Munich, Germany) that was interfaced with a cardiotachometer (1,000 Hz sampling rate, CWE, Ardmore, PA). After instrumentation, subjects rested in the supine position for 30 min to allow for the stabilization of fluid shifts. Baseline data were subsequently obtained.

Subjects were then exposed to three randomized and counterbalanced trials separated by at least 3 days. During one trial, each subject donned a water-perfused tube-lined suit (Med-Eng, Ottawa, Canada) that covered their entire body except for the head, hands, and feet. The suit permitted the control of whole body skin and internal temperatures by adjusting the temperature of the water perfusing the suit. Subjects were exposed to whole body heating by perfusing 48–50°C water through the suit to elevate core temperature by ~1.2°C (Passive). During the other two trials, subjects exercised on an upright cycle ergometer at 50% of their predetermined peak power output in an environmental chamber set to 40°C and 30% relative humidity until core temperature increased by ~1.2°C. After achieving the desired increase in core temperature, the subject rapidly donned the aforementioned water-perfused suit. The temperature of the water perfusing the suit was adjusted such that mean skin temperature was clamped at ~36.5°C to match skin temperature at the end of exercise (i.e., *active 36*) or clamped at ~38.0°C to match skin temperature during the passive heat stress trial (i.e., *active 38*). These two trials (*active 36* and *active 38*) were designed to address the influence of skin temperature upon LBNP tolerance following exercise-induced heat stress. Given the influence of heighted skin temperatures in compromising cutaneous vasoconstriction to LBNP (11, 38), clamping skin temperature in the *active 38* trial was an important control measure to ensure appropriate comparison with the passive heat stress trial.

Participants were encouraged to ingest 7 ml/kg body mass of warm water (37.1 ± 1.2°C) in all trials during the thermal provocation before LBNP. The volume of water ingested during the passive heat stress trial (403 ± 238 ml) was slightly less than the volume ingested in both *active 36* and *active 38* trials (551 ± 264 and 575 ± 256 ml respectively, both $P \leq 0.05$), whereas fluid ingestion was not different between *active 36* and *active 38* trials ($P > 0.05$).

In all trials, following the desired increase in core and mean skin temperatures, subjects underwent a supine LBNP tolerance test to the onset of presyncope. LBNP began at 20 mmHg for 3 min, followed by increasing negative pressure by 10 mmHg in 3-min stages until presyncope. The termination of LBNP was based upon subject self-reporting of feeling faint and/or nauseous, a rapid and progressive decrease in blood pressure resulting in sustained systolic blood pressure being ≤80 mmHg, and/or a relative and pronounced bradycardia. Throughout LBNP, arterial blood pressures were also measured at the brachial artery by auscultation (Tango, Suntech Medical Instruments, Raleigh, NC). Tolerance to LBNP was quantified using the cumulative stress index (CSI) (33), calculated by summing the time at each level of LBNP multiplied by that level (i.e., 20 mmHg·3 min + 30 mmHg·3 min + 40 mmHg·3 min, etc.) until presyncope. Nude body mass was obtained before any provocation and after the LBNP tolerance test.

Data analysis. Temperature and hemodynamic data were collected via a data-acquisition system (Biopac System, Santa Barbara, CA). Data were averaged across 60 s at baseline and after the desired increase in core and mean skin temperatures before LBNP. During LBNP, data were averaged over a 30-s period immediately preceding 20%, 40%, 60%, and 80% of the maximal CSI, and also during the 15 s immediately preceding the termination of LBNP (i.e., presyncope). Data were statistically analyzed using a two-way analysis of variance with repeated measures, with main factors of thermal condition (levels: passive, *active 36*, *active 38*) and time (levels: baseline, pre-LBNP, 20%, 40%, 60%, 80% max CSI, and presyncope). For CSI, data were analyzed via one-way repeated measures ANOVA across the three perturbations. Post-hoc analyses were performed using paired *t*-tests with a Bonferroni correction when a significant main effect or interaction was identified. Data are reported as means ± SD.

RESULTS

Cardiovascular and temperature variables were not different at baseline between trials (Table 1). Mean skin temperature was elevated from baseline due to both passive and active heat stress perturbations before LBNP (all $P \leq 0.05$ within trials relative to baseline, Fig. 1). Before LBNP, mean skin temperatures were not different between passive heat stress and *active 38* trials, but both were higher relative to the *active 36* trial (both $P \leq 0.05$). Core temperature increased in all trials ($P \leq 0.05$), with this measure not being different between trials immediately before LBNP. Relative to baseline, heart rate increased and blood pressure decreased at pre-LBNP in all trials (all $P \leq 0.05$), but both the absolute and the change in these measures to the heating stimuli were not different between trials (Fig. 2).

The duration of passive heat stress (39 ± 9 min) before achieving a 1.2°C increase in core temperature was shorter than the exercise duration in both *active 36* and *active 38* trials (48 ± 11 and 47 ± 12 min, respectively, both $P \leq 0.05$ relative

Table 1. *Thermal and hemodynamic measures during baseline, pre-LBNP, 80% CSI, and at presyncope for all three trials*

	Passive Heat Stress				Active 36				Active 38			
	Baseline	Pre-LBNP	80% CSI	Presyncope	Baseline	Pre-LBNP	80% CSI	Presyncope	Baseline	Pre-LBNP	80% CSI	Presyncope
T_{core}, °C	36.8 ± 0.4	38.0 ± 0.4*	38.3 ± 0.5*†	38.3 ± 0.5*†	37.0 ± 0.3	38.1 ± 0.4*	38.1 ± 0.4*	38.1 ± 0.5*	36.9 ± 0.3	38.0 ± 0.2*	38.3 ± 0.3*†	38.3 ± 0.3*†
T_{sk}, °C	32.9 ± 0.7	38.2 ± 0.5*	37.9 ± 0.6*	37.9 ± 0.6*	33.2 ± 1.4	36.6 ± 0.5*‡	36.4 ± 0.4*‡	36.4 ± 0.4*‡	33.4 ± 0.6	38.2 ± 0.7*	37.9 ± 0.8*	38.0 ± 0.8*
MAP, mmHg	82 ± 8	79 ± 13*	71 ± 16*	59 ± 11*§	91 ± 8	75 ± 8*	73 ± 10*	56 ± 13*§	91 ± 7	75 ± 11*	70 ± 6*	56 ± 5*§
HR, beats/min	53 ± 8	99 ± 12*	140 ± 15*	123 ± 32*§	59 ± 10	101 ± 14*	138 ± 16*	129 ± 16*§	58 ± 10	109 ± 13*	145 ± 14*	136 ± 14*§

Values are means ± SD for 8 participants. CSI, cumulative stress index; LBNP, lower body negative pressure; T_{core}, body core temperature; T_{sk}, mean skin temperature; MAP, mean arterial pressure; HR, heart rate. *Different from baseline within trial ($P \leq 0.05$). †Different from pre-LBNP within trial ($P \leq 0.05$). ‡Different from passive heat stress and active 38 trials ($P \leq 0.05$). §Different from 80% CSI within trial ($P \leq 0.05$).

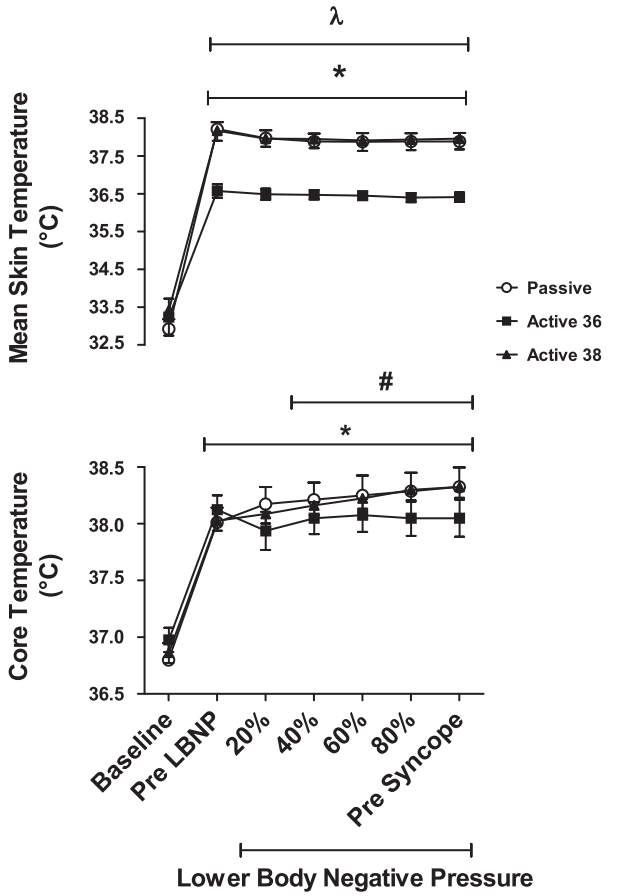

Fig. 1. Core and skin temperatures before and during lower body negative pressure (LBNP) to presyncope in all conditions. Mean skin and core body temperatures increased with all methods of heat stress before LBNP. However, by design, mean skin temperatures were higher after passive heat stress and *active 38* (~38°C, *active 38*) trials compared with *active 36* (36°C, *active 36*) (both $P \leq 0.05$), and remained higher throughout LBNP to presyncope. At presyncope, mean skin temperature was unchanged ($P > 0.05$), whereas core temperature was slightly elevated in the passive heat stress and *active 38* trials. Data are means ± SD at baseline, immediately before LBNP (pre-LBNP), throughout LBNP at 20, 40, 60, and 80% of maximal cumulative stress index (CSI), and at presyncope. *Different from baseline in all trials ($P \leq 0.05$). #Different from pre-LBNP in passive heat stress and *active 38* trials only ($P \leq 0.05$). λDifferent from *active 36* ($P \leq 0.05$).

to passive heat stress), whereas exercise time was not different between *active 36* and *active 38* trials ($P > 0.05$). The magnitude of the reduction in body mass was not different between trials (passive: 1.1 ± 0.3, *active 36*: 1.4 ± 0.7 and *active 38*: 1.4 ± 0.5 kg, respectively, $P > 0.05$).

Regardless of the trial, mean skin temperature did not change during LBNP (Fig. 1). However, during this period core temperature increased ~0.3°C in the passive and *active 38* trials ($P \leq 0.05$) but did not change in the *active 36* trial (Table 1). During LBNP heart rate increased relative to baseline ($P \leq 0.05$) but then declined from 80% CSI to presyncope in all trials ($P \leq 0.05$, Fig. 2). Arterial blood pressure declined in all trials during LBNP through presyncope ($P \leq 0.05$), with the magnitude of this reduction not being different between trials. Despite differing modes of heating, LBNP tolerance was not different between passive heat stress (340 ± 204 CSI units) and *active 38* (346 ± 167 CSI units, $P = 0.119$) trials, whereas LBNP tolerance during the *active 36* trial (513 ± 188 CSI units) was greater relative to both passive and *active 38* trials ($P \leq 0.05$, Fig. 3). The lower LBNP tolerance in the *active 38* trial relative to the *active 36* trial was evident in seven of eight subjects, with difference in tolerance of 201 ± 95 CSI units. In the one subject where LBNP tolerance was not reduced in the *active 38* relative to the *active 36* trial, LBNP tolerance was 313 and 241 CSI units, respectively.

DISCUSSION

Given that both passive heat stress (2, 26, 29, 31, 48, 51) and exercise in a thermoneutral environment (6) impair tolerance to a hypotensive challenge, we hypothesized that the combination of exercise in the heat would further reduce LBNP tolerance, relative to passive heat stress alone, when controlling for internal and mean skin temperatures. Counter to that hypothesis, LBNP tolerance was not different between these two perturbations when mean skin temperatures were clamped at similar levels. A secondary objective tested the hypothesis that mean skin temperature influences LBNP tolerance following exercise in a hot environment. Consistent with that hypothesis,

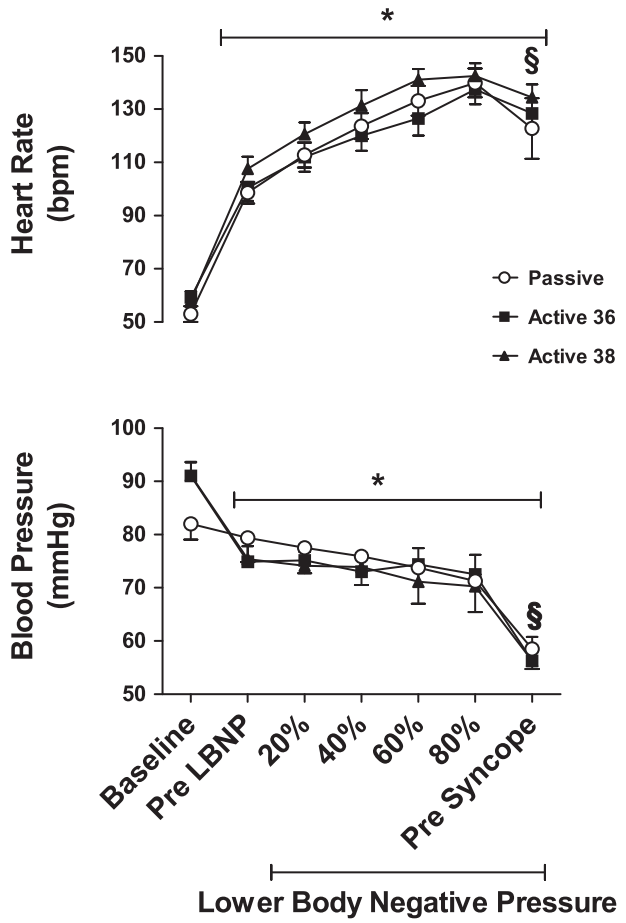

Fig. 2. Heart rate and blood pressure responses before and during LBNP to presyncope in all conditions. When expressed relative to a percentage of maximal CSI, heart rate and mean arterial pressure were not different between trials at any point. In all trials, blood pressure and heart rate decreased at presyncope relative to 80% CSI. Data are means ± SD at baseline, immediately before LBNP (pre-LBNP), throughout LBNP at 20, 40, 60, and 80% of maximal CSI and at presyncope. *Different from baseline in all trials ($P \leq 0.05$). §Different from 80% CSI in all trials ($P \leq 0.05$).

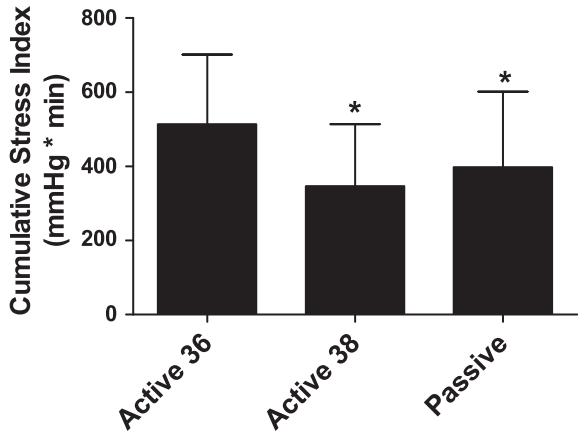

Fig. 3. Cumulative stress index in all trials. Tolerance to simulated hemorrhage (expressed as CSI) was similarly reduced in passive heat stress and *active 38* trials relative to *active 36* ($P \leq 0.05$). Data are means ± SD. *Different from *active 36* ($P \leq 0.05$).

LBNP tolerance following exercise in the heat was influenced by the magnitude of the elevation in mean skin temperature.

During a simulated hemorrhagic challenge, such as LBNP, central blood volume is reduced and the drive for neurally mediated vasoconstriction increases (5, 18, 43, 45). Given that skin and muscle vascular conductance increase in hyperthermic humans (22, 30, 35, 37, 42), the ability to vasoconstrict appropriately in these vascular beds is important for blood pressure control during a subsequent hypotensive challenge. Vascular control is impaired following exercise in thermoneutral conditions (i.e., in the absence of appreciable increases in core and/or skin temperatures) (19, 34), evidenced by a reduction in baroreflex sensitivity (40, 49) and mean arterial pressure (34), a reduced transduction of sympathetic outflow into vascular resistance, and lower sympathetic outflow for any given blood pressure (20). Consistent with these responses, orthostatic tolerance is impaired after a short-term bout of exercise in a thermoneutral environment (6). Passive heat stress places a significant burden on the cardiovascular system, which in part is due to pronounced increases in systemic vascular conductance (42) and reductions in central blood volume (13). Vascular control is impaired following passive heat stress through a decreased vasoconstrictor responsiveness to reductions in central blood volume (11). Given the influences of passive heat stress and exercise in altering vascular control via unique mechanisms, we expected an additive effect resulting in lower tolerance to LBNP after exercise in a hot environment, relative to passive heat stress, when controlling for the elevation in core and skin temperatures. However, counter to that hypothesis, LBNP tolerance was not different between passive heat stress and *active 38* trials (Fig. 3).

The lack of difference in LBNP tolerance between these two trials may be explained by two possibilities. First, it is possible that increases in muscle vascular conductance associated with dynamic exercise decreased to levels similar to passive heat stress (22, 30, 37) during the period between the cessation of exercise and the onset of LBNP (14.7 ± 3.4 min). However, femoral vascular conductance remains elevated for up to 90 min following cycling exercise in a warm environment (34). It is therefore unlikely that exercise-induced elevations in leg vascular conductance had completely returned to baseline values before the onset of LBNP. Second, a more likely explanation is that similar increases in cutaneous vascular conductance, owing to similar increases in mean skin temperature (1, 4, 25), between the passive heat stress and *active 38* trials contributed to comparable LBNP tolerances. This argument is strengthened by findings that such elevations in mean skin temperature and cutaneous vascular conductance are associated with an inadequate cutaneous vasoconstrictor response (38), which contributes to reduced tolerance to LBNP (11). Therefore, the present results suggest that LBNP tolerance is more closely related to the elevation in mean skin temperature rather than the methodology of increasing core temperature (e.g., passive vs. exercise-induced), and that vascular responses postexercise do not have an additive effect in contributing to compromised tolerance to central hypovolemia. Thus exercise itself does not further compromise tolerance to a simulated hemorrhagic challenge relative to passive heat stress, when elevations in mean skin temperature are similar between conditions.

LBNP tolerance was attenuated in the *active 38* trial relative to the *active 36* trial. The most likely explanation for this observation is the difference in mean skin temperature between these trials, which affected tolerance perhaps via two unique mechanisms. First, the extent of cutaneous vasodilation under the water-perfused suit, and thus presumably the reduction in central blood volume (13) before LBNP, would be greater in the *active 38* trial relative to the *active 36* trial. Consistent with this hypothesis, decreasing mean skin temperature by actively cooling the skin of otherwise hyperthermic individuals increases central blood volume and greatly improves tolerance to an orthostatic stress (14, 52). Second, elevated skin temperatures attenuate cutaneous vasoconstrictor responses to a hypotensive challenge (38), perhaps through nitric oxide mechanisms (15, 24, 46, 47, 53). The extent of cutaneous vasoconstriction at presyncope via LBNP is greatly attenuated in skin heated to 38°C relative to skin heated to 35°C (38). Therefore, differences in LBNP tolerance between *active 38* and *active 36* may be due, in part, to both: *1*) local temperature-induced differences in the magnitude of cutaneous vasodilation before LBNP and *2*) differences in the extent of cutaneous vasoconstriction under the water-perfused suit during LBNP between trials. Those mechanisms aside, throughout LBNP core temperature, slightly increased in the *active 38* trial (~Δ0.3°C) but was unchanged in the *active 36* trial. One may propose that such differences in core temperature could have contributed to the observed differences in LBNP tolerance. However, the magnitude of increase in core temperature during heat stress, between approximately Δ0.9 and 1.8°C, is not associated with differences in LBNP tolerance (17). It is therefore unlikely that a relatively small difference in core temperature during LBNP in the *active 38* trial contributed to reduced LBNP tolerance relative to the *active 36* trial; rather such tolerance differences were likely attributed to differences in skin temperature.

Limitations and considerations to the interpretation of the findings. The present study did not include a normothermic LBNP challenge. Such a challenge was not necessary to address the proposed hypotheses, and thus inclusion of a normothermic LBNP challenge would expose subjects to an unnecessary procedure and therefore some level of risk. That said, using a similar LBNP ramp, as well as CSI criteria to evaluate LBNP tolerance, we consistently observe CSI mean values in the ~900–1,100 mmHg·min range in normothermic

subjects (7, 28, 29, 32), which is well above what was observed in any of the three trials in the present experiment.

In *active 36* and 38 trials, after obtaining the appropriate increase in core temperature, participants donned the water-perfused suit for the control of skin temperature and were transferred into position for LBNP. The duration of this process varied between ~8 and 15 min. Despite this time delay, exercise-induced alterations in baroreflex sensitivity (40, 49), blood pressure (16, 21), sympathetic nerve activity (20), and limb blood flow (34) are evident for at least 60 min after the cessation exercise. It is therefore unlikely that the influence of exercise-induced neural and cardiovascular alterations upon LBNP tolerance diminished due to this period between the cessation of exercise and the onset of LBNP.

The duration of the heat stress perturbation was not different between *active 36* and *38* trials but was lower in the passive heat stress trial. Methodologically, it may have been more appropriate to clamp the heat stress duration between trials, though this would be very challenging given a variety of factors that influence the rate of heating during passive heat stress (e.g., size of the individual, water temperature, and perfusion rate of the suit, etc.), resulting in multiple passive heat stress trials to achieve a desired temperature within a specified duration. Nevertheless, there is currently no evidence to suggest that the duration of heat stress per se is a significant contributor to LBNP tolerance. However, it is recognized that longer heating periods may lead to more pronounced dehydration, which could influence LBNP tolerance, yet in the present protocol the reduction in body mass (i.e., fluid loss) was similar between trials.

In both active trials subjects exercised at an intensity equal to 50% of their peak power output. This workload was selected for two reasons: *1*) it is a similar intensity to that commonly occurring during routine military foot/reconnaissance patrols (3, 23), and *2*) it is a workload that can be maintained for sufficient period of time in relatively nontrained subjects to achieve the desired increases in core temperature. While it may be insightful to identify the combined influence of heat stress and exercise at substantially higher (or even maximal) intensities on LBNP tolerance, the subjects may not have been able to tolerate the workload for a sufficient duration to achieve the required increases in core temperature. That said, the responses observed in the present observation should not be extrapolated to what may occur following high-intensity exercise in hot environmental conditions.

Perspectives and Significance

These data have implications for individuals who become hyperthermic through either passive heat stress or exercise, and who are at risk for a hemorrhagic injury, such as firefighters and soldiers. For example, military personnel are often deployed in warm environments where they are exposed to both passive (i.e., snipers, turret gunners, etc.) and active heat stresses (i.e., foot patrols) while wearing body armor. Buller et al. (8) reported comparable increases in both skin and core temperatures during military procedures in Iraq relative to the present observations. The present data indicate that the implications of a hemorrhagic injury are similarly dire between actively and passively heat-stressed individuals, when skin temperatures are equally elevated. Furthermore, given that LBNP tolerance was prolonged following active heat stress when mean skin temperature was ~2°C lower, reducing skin temperature of hyperthermic and hemorrhaging individuals could prove beneficial toward survival. This small reduction in skin temperature may be achieved without cooling the skin with ice or related modalities. Therefore, identification of a light weight non-ice-dependent cooling modality to decrease skin temperature ~2°C may be beneficial in the treatment of a hemorrhaging hyperthermic soldier in the prehospital setting. Finally, it is noteworthy that soldiers are currently warmed following a hemorrhagic injury (10). Based upon the present findings, this action may actually be harmful for the soldier who is not hypothermic, as recently proposed (10).

Conclusions. The present results show that tolerance to a simulated hemorrhagic challenge resulting in central hypovolemia and accompanying hypotension is not different between actively and passively heat-stressed individuals, when internal and mean skin temperatures are controlled for. Second, during active heat stress resulting in comparable increases in internal temperatures, relatively small differences in mean skin temperature can appreciably affect tolerance to a hypotensive challenge. These data suggest that exercise itself does not further decrease tolerance to a simulated hemorrhagic challenge compared with passive heat stress, which may have important implications toward the treatment of a hyperthermic individual who has experienced a hemorrhagic injury.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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HEMODYNAMIC STABILITY TO SURFACE WARMING AND COOLING DURING SUSTAINED AND CONTINUOUS SIMULATED HEMORRHAGE IN HUMANS

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ABSTRACT—One in 10 deaths worldwide is caused by traumatic injury, and 30% to 40% of those trauma-related deaths are due to hemorrhage. Currently, warming a bleeding victim is the standard of care due to the adverse effects of combined hemorrhage and hypothermia on survival. We tested the hypothesis that heating is detrimental to the maintenance of arterial pressure and cerebral perfusion during hemorrhage, while cooling is beneficial to victims who are otherwise normothermic. Twenty-one men (31 ± 9 y) were examined under two separate protocols designed to produce central hypovolemia similar to hemorrhage. Following 15 min of supine rest, 10 min of 30 mm Hg of lower body negative pressure (LBNP) was applied. On separate randomized days, subjects were then exposed to skin surface cooling (COOL), warming (WARM), or remained thermoneutral (NEUT), while LBNP continued. Subjects remained in these thermal conditions for either 40 min of 30 mm Hg LBNP (N = 9), or underwent a continuous LBNP ramp until hemodynamic decompensation (N = 12). Arterial blood pressure during LBNP was dependent on the thermal perturbation as blood pressure was greater during COOL ($P > 0.001$) relative to NEUT and WARM for both protocols. Middle cerebral artery blood velocity decreased ($P < 0.001$) from baseline throughout sustained and continuous LBNP, but the magnitude of reduction did not differ between thermal conditions. Contrary to our hypothesis, WARM did not reduce cerebral blood velocity or LBNP tolerance relative to COOL and NEUT in normothermic individuals. While COOL increased blood pressure, cerebral perfusion and time to presyncope were not different relative to NEUT or WARM during sustained or continuous LBNP. Warming an otherwise normothermic hemorrhaging victim is not detrimental to hemodynamic stability, nor is this stability improved with cooling.

KEYWORDS—Cerebral perfusion, compensatory reserve index, lower body negative pressure, mean arterial pressure, skin surface warming and cooling

INTRODUCTION

One in 10 deaths worldwide is caused by traumatic injury, with 30% to 40% of trauma-related deaths due to hemorrhage (1). Hemorrhage from major trauma is the predominant mechanism of death on the battlefield and in potentially survivable casualties (2). Among trauma patients who do not die immediately, hemorrhage-induced hypotension (systolic blood pressure ≤90 mm Hg) often occurs (3) that can contribute to cerebral hypoperfusion (4, 5). Severe hypothermia (core body temperature <35°C) can accompany traumatic injuries and is associated with increased mortality rates (6, 7). In fact, mortality rate approaches 100% in trauma patients who are severely hypothermic, while a similar degree of hypothermia in the absence of trauma has a mortality rate of only 23% (8). These detrimental effects of hypothermia accompanying trauma are likely the primary justification for ''the practice in emergency medicine of warming patients with hemorrhage'' (9, 10). However, the use of this practice in all trauma victims has been questioned (10), particularly if the patient is not hypothermic (e.g., normothermic) since heating has the potential to prolong clotting time and reduces arterial blood pressure (11, 12).

Whole-body heating during simulated hemorrhage (e.g., lower body negative pressure, head up tilt test) greatly compromises blood pressure and cerebral perfusion (13–16), culminating in an early onset of symptoms (attenuated mental status) and/or hemodynamic decompensation (16–18). Based on these studies, heating may not be conducive, and may even be detrimental, to a hemorrhaging victim. Thus, identification of more effective therapies that can prevent or attenuate hemorrhage-induced hypotension and cerebral hypoperfusion, and/or prolong hemorrhagic tolerance is an important undertaking.

Cooling interventions have been proposed as a potential therapy to treat hemorrhagic injuries, particularly when the individual is hyperthermic (10, 19). When skin surface cooling is applied during simulated hemorrhage, mean arterial pressure

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All experiments were performed at the Institute for Exercise and Environmental Medicine. All authors contributed to the conception and design of the experiment, as well as data collection. All authors contributed to the interpretation of the data and to revision the manuscript for important intellectual content. All authors have approved the final version of the manuscript. 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.

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increases while the decrease in cerebral blood flow velocity is attenuated, suggesting better control, or maintenance of blood pressure and cerebral perfusion (20–23). The current study examined the effects of skin surface warming and cooling during sustained and continuous simulated hemorrhage in otherwise normothermic individuals. It was hypothesized that arterial blood pressure and cerebral perfusion would be compromised (e.g., lowered) during simulated hemorrhage while being heated, resulting in reduced tolerance. Alternatively, skin surface cooling during simulated hemorrhage would elevate blood pressure and cerebral perfusion, resulting in prolonged tolerance.

PATIENTS AND METHODS

Two separate experimental protocols were performed to examine the effects of warming and cooling during sustained (Protocol 1) and continuous (Protocol 2) simulated hemorrhage.

Subjects

Nine men volunteered to participate in Protocol 1, and 12 men volunteered to participate in Protocol 2; six men participated in both protocols. Descriptive characteristics (mean ± SD) for Protocol 1: age, 30 ± 8 y; height, 177 ± 11 cm; body mass, 79 ± 15 kg, and Protocol 2: age, 29 ± 8 y; height, 179 ± 10 cm; body mass, 82 ± 16 kg. Subjects were nonsmokers, were free of any known cardiovascular, respiratory, neurological, or metabolic diseases, and were not taking any medications. For both experimental protocols, randomized trials were performed at the same time of day within subject and each trial was separated by an average of 1 week. Subjects were asked to refrain from strenuous physical activity for 24 h, as well as from caffeine and alcohol for 12 h prior to the experimental visits. The informed consent and all experimental procedures were approved by the following Institutional Review Boards for Human Subjects: U.S. Army Medical Research and Materiel Command Human Research Protection Office, University of Texas Southwestern Medical Center, and Texas Health Presbyterian Hospital of Dallas. Written informed consent was obtained from all subjects prior to their participation in the study.

Measurements

Measurements were identical for both protocols. Subjects were dressed in a two-piece, tube-lined suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body, except for the head, hands, feet, and one arm. Core temperature was measured with a telemetric pill (HQ Inc, Palmetto, FL) that was swallowed a minimum of 1 h prior to the onset of data collection. Mean skin temperature was measured as the weighted average of six thermocouples attached to the skin surface on the abdomen (14%), calf (11%), chest (22%), lower back (19%), thigh (13%), and upper back (21%). Heart rate was obtained from an electrocardiogram (GE Healthcare, Milwaukee, WI) that was interfaced with a cardiotachometer (CWE, Ardmore, PA). Blood pressure measurements were obtained by automated auscultation of the brachial artery (Tango+; SunTech Medical, Morrisville, NC). Continuous blood pressure measurements were obtained noninvasively using photoplethysmography (Finometer Pro, FMS, Amsterdam, The Netherlands) with the hydrostatic height at the finger corrected with respect to the heart level. Cerebral blood velocity was evaluated via transcranial Doppler of the middle cerebral artery, which is capable of detecting changes in blood velocity with high (>90%) sensitivity and specificity (24).

The physiological reserve to compensate for reductions in central blood volume was determined via continuous beat-by-beat recordings of arterial pressure waveforms from a noninvasive photoplethysmographic device with a finger pulse oximeter (Flashback Technologies, Boulder, CO). The compensatory reserve was subsequently quantified using a machine learning algorithm, resulting in a compensatory reserve index (CRI) output (25). CRI provides a normalized value on a scale of 1 to 0, where ''1'' reflects the maximal capacity of the sum total of physiological mechanisms to compensate for deficits in central blood volume, and ''0'' implies imminent cardiovascular instability and decompensation (26).

Experimental procedures common to both protocols

Upon arrival at the laboratory, participants ingested the telemetric temperature-sensing pill. Subjects then provided a urine sample to ensure proper hydration, and weighed themselves nude prior to instrumentation for measurements of mean skin temperature, heart rate, and blood pressure. After donning the tube-lined suit, subjects laid in the supine position with the lower half of their body in a lower body negative pressure (LBNP) box, sealed at the level of the iliac crest. Mean skin temperature was initially clamped at ~33 to 34°C by adjusting the temperature of the water circulating through the suit. Subjects were then instrumented for the measurement of cerebral perfusion.

Experimental protocol 1

Procedures for this protocol are graphically illustrated in Figure 1A. Following a 15 min period of baseline measurements, 10 min of 30 mm Hg LBNP was applied to simulate a mild hemorrhage that might occur upon stopping active bleeding (e.g., tourniquet applied), without causing arterial hypotension (e.g., reduction >10 mm Hg in mean arterial blood pressure) while subjects remained normothermic. This approach has previously been verified to simulate central hypovolemia accompanying actual hemorrhage (27, 28). With this level of LBNP continuing, participants were then exposed to 40 min of the following thermal perturbations (LBNP+thermal):

1. COOL: 15 to 17°C water perfused through the tube-lined suit to decrease mean skin temperature from normothermia (~34°C) to as low as possible, without causing shiver (~30°C). Water bath temperature was slightly elevated if the participant began to shiver, or if a 0.5°C decrease in core temperature was observed.
2. WARM: 40 to 44°C water perfused through the tube-lined suit to elevate mean skin temperature from ~34°C (normothermia) to ~37°C, and caused not more than a 0.5°C increase in intestinal temperature. This thermal condition was used to simulate the current medical practice of warming patients with blankets. Water bath temperature was adjusted if the participant's intestinal temperature surpassed a 0.5°C increase.
3. NEUT: 33 to 34°C water continued to perfuse the tube-lined suit to maintain a normothermic mean skin temperature (~34°C).

Experimental protocol 2

Procedures for this protocol are depicted in Figure 1B. Following 15 min of baseline measurements, 10 min of 30 mm Hg LBNP was applied, while subjects remained normothermic (LBNP only). With LBNP continuing, subjects were then exposed to 5 min of either COOL, WARM, or NEUT, as described above. The pressure within the LBNP device was then progressively decreased at a rate of 5 mm Hg min^{-1} (to replicate continuous hemorrhage) until hemodynamic decompensation. Criteria for determining presyncope included: self-reporting by the subject of feeling faint, sustained nausea; rapid and progressive decrease in blood pressure resulting in a sustained systolic blood pressure <80 mm Hg; and/or relative bradycardia accompanied by a narrowing of pulse pressure. The orders of the thermal challenges for both Protocols 1 and 2 were randomized between experimental days, with a minimum of 24 h between each trial. Randomization was determined by using a random assignment technique where thermal conditions were coded with a number (i.e., 1 = COOL, 2 = WARM, 3 = NEUT), and randomly selected for each participant.

Data analysis

Data were collected with data acquisition software (Biopac MP150; Santa Barbara, CA) at a sampling frequency of 50 Hz and subsequently converted into 1 min averages for data analysis. For Protocol 1, data were analyzed at five time points: Baseline, LBNP only, 5 min into LBNP+thermal (early), 30 min into LBNP+thermal (middle), and 40 min into LBNP+thermal (later). For Protocol 2, data were analyzed at the following eight time points: Baseline, LBNP only, LBNP+thermal, 20%, 40%, 60%, and 80% of completed LBNP ramp, and at the time of decompensation.

Statistical analysis

Mixed effect repeated measures models were used to conduct the analysis of the crossover design of Protocols 1 and 2. For Protocol 1, three conditions and five time points were evaluated for thermal and hemodynamic variables. For Protocol 2, three conditions and eight time points were evaluated for thermal and hemodynamic variables, and tolerance time. For all crossover end points, the analysis was performed on an intention-to-treat basis including all available measurements. The mixed models include the condition effect, time effect, and condition × time interaction fixed effects, in addition to incorporating the study period of the crossover design as a fixed effect. The study participant was modeled as a random effect and covariance structure was selected on the basis of Akaike information criterion and Bayesian information criterion as well as model parsimony. Following a significant condition × time interaction, pairwise comparisons were made from the least square means differences derived

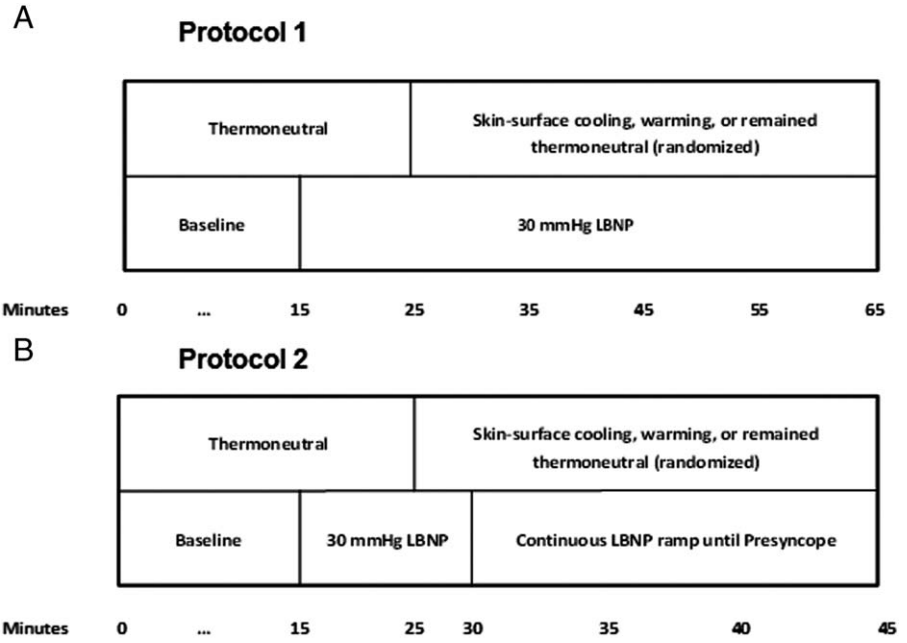

FIG. 1. **Experimental schematic for Protocol 1 (A) and Protocol 2 (B).** Subjects were supine within the lower-body negative pressure (LBNP) device under thermoneutral conditions but without LBNP (15 min Baseline). Subjects were then exposed to 10 min of LBNP (30 mm Hg) while remaining thermoneutral. For Protocol 1: with LBNP continuing, subjects remained thermoneutral, underwent warming, or skin surface cooling (each on a different day and randomized) for 40 min. For Protocol 2: with LBNP continuing, subjects were exposed to 5 min of one of the thermal manipulations, LBNP then continuously increased at 5 mm Hg min^{-1} until presyncope while the applied thermal manipulation continued. Thermal and hemodynamic variables were continuously measured throughout both protocols.

from the mixed models at an adjusted alpha = 0.01. Kaplan–Meier analysis with a log rank test to compare the "survival" distributions was used to depict the number of remaining subjects in each thermal condition during the LBNP ramp portion of Protocol 2. The "survival" probability at each minute, beginning at the start of the LBNP ramp, was calculated by dividing the number of subjects remaining by the population.

Statistical analyses were performed using SigmaPlot (Systat Software Inc, San Jose, CA) and SAS Proc Mixed version 9.4 (SAS Institute, Cary, NC). Results are reported as mean ± SD, and a two-sided P value <0.05 was considered the statistical probability of concluding wrongly that the means of the three thermal conditions during the protocols were in fact due to true differences, and did not arise from random variability for the given sample size of this experiment.

RESULTS

Experimental protocol 1

Post hoc tests revealed that mean skin temperature was elevated throughout WARM, whereas it was decreased throughout COOL (Table 1). Core temperature was maintained throughout all trials and no interaction between LBNP time and thermal condition was observed ($P = 0.07$). A main effect of time for core temperature ($P < 0.0001$) was detected, with *post hoc* tests revealing that core temperature increased 0.1°C to 0.2°C throughout the protocol, regardless of thermal conditions.

As shown in Table 2, both heart rate and mean arterial pressure responses during sustained LBNP were dependent on the thermal perturbations applied ($P < 0.0001$). The increase in heart rate was greatest during WARM ($\Delta_{Baseline} = 19$ beats min^{-1}), while COOL had the smallest increase ($\Delta_{Baseline} = 6$ beats min^{-1}). *Post hoc* tests revealed that mean arterial pressure during WARM was reduced ($\Delta_{Baseline} = -7 \pm 1$ mm Hg) throughout LBNP ($P < 0.0001$), whereas COOL elevated mean arterial pressure ($\Delta_{Baseline} = +4 \pm 1$ mm Hg; $P = 0.0001$). Blood pressure responses during LBNP were unchanged during NEUT ($P = 0.38$).

A main effect of time was detected for cerebral perfusion ($P = 0.004$) and CRI ($P < 0.0001$), revealing that cerebral blood velocity and compensatory reserve decreased from Baseline throughout sustained LBNP (-4.9 ± 0.1 cm·s^{-1} and -0.4 ± 0.1, respectively). These decreases were independent of

TABLE 1. **Thermal variables for Protocol 1**

		Baseline	LBNP only	LBNP+thermal (early)	LBNP+thermal (middle)	LBNP+thermal (later)
Tsk (°C)	*COOL*	34.0 ± 0.7	33.8 ± 0.7	30.7 ± 0.9†	29.8 ± 0.9†	29.6 ± 1.0†
	NEUT	33.8 ± 0.4	33.5 ± 0.4	33.5 ± 0.5	33.5 ± 0.6	33.5 ± 0.3
	WARM	34.0 ± 0.3	33.7 ± 0.4	36.1 ± 0.4*	36.6 ± 0.4*	36.7 ± 0.5*
Tcore (°C)	*COOL*	37.0 ± 0.3	36.9 ± 0.2	37.3 ± 0.6*	37.3 ± 0.2*	37.3 ± 0.2*
	NEUT	36.8 ± 0.3	36.9 ± 0.2	37.1 ± 0.4*	37.0 ± 0.3*	37.1 ± 0.3*
	WARM	36.8 ± 0.2	36.9 ± 0.2	37.0 ± 0.3*	37.0 ± 0.3*	37.1 ± 0.3*

Mean (± SD) skin temperature (Tsk) and core temperature (Tcore) for Protocol 1 during Baseline, lower body negative pressure (LBNP only), and following 5 (early), 30 (middle), and 40 (later) min of combined LBNP and thermal perturbation (LBNP+thermal).
*Greater than Baseline and LBNP.
†Less than Baseline and LBNP.

TABLE 2. **Hemodynamic variables for Protocol 1**

		Baseline	LBNP only	LBNP+thermal (early)	LBNP+thermal (middle)	LBNP+thermal (later)
HR (beats·min^{-1})	*COOL*	55 ± 8	62 ± 10	59 ± 11*	60 ± 13*	60 ± 14*
	NEUT	57 ± 8	62 ± 11	62 ± 11*	63 ± 11*	66 ± 11*
	WARM	56 ± 11	61 ± 10	67 ± 11*	72 ± 12*	75 ± 12*
MAP (mm Hg)	*COOL*	91 ± 4	88 ± 5	95 ± 5*	95 ± 3*	95 ± 55*
	NEUT	90 ± 6	88 ± 7	89 ± 7	89 ± 8	90 ± 9
	WARM	93 ± 7	91 ± 7	87 ± 7†	87 ± 8†	86 ± 7†
MCA Vmean (cm·s^{-1})	*COOL*	54.4 ± 10.9	52.2 ± 12.2‡	51.6 ± 10.2‡	49.4 ± 10.8‡	50.7 ± 10.7‡
	NEUT	52.2 ± 12.0	51.7 ± 9.6‡	50.9 ± 9.7‡	51.4 ± 11.2‡	50.2 ± 10.5‡
	WARM	56.1 ± 14.2	52.4 ± 13.7‡	51.9 ± 13.1‡	51.0 ± 14.9‡	47.2 ± 12.2‡
CRI	*COOL*	0.9 ± 0.1	0.6 ± 0.1‡	0.7 ± 0.1‡	0.6 ± 0.2‡	0.6 ± 0.2‡
	NEUT	0.9 ± 0.1	0.6 ± 0.1‡	0.6 ± 0.1‡	0.6 ± 0.2‡	0.5 ± 0.1‡
	WARM	0.9 ± 0.1	0.6 ± 0.1‡	0.6 ± 0.1‡	0.6 ± 0.1‡	0.5 ± 0.1‡

Mean (± SD) heart rate (HR), blood pressure (MAP), cerebral blood velocity (MCA Vmean), and compensatory reserve index (CRI) for Protocol 1 during Baseline, lower body negative pressure (LBNP only), and following 5 (early), 30 (middle), and 40 (later) min of combined LBNP and thermal perturbation (LBNP+thermal).
*Greater than Baseline and LBNP.
†Less than Baseline and LBNP.
‡Less than Baseline.

thermal status, as no interaction between thermal condition and LBNP time was observed for cerebral perfusion ($P = 0.82$) or CRI output ($P = 0.12$; Table 2).

Experimental protocol 2

By design, mean skin temperature during the continuous LBNP ramp was dependent on the thermal perturbation applied ($P < 0.0001$; Fig. 2, top panel), such that it was elevated during WARM (36.4 ± 0.5°C), and lowered during COOL (30.5 ± 0.9°C). Core temperature was elevated throughout the LBNP ramp by 0.2°C to 0.3°C similarly for all three thermal conditions ($P = 0.01$; Fig. 2, bottom panel).

Heart rate ($P = 0.0004$; Fig. 3) and arterial blood pressure ($P < 0.0001$; Fig. 4) responses were dependent on the thermal perturbation applied during the continuous LBNP ramp. The increase in heart rate from Baseline throughout the LBNP until Presyncope was greatest during WARM ($\Delta_{Baseline} = 53$ beats min^{-1}), while COOL had the smallest increase ($\Delta_{Baseline} = 45$ beats min^{-1}), followed by NEUT ($\Delta_{Baseline} = 49$ beats min^{-1}). *Post hoc* tests revealed that mean arterial blood pressure was elevated (Δ +7 ± 3 mm Hg; $P < 0.0001$) during COOL across the LBNP ramp. During WARM, blood pressure at 60 and 80% of the ramp was reduced ($\Delta_{Baseline} = -4 \pm 1$ mm Hg; $P < 0.0001$) relative to Baseline. For all thermal conditions, blood pressure at the time of decompensation was reduced from Baseline ($P < 0.0001$).

Cerebral blood velocity ($\Delta_{Baseline} = 26 \pm 1$ cm·s^{-1}) and CRI ($\Delta_{Baseline} = 0.7 \pm 0.1$) decreased throughout the LBNP ramp protocol ($P < 0.0001$), which were independent of the thermal conditions (cerebral blood velocity: $P = 0.69$, Fig. 5; CRI: $P = 0.63$, Fig. 6). Tolerance to LBNP, as defined by time to cardiovascular decompensation (12.7 ± 3.4 min; $P = 0.14$), or the LBNP level achieved at decompensation (87.8 ± 11.2 mm Hg; $P = 0.09$), was similar between thermal conditions. Kaplan–Meier and log rank tests confirmed that LBNP tolerance was similar between trials, as ''survival'' curves were not shifted by the applied thermal conditions ($P = 0.57$; Fig. 7; average time to hemodynamic decompensation,

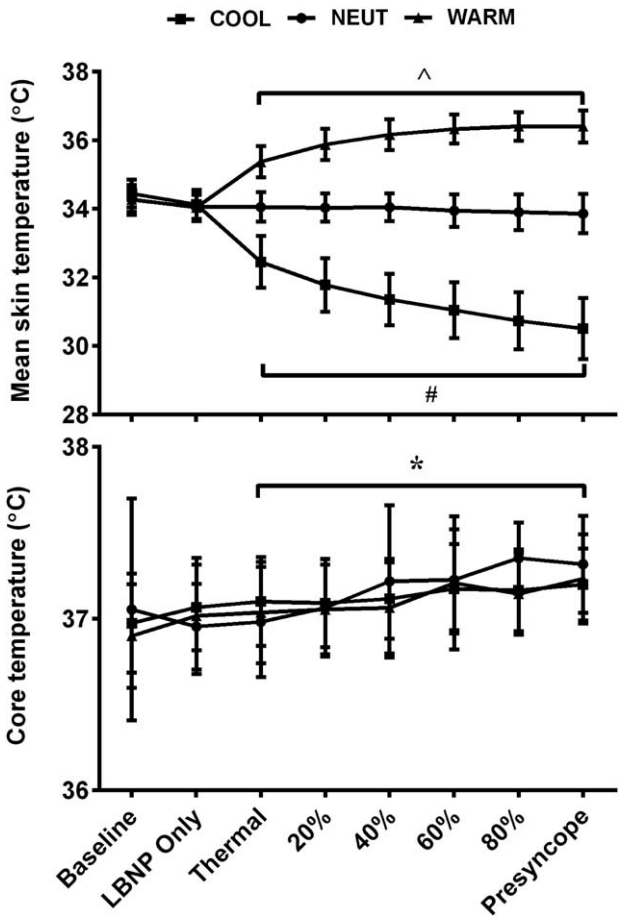

FIG. 2. **Mean (± SD) skin temperature (top panel) and core temperature (bottom panel) for Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60%, and 80% of LBNP ramp, and Presyncope; squares- COOL, circles- NEUT, triangles- WARM; ^, greater than Baseline and LBNP only for the WARM condition; #, less than Baseline and LBNP only for the COOL condition; *, greater than Baseline and LBNP only for all thermal conditions.**

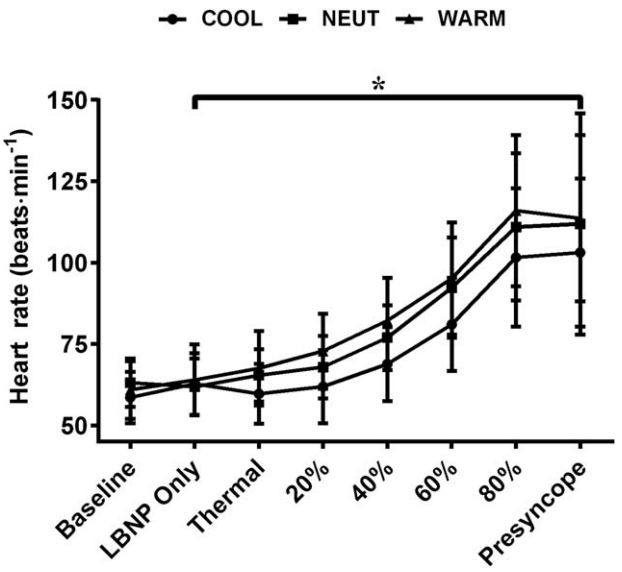

FIG. 3. **Mean (± SD) heart rate for Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60%, and 80% of LBNP ramp, and Presyncope; squares- COOL, circles- NEUT, triangles- WARM; *, greater than Baseline for all thermal conditions.**

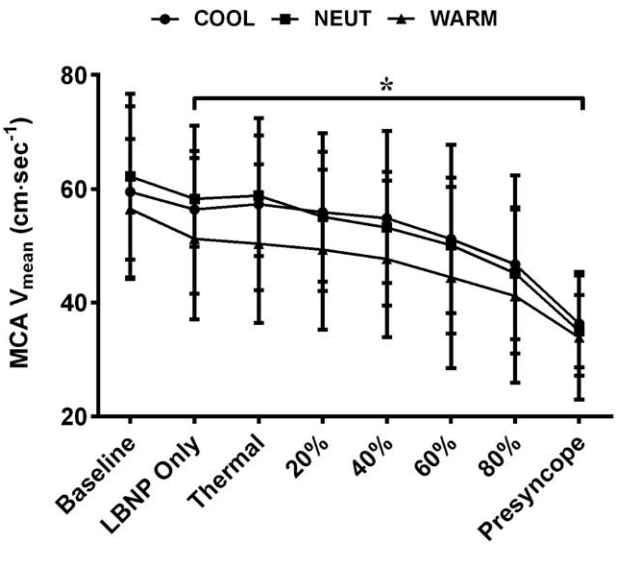

FIG. 5. **Mean (± SD) cerebral blood flow velocity for Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60%, and 80% of LBNP ramp, and Presyncope; squares- COOL, circles- NEUT, triangles- WARM; *, less than Baseline for all thermal conditions.**

COOL: 13.0 ± 2.8 min, NEUT: 13.1 ± 3.9 min, WARM: 11.9 ± 3.5 min).

DISCUSSION

Specific physiological effects of skin surface warming and cooling during two distinct simulated hemorrhagic challenges (sustained and continuous ramp) were examined in the present set of experiments. Our findings revealed that contrary to our hypothesis, cerebral blood velocity was unaffected by these thermal perturbations, despite a reduction and increase in arterial blood pressure during warming and cooling, respectively. Additionally, the physiological reserve to compensate for reductions in central blood volume (CRI) and subsequent tolerance to simulated hemorrhage (Protocol 2 only) were unaffected by either thermal manipulation. Overall, these data suggest that external body warming within the temperature limits applied in the present experiment, of an otherwise normothermic hemorrhaging victim is no more detrimental to hemodynamic stability or the reserve to compensate for

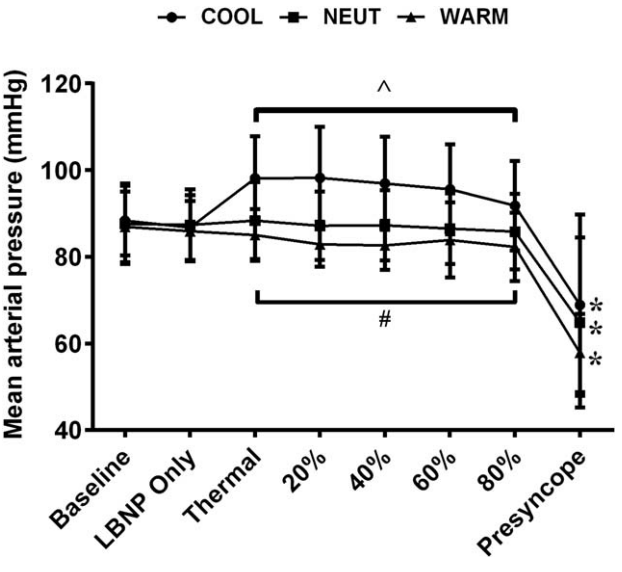

FIG. 4. **Mean (± SD) arterial pressure for Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60%, and 80% of LBNP ramp, and Presyncope; squares- COOL, circles- NEUT, triangles- WARM; ^, greater than Baseline for the COOL condition; #, less than Baseline for the WARM condition; *, less than Baseline and LBNP ramp.**

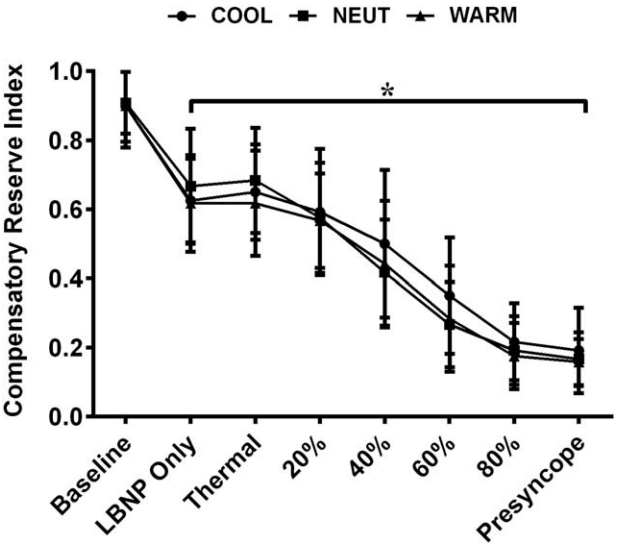

FIG. 6. **Mean (± SD) compensatory reserve index Protocol 2 during Baseline, lower body negative pressure (LBNP only), LBNP with the thermal perturbation (Thermal), 20%, 40%, 60%, and 80% of LBNP ramp, and Presyncope; squares- COOL, circles- NEUT, triangles- WARM; *, less than Baseline for all thermal conditions.**

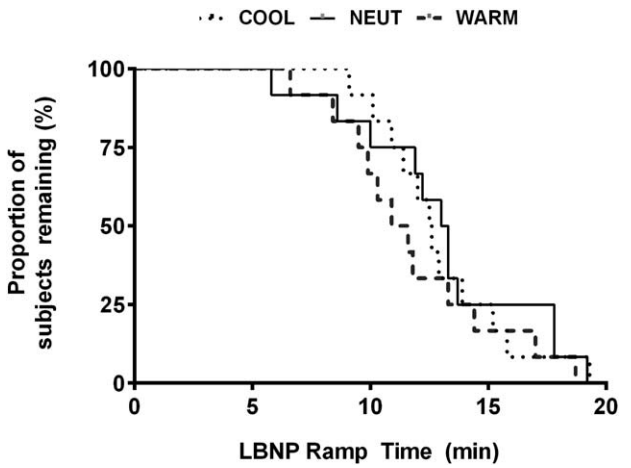

Fig. 7. **Proportion of subjects remaining during lower body negative pressure ramp for COOL (dotted line), NEUT (solid line), and WARM (dashed line).**

reduced circulating central blood volume than that of a normothermic individual.

Despite differences in arterial pressure during the thermal provocations (Table 2; Fig. 4), cerebral blood velocity responses were similar throughout LBNP between thermal conditions (Table 2; Fig. 5). A possible explanation for this observation is that cerebral autoregulation maintained constant cerebral blood flow despite subtle deficits in central blood volume and corresponding reductions in arterial blood pressure (4, 5, 27). However, given recent challenges to the existence of cerebral autoregulation (29, 30), another explanation for the apparent uncoupling of cerebral blood flow with changing arterial blood pressures is the possibility that the thermal provocations caused cerebral vascular resistance and intracranial pressure (ICP) changes proportionate to and in the same direction as arterial pressures (i.e., COOL increased ICP, WARM decreased ICP), resulting in a constant cerebral perfusion pressure. The latter possibility is consistent with similar patterns of response in CRI (Fig. 6), suggesting that similar ''taxing'' of compensatory mechanisms was required to maintain similar cerebral perfusion. That said, perhaps cerebral blood velocity would have directionally followed mean arterial pressure if greater cardiovascular instability (e.g., greater reductions in arterial pressure) occurred during the thermal provocations.

In Protocol 1, it is clear that an applied level of sustained stimulated hemorrhage of less than ~500 mL (30 mm Hg LBNP) did not substantially alter cerebral perfusion, regardless of the thermal perturbations applied. Continuously increasing the level of LBNP (performed in Protocol 2), to replicate continuous bleeding, elicited greater reductions in arterial blood pressure, which was reflected in the average rate of decrease in CRI, and subsequently cerebral blood flow (Figs. 4–6). However, there was no effect of mean skin temperature on hemorrhagic tolerance. Perhaps the lack of effectiveness of the thermal provocations on cerebral perfusion and tolerance to LBNP is due to the moderate level of heating and cooling applied, as well as the relatively short duration (5 min) of the thermal provocation prior to the onset of simulated hemorrhage. That said, the objective of the selected thermal provocations was to simulate the level and duration of heating and cooling that may occur in the field when treating a hemorrhaging victim. While 5 min may seem unrealistic, the thermal application continued throughout the simulated hemorrhage, which would normally occur (i.e., continually applying treatment) until emergency personnel begins treatment and/or the individual is transported to a field center.

Previous investigations showing that whole-body heat stress profoundly compromises tolerance to simulated hemorrhage utilized a substantially greater hyperthermic stress that increased core and skin temperatures to a much greater extent (~39°C for both measurements; 11, 16-18), relative to that which occurred in the present protocols. During profound hyperthermic stress, the cutaneous sympathetic vasodilator system is activated (as well as locally induced vasodilation) to appreciably increase skin blood flow. This response has the potential to compromise tolerance to simulated hemorrhage as blood is displaced to the periphery and central blood volume is reduced (11). Another factor that can negatively influence hemorrhagic tolerance is dehydration; Lucas et al. (31) reported that fluid loss during heat stress impaired LBNP tolerance by 64%. Notably, the onset for cutaneous vasodilation and sweating occurs only after a core temperature threshold is surpassed, which may vary individually (32, 33). The level of heating applied in the present studies, and likely what is being delivered on the battlefield, did not elicit a substantial change in core or mean skin temperatures. Further, the small sustained increase in core temperature across all conditions may have been due to reductions in skin blood flow due to the hypotensive challenge. Thus, it is unlikely that substantial cutaneous vasodilation or sweating occurred, which may explain why the heating stimulus did not compromise tolerance to LBNP.

Skin surface cooling elicited similar blood pressure responses relative to what has been previously reported (20–23). Although no change in tolerance to reduced central blood volume was observed during Protocol 2 with the cooling stimulus, other studies show that cooling improves LBNP tolerance and cerebral blood velocity (23) in normothermic subjects (21, 34). Differences between these studies and the present one are likely due to differences in methodology. Durand et al. applied skin cooling for 30 min prior to the onset of a step-wise LBNP protocol, while Kean et al. (34) utilized environmental cooling for 100 min prior to sustained subhypotensive LBNP. In Protocol 2, cooling was imposed for only 5 min before a hemorrhagic ramp challenge. Although the time of exposure may have been insufficient to cause appreciable changes in skin temperature, the cooling challenge continued throughout the hemorrhagic ramp, which may be more applicable to what may occur in the current battlefield and civilian prehospital settings. However, with anticipated requirements for prolonged field care (e.g., as much as 72 h), skin cooling at levels that does not compromise normal coagulation function may prove to be an important adjunct intervention that can delay the onset of life-threatening circulatory shock.

Wilson et al. (23) demonstrated that if individuals are sufficiently hyperthermic, such as that achievable in the military setting (i.e., elevated environmental temperatures, physical exertion, body armor), skin surface cooling improves the

maintenance of cerebral perfusion and presumably tolerance during simulated hemorrhage. Given that observation, it is likely that skin surface cooling is most effective if the individual is sufficiently hyperthermic prior to the simulated hemorrhage relative to being normothermic as in the present studies. That is, we proposed that an applied thermal provocation when administered to a hyperthermic hemorrhaging individual will be beneficial (with cooling) or detrimental (with warming) to hemorrhagic tolerance, relative to if that individual is normothermic; conclusions consistent with prior observations (11, 16–18, 20–23). Additional experiments will be required to test this hypothesis and provide evidence to support direction of such a clinical practice guideline.

CONCLUSION

The present findings demonstrate that skin surface cooling is not beneficial, while skin surface warming is not detrimental to the maintenance of cerebral perfusion and hemorrhagic tolerance during simulated hemorrhage in normothermic individuals within the temperature limits applied in the present investigation. While we acknowledge the limitation that the LBNP model does not encompass the complete cascade resulting from hemorrhage (e.g., there is no activation of hemostasis mechanisms or altered metabolic state), LBNP does mimic the integrative cardiovascular responses similar to an actual hemorrhagic insult (i.e., central hypovolemia, reduced blood pressure, and increased heart rate; 27, 28). Thus, the obtained results are translatable to noninvasive treatment of bleeding trauma patients and combat casualties in the prehospital setting. Moreover, we recognize that the present findings do not address a variety of other conditions and factors (e.g., sex, age, health status, medications, menstrual cycle, pre- and post-menopausal status, etc.) that may influence the outcomes. Further research will be necessary to address the potential impact of such factors on the observed findings.

Information from these studies can assist in the modification of clinical practice guidelines and identification of treatment(s) that may be beneficial to the care of hemorrhaging victims who are young (to middle-aged) and relatively healthy—particularly those patients with relatively short transport times to higher levels of care (i.e., Role 3). While the present data do not support the hypothesis that acute ($\leq$5 min) cooling is beneficial for a normothermic hemorrhaging victim, these data indicate that warming would not be harmful during this short time frame.

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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).

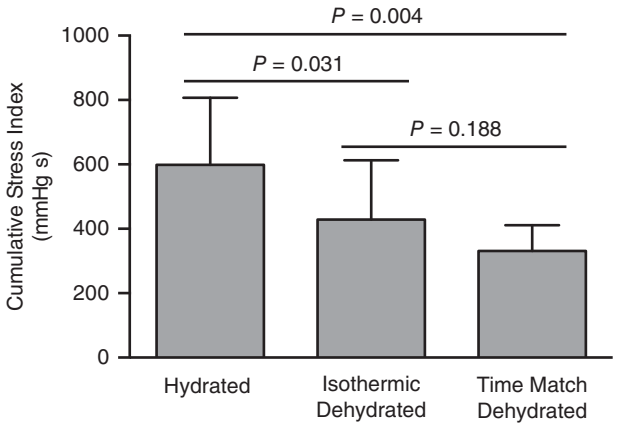

Figure 1. Lower body negative pressure (LBNP) tolerance, expressed as the cumulative stress index, following exercise in a hot environment during which: (i) water was ingested to offset sweat losses (Hydrated, $n = 11$); (ii) water was withheld, and exercise was terminated upon the same increase in intestinal 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

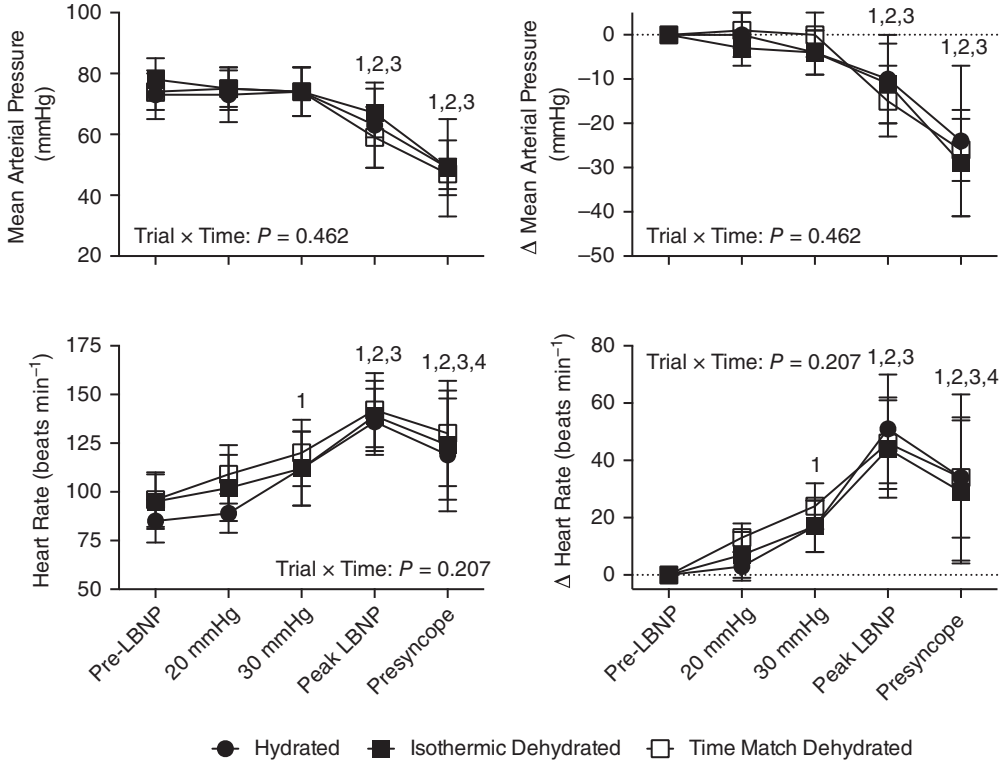

Figure 2. Absolute values (left panels) and the change (Δ; right panels) from immediately prior to lower body negative pressure (pre-LBNP) in mean arterial pressure (top panels) and heart rate (bottom panels) at pre-LBNP, 20 mmHg LBNP, 30 mmHg LBNP, upon the attainment of the highest heart rate achieved 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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Tissue oxygen saturation during hyperthermic progressive central hypovolemia

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Schlader ZJ, Rivas E, Soller BR, Convertino VA, Crandall CG. Tissue oxygen saturation during hyperthermic progressive central hypovolemia. *Am J Physiol Regul Integr Comp Physiol* 307: R731–R736, 2014. First published July 16, 2014; doi:10.1152/ajpregu.00190.2014.—During normothermia, a reduction in near-infrared spectroscopy (NIRS)-derived tissue oxygen saturation (So_2) is an indicator of central hypovolemia. Hyperthermia increases skin blood flow and reduces tolerance to central hypovolemia, both of which may alter the interpretation of tissue So_2 during central hypovolemia. This study tested the hypothesis that maximal reductions in tissue So_2 would be similar throughout normothermic and hyperthermic central hypovolemia to presyncope. Ten healthy males (means ± SD; 32 ± 5 yr) underwent central hypovolemia via progressive lower-body negative pressure (LBNP) to presyncope during normothermia (skin temperature ≈34°C) and hyperthermia (+1.2 ± 0.1°C increase in internal temperature via a water-perfused suit, skin temperature ≈39°C). NIRS-derived forearm (flexor digitorum profundus) tissue So_2 was measured throughout and analyzed as the absolute change from pre-LBNP. Hyperthermia reduced ($P < 0.001$) LBNP tolerance by 49 ± 33% (from 16.7 ± 7.9 to 7.2 ± 3.9 min). Pre-LBNP, tissue So_2 was similar ($P = 0.654$) between normothermia (74 ± 5%) and hyperthermia (73 ± 7%). Tissue So_2 decreased ($P < 0.001$) throughout LBNP, but the reduction from pre-LBNP to presyncope was greater during normothermia (−10 ± 6%) than during hyperthermia (−6 ± 5%; $P = 0.041$). Contrary to our hypothesis, these findings indicate that hyperthermia is associated with a smaller maximal reduction in tissue So_2 during central hypovolemia to presyncope.

lower body negative pressure; heat stress; simulated hemorrhage; syncope

HEMORRHAGE, AND SUBSEQUENT cardiovascular decompensation, is a leading cause of death in both civilian and military settings (3, 14). That said, up to 25% of battlefield deaths are potentially survivable if adequate detection, intervention, and treatment is provided, with ~85% of those being hemorrhage-related (8, 16). Surviving a hemorrhagic injury is extremely time-sensitive (3). Thus, early recognition of the severity of the injury and rapid medical intervention is vital to patient survival (17). Unfortunately, changes in traditional hemodynamic markers (e.g., blood pressure and heart rate) during a hemorrhagic event are often late indicators of cardiovascular instability and are, therefore, poor survival prognosticators (5). Interestingly, Soller et al. (21, 22) identified that tissue oxygen saturation (So_2), in the region of skeletal muscle, determined noninvasively via near-infrared spectroscopy (NIRS), is reduced during the initial stages of graded lower body negative pressure (LBNP), a hemorrhage model (11). In the absence of changes in metabolism, when blood flow under the measurement area is reduced, an increase in oxygen extraction ensues that is reflected in proportional reductions in tissue So_2 (2). Thus, reductions in tissue So_2 during LBNP reflect the magnitude of reductions in muscle blood flow in the measurement area (21, 22). Importantly, these LBNP-induced reductions in tissue So_2 occur prior to changes in blood pressure and heart rate and reflect the onset and the magnitude of reductions in stroke volume (21, 22). This is notable given that stroke volume is an index of the degree of central hypovolemia, but it is challenging to accurately measure in the field. Thus, noninvasive monitoring of tissue So_2 appears to be an early indicator of central hypovolemia in humans, suggesting it may be a valuable tool for monitoring the severity of blood loss during a hemorrhagic injury in prehospital and/or field settings.

Hyperthermia (i.e., increases in internal and skin temperatures) universally decreases tolerance to a simulated hemorrhagic insult (19), suggesting that the timeline to begin treatment is shortened during such conditions. Notably, early, noninvasive indicators of central hypovolemia during hyperthermia have not been determined. Therefore, the objective of this study was to test the hypothesis that, relative to that occurring during normothermia, hyperthermia will not affect the magnitude of maximal reductions in tissue So_2 occurring during LBNP to presyncope. The testing of this hypothesis will provide data regarding the utility of tissue So_2 as an early indicator of the severity of hemorrhage-induced central hypovolemia while hyperthermic. Such information could dictate medical treatment decisions made in prehospital and/or field settings. These findings have implications for conditions in which individuals are often hyperthermic and at an increased risk of a hemorrhagic injury (e.g., soldiers, miners, and firefighters).

METHODS

Subjects. Ten healthy, physically active, males participated in this study. The subject characteristics were (means ± SD) the following: age, 32 ± 5 yr; height, 183 ± 9 cm; and weight, 85.1 ± 12.5 kg. All subjects were nonsmokers, 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. This protocol and informed consent were approved by the Institutional

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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. Subjects arrived at the laboratory euhydrated (confirmed via a urine-specific gravity <1.025) and having refrained from strenuous exercise, alcohol, and caffeine for a period of 24 h. Testing was completed in the northern hemisphere (Dallas, Texas) during fall, winter, and spring months.

Instrumentation and measurements. Approximately 60 min prior to experimental testing, each subject swallowed a telemetry pill (HQ, Palmetto, FL) for the measurement of intestinal temperature. Mean skin temperature was measured as the weighted average of six thermocouples attached to the skin. Body temperature was controlled via a water-perfused tube-lined suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body except the head, hands, one forearm, and the feet. Heart rate was continually recorded from an electrocardiogram (HP Patient Monitor, Agilent, Santa Clara, CA) interfaced with a cardiotachometer (CWE, Ardmore, PA). Beat-to-beat blood pressure was continuously measured via the Penaz method (Finometer Pro, FMS, Amsterdam, The Netherlands), which was confirmed intermittently via auscultation of the brachial artery by electrosphygmomanometry (Tango+, SunTech, Raleigh, NC). Tissue So_2 was measured noninvasively using NIRS (CareGuide 1100, Reflectance Medical, Westborough, MA). This NIRS device uses a novel sensor design and mathematical preprocessing techniques to correct spectra for variations in skin pigment and fat prior to the calculation of tissue So_2 (9). The NIRS sensor was placed on the left forearm over the flexor digitorum profundus in the longitudinal axis with the head of the sensor nearest to the olecranon process and secured against the skin with custom adhesive pads.

Experimental protocol. Subjects visited the laboratory on two separate occasions, separated by at least 8 wk, but completed at the same time of day (within a subject). During both trials, following instrumentation, subjects rested quietly in the supine position for at least 45 min, while normothermic water (34°C) perfused the suit. After baseline data collection, subjects underwent either whole body passive heat stress or a normothermic time control period, the latter of which was 40–60 min in duration. Whole body passive heat stress was induced by perfusing 49°C water through the suit that was sufficient to increase internal temperature ~1.2°C above baseline, while 34°C water was perfused through the suit throughout the normothermic, time control trial. The subjects were not allowed to drink fluids at any time during either trial. Both trials were conducted in a randomized, counterbalanced manner. Immediately following the heating/time control period, the subjects underwent progressive LBNP to presyncope. 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: 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 hemodynamically identified syncopal signs.

Data and statistical analyses. Most data were collected at 50 Hz via a data acquisition system (Biopac System, Santa Barbara, CA), the exception being the NIRS tissue So_2 data, which were sampled every 30 s. Steady-state data (3-min average) were analyzed at baseline (i.e., prethermal perturbation) and just prior to commencing LBNP (i.e., postthermal perturbation or time control period; Pre-LBNP). During LBNP, data (2-min average) were statistically compared between thermal conditions at 20 mmHg LBNP ($n = 10$), 30 mmHg LBNP ($n = 9$, due to poor NIRS signal in one subject), and 40 mmHg LBNP ($n = 5$, due to presyncope occurring at 30 mmHg during hyperthermia in an additional four subjects). Data were also analyzed at presyncope in both trials, regardless of the LBNP stage (1-min average; presyncope; $n = 9$) and at the same level of normothermia LBNP as that occurring at hyperthermia presyncope (mean LBNP level: 40 ± 10 mmHg; $n = 9$), i.e., the highest common LBNP stage between thermal conditions for each subject. Muscle metabolism changes minimally during hyperthermia under resting conditions (18), and therefore, changes in NIRS tissue So_2 reflect the magnitude and direction of changes in tissue perfusion (2). Since perfusion through a vascular bed is governed, in part, by perfusion pressure, an index of tissue vascular conductance (tVC) was calculated as the quotient of tissue So_2 and mean arterial pressure. To evaluate the isolated effect of LBNP, both with and without hyperthermia, data were also analyzed as the change from Pre-LBNP.

Baseline, Pre-LBNP, and during LBNP (20–40 mmHg LBNP) data were analyzed using two-way (main effects: trial × time) repeated-measures ANOVA. Data at normothermia presyncope, hyperthermia presyncope, and normothermia 40 ± 10 mmHg LBNP (i.e., the same normothermia LBNP as that occurring at hyperthermia presyncope) were analyzed using one-way repeated-measures ANOVA. Where appropriate, post hoc Holm-Sidak pair-wise comparisons were made. Data were analyzed using SigmaPlot (v.12, Systat Software, Chicago, IL) with a priori statistical significance set at $P \leq 0.05$. All data are reported as means ± SD.

RESULTS

Responses to hyperthermia alone. Baseline internal and mean skin temperatures were not different ($P \geq 0.498$) between trials (Table 1). Whole body passive heat stress increased ($P < 0.001$) both intestinal and mean skin temperatures by 1.2 ± 0.1°C and 5.1 ± 0.1°C, respectively, whereas temperatures remained unchanged throughout the normothermia trial (0.0 ± 0.2°C and 0.0 ± 0.7°C, $P \geq 0.217$). Mean arterial pressure slightly increased ($P = 0.013$) from baseline to Pre-LBNP during the normothermia trial, but was unchanged ($P = 0.097$) during the hyperthermia trial (Table 1). Tissue So_2 increased ($P = 0.009$) from baseline to Pre-LBNP in both trials (Table 1), while the magnitude of this increase was not different ($P = 0.593$) between trials. During normothermia, tVC did not change ($P = 0.994$) from baseline to

Table 1. *Thermal and hemodynamic responses from baseline to Pre-LBNP during the normothermia and hyperthermia trials*

	Normothermia		Hyperthermia	
	Baseline	Pre-LBNP	Baseline	Pre-LBNP
Intestinal temperature, °C	37.0 ± 0.2	37.0 ± 0.3	36.9 ± 0.1	38.1 ± 0.1*†
Mean skin temperature, °C	34.1 ± 0.5	34.2 ± 0.7	34.0 ± 0.3	39.1 ± 0.7*†
Heart rate, bpm	58 ± 8	62 ± 9	59 ± 8	99 ± 16*†
Mean arterial pressure, mmHg	82 ± 6	87 ± 8†	80 ± 7	77 ± 6
Tissue So_2, %	70 ± 4	74 ± 5†	68 ± 4	73 ± 7†
tVC (%/mmHg)	0.86 ± 0.07	0.86 ± 0.11	0.86 ± 0.10	0.95 ± 0.11†#

Tissue So_2, near infrared spectroscopy-derived tissue oxygen saturation; tVC, tissue vascular conductance. *Significantly different from normothermia ($P < 0.001$). †Significantly different from baseline ($P \leq 0.047$). #Significantly different from normothermia ($P = 0.054$).

Pre-LBNP, but increased ($P = 0.047$) during this period during hyperthermia.

Responses to hyperthermic LBNP. LBNP time to tolerance (normothermia: 16.7 ± 7.9 min; hyperthermia: 7.2 ± 3.9 min) and the final LBNP stage reached (normothermia: 70 ± 20 mmHg; hyperthermia: 40 ± 10 mmHg) were higher ($P < 0.001$) during the normothermia trial. During hyperthermia, mean arterial pressure decreased ($P < 0.001$) during 20 through 40 mmHg LBNP, but not at these LBNP stages during normothermia (Fig. 1). Absolute mean arterial pressure and reductions (relative to Pre-LBNP) in mean arterial pressure at presyncope were not different ($P \geq 0.121$) between thermal conditions (Fig. 1). However, mean arterial pressure at normothermia 40 ± 10 mmHg LBNP was higher ($P \leq 0.007$) than this value when subjects were at presyncope during both normothermia and hyperthermia (Fig. 1). Heart rate was 30–40 bpm higher ($P < 0.001$) throughout hyperthermia prior to LBNP and increased ($P < 0.001$) during LBNP in both trials (change from Pre-LBNP to presyncope: normothermia: +38 ± 32 bpm; hyperthermia: +27 ± 20 bpm). In both trials, tissue So_2 progressively decreased ($P < 0.001$) throughout LBNP (Fig. 2). However, the magnitude of this reduction was greater ($P = 0.041$) at presyncope during normothermia (−10 ± 6%) than during hyperthermia (−6 ± 5%) (Fig. 2). At 40 ± 10 mmHg LBNP, the reduction in tissue So_2 was not different ($P = 0.803$) between trials, despite this being the average LBNP level at which presyncope occurred during hyperthermia, while tissue So_2 continued to further decrease ($P = 0.028$) through presyncope in the normothermic trial (Fig. 2). Throughout LBNP, tVC was higher ($P \leq 0.042$) during hyperthermia than during normothermia, and this persisted through presyncope (Fig. 3).

DISCUSSION

The primary objective of this study was to test the hypothesis that hyperthermia would not affect changes in tissue So_2 during LBNP to presyncope. Consistent with this hypothesis, reductions in tissue So_2 were similar at each absolute level of LBNP in both normothermia and hyperthermia (Fig. 2). Counter to our expectations, however, tissue So_2 was lower at presyncope during normothermia compared with during hyperthermia (Fig. 2). Furthermore, hyperthermia, alone, increased tissue So_2 independent of changes in blood pressure, as evidenced by an increase in tVC (Table 1). These findings indicate that hyperthermia, alone, influences tissue So_2 under the measurement area. Furthermore, these data also indicate that, despite tissue So_2 being similar at absolute levels of LBNP during both conditions, the magnitude of maximal reductions in tissue So_2 were smaller at presyncope during hyperthermia, compared with during normothermia. These data suggest that tissue So_2 underestimates the relative magnitude of central hypovolemia during hyperthermia.

Tissue So_2 during hyperthermia alone. In the absence of changes in metabolic rate, changes in tissue So_2 indicate the magnitude and direction of changes in tissue blood flow (2). In the current study, tissue So_2 increased in a similar fashion in both hyperthermic and normothermic conditions following 40–60 min of supine rest (Table 1). During normothermia, increases in tissue So_2 were likely driven largely by increases in tissue perfusion pressure rather than alterations in vascular resistance, as indicated by elevated blood pressure without a change in tVC (Table 1). By comparison, increases in tissue So_2 during hyperthermia were likely the result of reduced vascular resistance under the evaluated area rather than increased tissue perfusion pressure, as indicated by an elevated tVC in this thermal condition (Table 1). A possible explanation for these findings is a large (upward to 6–10-fold) hyperthermia-induced increase in skin blood flow (4, 7). Increases in muscle blood flow during hyperthermia may also contribute (18), but this finding is not always observed (10). Furthermore, a temperature-induced rightward shift in the oxygen dissociation curve cannot be discounted as a potential mechanism for

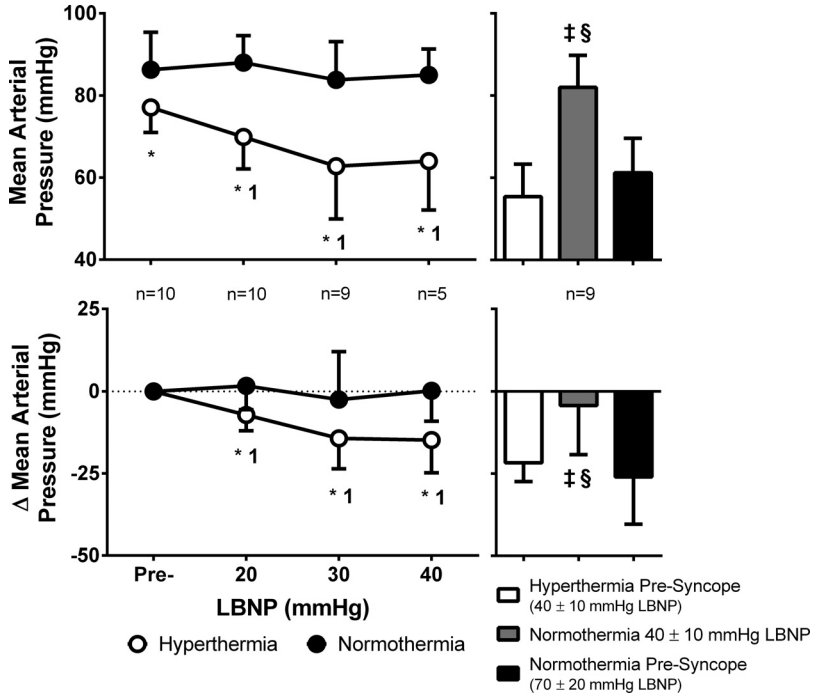

Fig. 1. Mean arterial pressure, expressed as absolute (*top*) and the change (Δ) from pre-lower body negative pressure (Pre-LBNP) (*bottom*), during normothermia and hyperthermia (means ± SD). On the left, data are presented from Pre-LBNP through 40 mmHg LBNP, while data on the right data are presented at presyncope during normothermia and hyperthermia and at the same level of normothermia LBNP as that occurring at hyperthermia presyncope, within a given subject (mean LBNP level: 40 ± 10 mmHg). *n* indicates the number of subjects included in the analysis at a given LBNP stage. [1]Significantly different from Pre-LBNP for the indicated thermal condition ($P \leq 0.005$). *Significantly different from normothermia ($P \leq 0.006$). ‡Significantly different from normothermia presyncope ($P \leq 0.001$). §Significantly different from hyperthermia presyncope ($P \leq 0.007$).

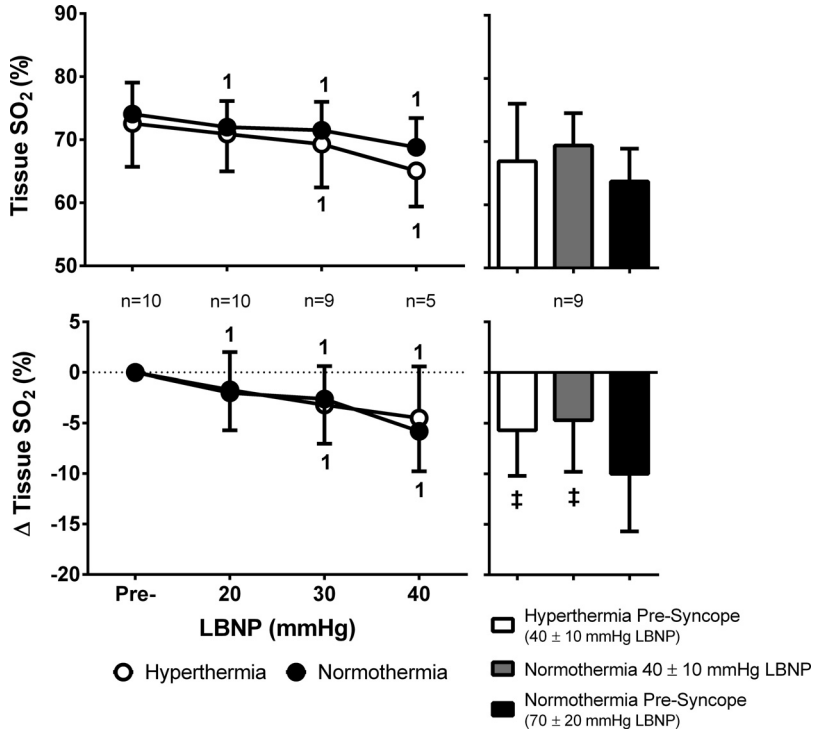

Fig. 2. Near infrared spectroscopy-derived tissue oxygen saturation (tissue So_2), expressed as absolute (*top*) and the change (Δ) from Pre-LBNP (*bottom*), during normothermia and hyperthermia (mean ± SD). On the left, data are presented from Pre-LBNP through 40 mmHg LBNP, while data on the right are presented at presyncope during normothermia and hyperthermia and at the same level of normothermia LBNP as that occurring at hyperthermia presyncope, within a given subject (mean LBNP level: 40 ± 10 mmHg). *n* indicates the number of subjects included in the analysis at a given LBNP stage. [1]Significantly different from Pre-LBNP for the indicated thermal condition ($P \leq 0.030$). ‡Significantly different from normothermia presyncope ($P \leq 0.041$).

hyperthermia-induced decreases in tissue So_2. However, any influence of temperature on the oxygen dissociation curve is likely small given the moderate level of hyperthermia in this study (i.e., ~1.2°C increase in intestinal temperature).

Tissue So_2 during hyperthermic LBNP. Tissue So_2 is as an early indicator of the magnitude of central hypovolemia occurring subsequent to simulated hemorrhage in normothermic individuals (21, 22). Consistent with those observations, tissue So_2 progressively decreased throughout LBNP in the normothermic trial of the present study (Fig. 2). During hyperthermia, tissue So_2 also decreased during LBNP, but at presyncope, the magnitude of the maximal reduction was lower than that occurring at presyncope during normothermia (Fig. 2). Interestingly, at the highest common LBNP stage between trials (i.e., 40 ± 10 mmHg LBNP), despite this being the level of LBNP that caused presyncope during the hyperthermic trial,

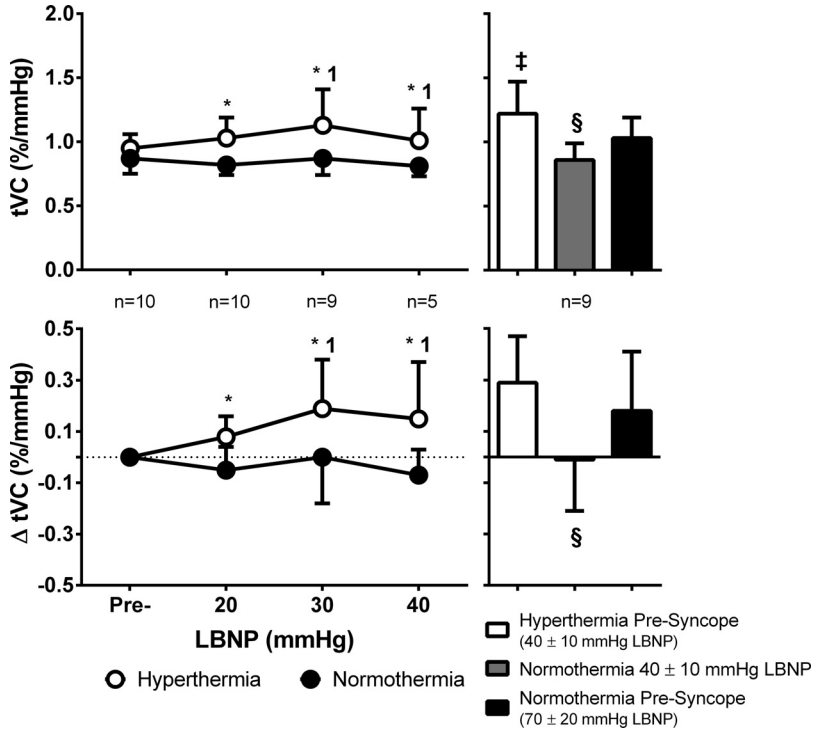

Fig. 3. Tissue vascular conductance (tVC), expressed as absolute (*top*) and the change (Δ) from Pre-LBNP (*bottom*), during normothermia and hyperthermia (means ± SD). On the left, data are presented from Pre-LBNP through 40 mmHg LBNP, while data on the right data are presented at presyncope during normothermia and hyperthermia and at the same level of normothermia LBNP as that occurring at hyperthermia presyncope, within a given subject (mean LBNP level: 40 ± 10 mmHg). *n* indicates the number of subjects included in the analysis at a given LBNP stage. [1]Significantly different from Pre-LBNP for the indicated thermal condition ($P \leq 0.004$). *Significantly different from normothermia ($P \leq 0.023$). ‡Significantly different from normothermia presyncope ($P \leq 0.042$). §Significantly different from hyperthermia presyncope ($P \leq 0.011$).

the magnitude of the reduction in tissue So_2 was similar between thermal conditions (Fig. 2). Given that at this point during hyperthermia, blood pressure was profoundly lower (Fig. 1) and the magnitude of central hypovolemia is greater (6), it may be that the tissue So_2 underestimates the relative magnitude of the central hypovolemic insult during hyperthermia. Furthermore, tVC was elevated throughout LBNP during hyperthermia (Fig. 3), suggesting that, compared with that occurring during normothermia, the vasculature under the measurement area was in a dilated state. One explanation for these observations may be due to hyperthermia-induced elevation in skin blood flow under the area of measurement (4, 7). Attenuated reductions in muscle blood flow during hyperthermic LBNP may also contribute. This contention is supported by evidence indicating that heated conduit blood vessels have attenuated vasoconstrictor capacity in vitro (12, 13) but is contrasted by in vivo evidence, indicating that muscle vasoconstrictor capacity is preserved during leg heating (15). Notably, however, the extent by which hyperthermia impacts muscle vasoconstrictor capacity currently remains unknown. Clearly, further research is required to address the mechanism(s) regarding the observed smaller reductions in tissue So_2 at presyncope during hyperthermia.

It is interesting to note that tVC increased during the latter stages of LBNP in both trials (Fig. 3). These observations corroborate other findings, indicating that muscle vasodilation commonly precedes syncope (1). That this apparent vasodilation occurred earlier during the hyperthermia trial (Fig. 3) can likely be explained by the closer proximity of a given level of LBNP to presyncope during the hyperthermia trial compared with the normothermia trial. Although intriguing, it is important to note that these findings should be interpreted with caution, given that the utility of tVC as an indicator of tissue vascular tone during LBNP and/or hyperthermia remains uncertain.

Methodological considerations. It should be noted that the findings presented herein are likely constrained to the NIRS technology used in this study (i.e., CareGuide 1100, Reflectance Medical) and may have been different had an alternative NIRS technology been applied. Likewise, it is also notable that the clinical applicability of this technology is in its infancy. That said, although not directly related to the present study, preliminary evaluation of this NIRS technology has found that tissue So_2 is an indicator of plasma leakage in patients with dengue hemorrhagic fever, highlighting tissue So_2's potential utility in a clinical setting (20).

Perspectives and Significance

Early recognition of the extent of a hemorrhagic injury and, thus, timely treatment is vital to surviving such an insult (3, 17). Noninvasive tissue So_2 has been proposed as a valuable tool for monitoring the severity of central hypovolemia during the early stages of a hemorrhagic injury in prehospital or field settings (21, 22). However, often those individuals who are at the highest risk of a hemorrhagic injury are also hyperthermic (e.g., soldiers, miners, and firefighters). Notably, the ability to tolerate a simulated hemorrhagic event is markedly reduced during hyperthermia (19), suggesting that the timeline to begin treatment is shortened during such conditions. Therefore, the present study evaluated whether LBNP-induced reductions in tissue So_2 were affected by hyperthermia. It is clear that hyperthermia impacts tissue So_2 during progressive central hypovolemia. Specifically, tissue So_2 appears to underestimate the relative magnitude of the central hypovolemic insult during hyperthermia. Thus, it remains unknown whether a noninvasive measurement of tissue So_2 generated from the use of NIRS technology will provide the medic with appropriate triage decision support regarding the severity of a patient's degree of central hypovolemia during hyperthermia. Further research is required.

Conclusions. Compared with that occurring during normothermia, the present study identified that reductions in tissue So_2 during LBNP are similar with hyperthermia, but that LBNP-induced maximal reductions in tissue So_2 are smaller at presyncope in this thermal condition. These data suggest that tissue So_2 underestimates the relative magnitude of central hypovolemia during hyperthermia. These observations can be explained by changes in muscle blood flow and/or hyperthermia-induced elevations in skin blood flow under the measurement area. Further studies are needed to better understand the application of NIRS-derived tissue So_2 as a noninvasive indicator of central hypovolemia during hyperthermia.

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DISCLOSURES

B.R.S. is an employee and officer of Reflectance Medical and holds stock and stock options in the company. There are no further conflicts of interest to report.

AUTHOR CONTRIBUTIONS

Author contributions: Z.J.S., E.R., and C.G.C. performed experiments; Z.J.S. analyzed data; Z.J.S., B.R.S., V.A.C., and C.G.C. interpreted results of experiments; Z.J.S. prepared figures; Z.J.S. drafted manuscript; Z.J.S., E.R., B.R.S., V.A.C., and C.G.C. edited and revised manuscript; Z.J.S., E.R., B.R.S., V.A.C., and C.G.C. approved final version of manuscript; B.R.S., V.A.C., and C.G.C. conception and design of research.

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RESEARCH ARTICLE

Normothermic central hypovolemia tolerance reflects hyperthermic tolerance

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Abstract
Purpose To test the hypothesis that those who are highly tolerant to lower body negative pressure (LBNP) while normothermic are also highly tolerant to this challenge while hyperthermic.
Methods Sixty pairs of normothermic and hyperthermic LBNP tests to pre-syncope were evaluated. LBNP tolerance was quantified via the cumulative stress index (CSI), which is calculated as the sum of the product of the LBNP level and the duration of each level until test termination (i.e., 20 mmHg × 3 min + 30 mmHg × 3 min, etc.). CSI was compared between normothermic and hyperthermic trials. Internal and skin temperatures, heart rate, and arterial pressure were measured throughout.
Results Hyperthermia reduced ($P < 0.001$) CSI from 997 ± 437 to 303 ± 213 mmHg min. There was a positive correlation between normothermic and hyperthermic LBNP tolerance ($R^2 = 0.38$; $P < 0.001$). As a secondary analysis, the 20 trials with the highest LBNP tolerance while normothermic were identified (indicated as the HIGH group; CSI 1,467 ± 356 mmHg min), as were the 20 trials with the lowest normothermic tolerance (indicated as the LOW group; CSI 565 ± 166 mmHg min; $P < 0.001$ between groups). While hyperthermia unanimously reduced CSI in both HIGH and LOW groups, in this hyperthermic condition CSI was ~threefold higher in the HIGH group (474 ± 226 mmHg min) relative to the LOW group (160 ± 115 mmHg min; $P < 0.001$).
Conclusions LBNP tolerance while hyperthermic is related to normothermic tolerance and, associated with this finding, those who have a high LBNP tolerance while normothermic remain relatively tolerant when hyperthermic.

Keywords Lower body negative pressure · Heat stress · Simulated hemorrhage · Syncope

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Introduction

The ability to tolerate central hypovolemia, induced by lower body negative pressure (LBNP), greatly varies between individuals [7–9, 16, 17, 21, 25]. Greater tolerance to LBNP is associated with a number of factors, including an augmented vasoactive hormone response [9, 16], higher increases in vascular resistance [7, 9, 26], greater increases in heart rate [7, 8, 26], enhanced protection of central blood volume and cerebral perfusion [21], and augmented oscillations in arterial pressure and cerebral perfusion [25].

Hyperthermia (i.e., increases in internal and skin temperatures) universally decreases LBNP tolerance [19, 28]. The mechanisms by which this occurs are numerous and likely involve insufficient increases in peripheral resistance during LBNP [12, 15, 24], hyperthermia-induced reductions in the central blood volume [13, 14] and accompanying decreases in ventricular filling pressures [14, 29], altered arterial baroreflex control of blood pressure [11], and reductions in cerebral perfusion [4, 23, 28]. Notably, substantial inter-individual differences in

LBNP tolerance likewise persist while hyperthermic [3, 12, 19, 20].

The mechanisms mediating variations in normothermic LBNP tolerance appear comparable to those mediating such variations while hyperthermic (e.g., altered vascular resistance, protection of central blood volume). Thus, the mechanisms underlying inter-individual variability in normothermic LBNP tolerance may explain such variations in tolerance while hyperthermic. If so, we would expect that those observed to be highly tolerant to LBNP while normothermic would also exhibit a high tolerance to this challenge while hyperthermic. In accordance, the primary objective of this study was to test the hypothesis that hyperthermic LBNP tolerance is related to normothermic LBNP tolerance, and by extension that those observed to have high normothermic LBNP tolerance will also be relatively tolerant during hyperthermic LBNP.

Methods

Subjects and study design

Data were retrospectively queried to identify subjects who had undergone progressive LBNP challenges to pre-syncope while both normothermic and hyperthermic. Only those in which the normothermic and hyperthermic trials were carried out in identical experimental conditions were selected, thereby allowing for a repeated measures experimental design. This query resulted in 79 pairs of observations from 60 different subjects. Given the focus on inter-individual variability, for the subjects with more than one pair of normothermic/hyperthermic trials, only one data set was included. In such instances, the paired trial that was included in the analysis was randomly decided via a coin toss. Therefore, the analysis comprised 60 pairs of observations from 60 different subjects (53 males). The subject characteristics were (mean ± SD): age 35 ± 8 years, height 178 ± 8 cm, and weight 83.7 ± 15.8 kg. All subjects were free of any known cardiovascular, neurological, or metabolic diseases. Each study protocol from which these data were obtained received institutional approval from the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas, and all subjects signed an approved informed consent form.

Under most circumstances ($n = 41$ pairs, 7 females), the order of the normothermic and hyperthermic trials were randomized, with the second trial being undertaken at least 24 h after the first (mean 33 ± 32 days), but at the same time of day. However, in a subset of trials ($n = 19$ pairs, all males), both the normothermic and hyperthermic trials were conducted on the same day, with the normothermic trial occurring first, separated by 140 ± 37 min. These data were included in the analysis given that the magnitude of the hyperthermia-induced reductions in LBNP tolerance in this group (−67 ± 16 %) were not different ($P = 0.768$) to that occurring in the group in which the trials were conducted on separate days (−69 ± 24 %), and the evidence indicating that plasma volume and leg interstitial fluid pressures are fully restored within 30 min following LBNP [1]. For the females, both trials were undertaken in the same phase of their menstrual cycle. Subjects arrived at the laboratory euhydrated, confirmed via urine specific gravity (1.013 ± 0.007), and having refrained from strenuous exercise, alcohol, and caffeine for 24 h. All procedures were undertaken in a temperature-controlled laboratory (~25 °C).

Instrumentation and measurements

Approximately 90 min prior to experimental testing, each subject swallowed a temperature pill (HQ Inc., Palmetto, FL, USA) to measure intestinal temperature. Mean skin temperature was measured from the weighted average of six thermocouples attached to the skin [27]. Body temperature was controlled via a water-perfused tube lined suit (Med-Eng, Ottawa, ON, Canada) that covered the entire body except the head, hands, and the feet. Heart rate was continually recorded from an electrocardiogram (HP Patient Monitor, Agilent, Santa Clara, CA, USA) interfaced with a cardiotachometer (CWE, Ardmore, PA, USA). Beat-to-beat arterial pressure was continuously measured via the Penaz method (Finometer Pro, FMS, Amsterdam, The Netherlands or NexFin HD, BMEYE B.V., Amsterdam, The Netherlands), with its readings confirmed intermittently via auscultation of the brachial artery by electrosphygmomanometry (Tango+, SunTech, Raleigh, NC, USA). During all experimental trials the subjects were placed into an LBNP box that was sealed at the level of the iliac crest, remaining supine for the duration of the protocol.

Experimental protocol

Following instrumentation and either a normothermic period or whole-body passive heat stress, all subjects underwent progressive LBNP to pre-syncope. During normothermia, 34 °C water perfused the suit throughout the experiment. During the hyperthermic trial, the subjects underwent whole-body passive heat stress by perfusing 46–50 °C water through the suit, with the LBNP test commencing when intestinal temperature was ~1.4 °C above baseline temperature. Under most circumstances

($n = 48$ pairs, 3 females), the progressive LBNP test started at 20 mmHg for 3 min, with the LBNP increasing by 10 mmHg every 3 min until the onset of syncopal signs and symptoms. In a subset of tests ($n = 12$ pairs, 4 females), the starting LBNP was 10 mmHg, and likewise increased by 10 mmHg every 3 min. These trials were included in the analysis given the repeated measures study design and that the magnitude of the hyperthermia-induced reductions in LBNP tolerance were not different ($P = 0.689$) whether the trials commenced at 10 mmHg LBNP (-66 ± 19 %) or 20 mmHg LBNP (-69 ± 22 %). In the event the LBNP level reached 100 mmHg, that stage was continued without further increasing LBNP until the onset of syncopal signs and symptoms. The criteria for LBNP termination were: continued self-reporting by the subject 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, save one, was terminated due to hemodynamically identified syncopal signs, with one trial being terminated due to syncopal symptoms expressed by the subject.

Data analysis

Data were sampled at a minimum of 50 Hz via a data acquisition system (Biopac System, Santa Barbara, CA, USA). Steady-state data (60 s average) were analyzed at normothermic baseline (i.e., pre-thermal perturbation; Baseline) and just prior to commencing LBNP (i.e., post-thermal perturbation during hyperthermia trials; Pre-LBNP). Data (10 s average) were also analyzed upon the attainment of the peak heart rate during the final 2 min of LBNP (Peak-LBNP) [12] and during the final 10 s of LBNP (Pre-Syncope). Heart rate data at Peak-LBNP and Pre-Syncope were also evaluated as the change (Δ) from Pre-LBNP. LBNP tolerance was quantified using the cumulative stress index (CSI) [22], 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 $\times$ 3 min + 30 mmHg $\times$ 3 min, etc.).

Given that hyperthermia unanimously reduces LBNP tolerance [19, 28], CSI data were 'standardized' to quantitatively identify whether those trials deemed highly tolerant during normothermia remained tolerant, relative to the entire data set, during hyperthermia. Therefore, a *Z*-score was calculated for each subject's LBNP trial in both thermal conditions as follows: CSI *Z*-score = $(CSI_{subject} - CSI_{mean})/CSI_{SD}$, where $CSI_{subject}$ is a subject's CSI, CSI_{mean} is the mean CSI of all subjects in a given thermal condition, and CSI_{SD} is the standard deviation of the CSI in the same thermal condition. Thus, within a given thermal condition an individual's CSI *Z*-score of '0' represents the average CSI of the data set, while a CSI *Z*-score of ± 1.0, ± 2.0, ± 3.0, etc. indicates CSI values that are 1, 2, 3, etc. standard deviations greater (+) or lower (−) than the mean CSI value. Subsequently, the 20 trials with the highest normothermic CSI *Z*-scores (designated as HIGH) and the 20 observations with the lowest normothermic CSI *Z*-scores (designated as LOW) were identified. The CSI *Z*-scores during the hyperthermic LBNP challenge were statistically compared between the 20 HIGH normothermic observations and the 20 LOW normothermic observations, irrespective of their ranking in the hyperthermic LBNP trial. The 'middle' 20 observations were not included in this sub-analysis.

Statistical analysis

Relationships between normothermic LBNP tolerance and hyperthermic LBNP tolerance across all subjects were identified via Pearson product moment correlation analysis. Data at Baseline, Pre-LBNP, Peak-LBNP, and Pre-Syncope, irrespective of group (i.e., HIGH or LOW), were analyzed using repeated measures analysis of variance (ANOVA; main effects temperature $\times$ time). Subject characteristics of the HIGH and LOW groups were compared using independent sample *t* tests. All other data in this HIGH vs. LOW analysis were analyzed using mixed-model repeated measures ANOVA (main effects group $\times$ temperature). Where appropriate, post hoc, pairwise, comparisons were made incorporating a Bonferroni adjustment. Data were analyzed using SigmaPlot (v.12, Systat Software Inc., Chicago, IL, USA) with a priori statistical significance set at $P \leq 0.05$. All data are reported as mean $\pm$ SD.

Results

Complete data set analysis

Baseline (i.e., pre-perturbation) internal (36.9 ± 0.3 °C) and mean skin (34.3 ± 0.5 °C) temperatures were similar ($P \geq 0.513$) between thermal conditions. Hyperthermia increased intestinal (to 38.3 ± 0.3 °C; $P < 0.001$) and mean skin (to 38.5 ± 0.8 °C; $P < 0.001$) temperatures, which remained elevated and stable throughout LBNP. LBNP time to tolerance (normothermia 19.8 ± 5.3 min, hyperthermia 9.1 ± 4.23 min) and the final LBNP stage reached (normothermia 80 ± 20 mmHg, hyperthermia 40 ± 10 mmHg) were higher ($P < 0.001$ for both) during

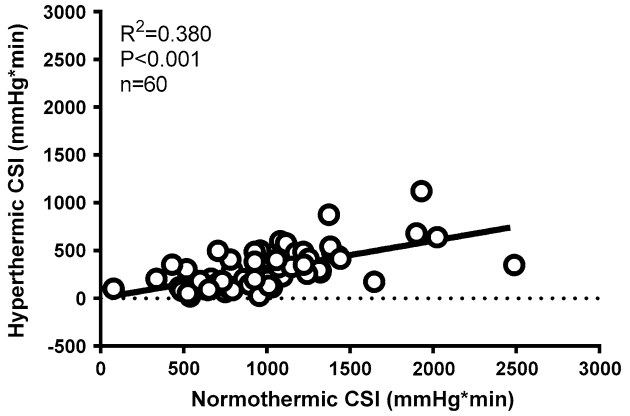

Fig. 1 Correlation between normothermic lower body negative pressure (LBNP) tolerance [i.e., the cumulative stress index (CSI)] and hyperthermic LBNP tolerance

normothermia. Consistent with those values, LBNP tolerance, as assessed with CSI, was unanimously higher during normothermia (997 ± 437 mmHg min) compared to hyperthermia (303 ± 213 mmHg min; $P < 0.001$). Furthermore, normothermic CSI was correlated ($R^2 = 0.380$; $P < 0.001$) with hyperthermic CSI (Fig. 1).

Hyperthermia slightly decreased ($P < 0.001$) mean arterial pressure and profoundly elevated ($P < 0.001$) heart rate (Fig. 2). However, in both thermal conditions heart rate progressively increased ($P < 0.001$) during LBNP, but the magnitude of the elevation in heart rate to LBNP, prior to any bradycardia associated with pre-syncope, was less ($P < 0.001$) during the hyperthermic trial (Fig. 2).

Assessment of HIGH vs. LOW groups

Subject characteristics of the LOW and HIGH groups are presented in Table 1. Intestinal and mean skin temperatures did not differ ($P \geq 0.319$) at any time points, inclusive of LBNP, between the LOW and HIGH groups and were similar to those values reported for the complete data set (see above). Normothermic LBNP time to tolerance (HIGH 25.3 ± 2.6 min, LOW 14.2 ± 3.1 min), the final LBNP stage reached (HIGH 90 ± 10 mmHg, LOW 60 ± 10 mmHg), and CSI (HIGH 1,467 ± 356 mmHg min, LOW 565 ± 166 mmHg min) were higher ($P < 0.001$ for all comparisons) in the HIGH group. Hyperthermia decreased, in both groups ($P < 0.001$), LBNP tolerance time (HIGH 12.6 ± 3.6 min, LOW 5.9 ± 2.9 min), the final LBNP stage reached (HIGH 60 ± 10 mmHg, LOW 30 ± 10 mmHg), and CSI (HIGH 474 ± 226 mmHg min, LOW 160 ± 115 mmHg min), with the hyperthermic value for each of these variables being lower ($P < 0.001$) in the LOW group. Notably, the HIGH group had a greater absolute reduction in LBNP tolerance from normothermia to hyperthermia (HIGH −992 ± 362 mmHg min, LOW −406 ± 193 mmHg min; $P < 0.001$).

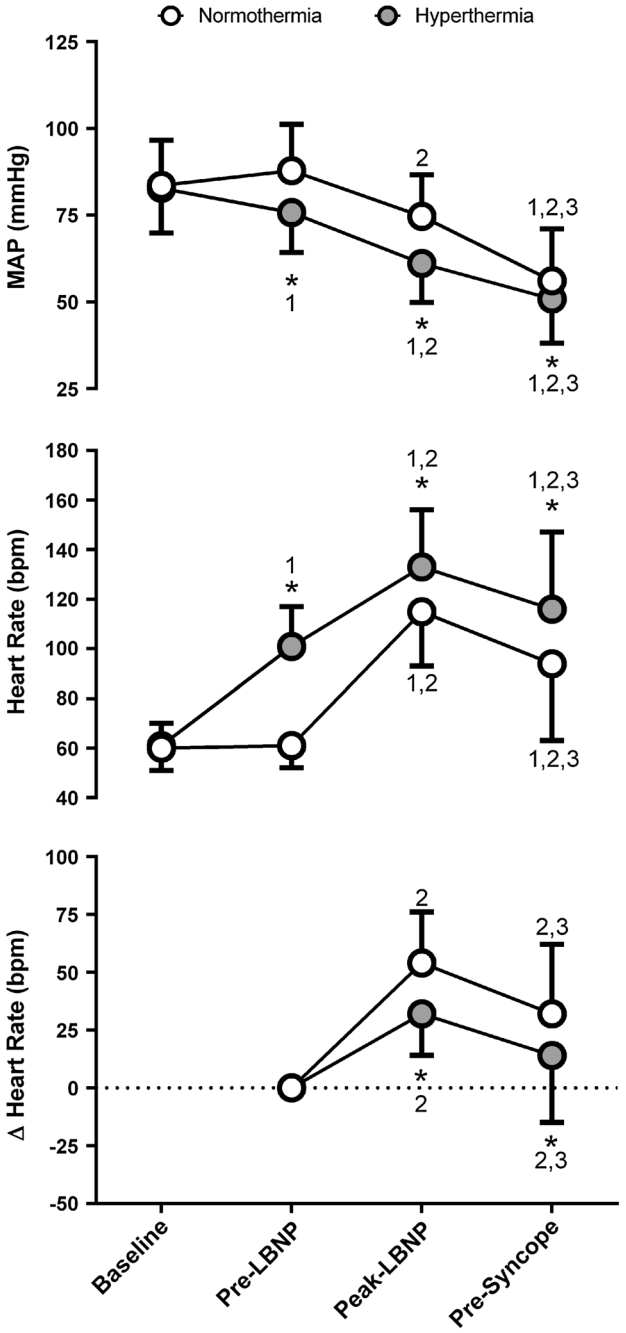

Fig. 2 Mean arterial pressure (MAP; *top*), heart rate (*middle*), and the change (Δ) in heart rate from Pre-LBNP (*bottom*) during normothermic and hyperthermic LBNP at Baseline, Pre-LBNP, Peak-LBNP, and immediately prior to LBNP termination (Pre-Syncope) (mean ± SD). *Asterisks* indicate different from normothermia ($P \leq 0.029$); *1*, *2*, and *3* indicate different from Baseline, Pre-LBNP, and Peak-LBNP, respectively ($P \leq 0.018$). Peak-LBNP is the period with the highest heart rate achieved during the final 2 min of LBNP (i.e., prior to any bradycardia associated with progressive LBNP)

Calculating the CSI *Z*-scores standardized the data such that CSI *Z*-scores for the complete data set ($n = 60$ pairs) during normothermia (0.0 ± 1.0 a.u.) and hyperthermia (0.0 ± 1.0 a.u.) were not different ($P = 0.495$; Fig. 3). By design, in normothermia the LOW group's CSI *Z*-score (−1.0 ± 0.4 a.u.) was lower ($P < 0.001$) than the HIGH group's CSI *Z*-score (1.1 ± 0.8 a.u.; Fig. 3). That during hyperthermia the CSI *Z*-score remained significantly lower in the LOW group (−0.7 ± 0.5 a.u.) compared to the HIGH group (0.8 ± 1.1 a.u.; $P < 0.001$) indicates that the LOW group remained relatively intolerant and the HIGH group remained relatively tolerant to LBNP while hyperthermic.

During normothermic LBNP trials, the HIGH group had a greater increase in heart rate ($P \leq 0.022$), despite no difference ($P \geq 0.395$) in mean arterial pressures between groups (Fig. 4). By contrast, during hyperthermic LBNP, heart rate and the magnitude of the increase in heart rate were not different ($P \geq 0.161$) between groups. Except for Pre-LBNP ($P = 0.020$), mean arterial pressure during hyperthermia was not different ($P \geq 0.347$) between LOW and HIGH groups (Fig. 4).

Table 1 HIGH vs. LOW subject characteristics (mean ± SD)

	LOW	HIGH
Age (years)	37 ± 9	36 ± 9
Height (cm)	178 ± 7	179 ± 8
Weight (kg)	87.5 ± 18.3	83.9 ± 17.4
Sex (male/female)	17/3	17/3

HIGH 20 observations with the highest normothermic lower body negative pressure tolerance, *LOW* 20 observations with the lowest normothermic lower body negative pressure tolerance

Discussion

The primary objective of this study was to test the hypothesis that hyperthermic LBNP tolerance is related to normothermic LBNP tolerance, and by extension that a group observed to have high normothermic LBNP tolerance will also have a relatively high hyperthermic LBNP tolerance. The data presented in this study support this hypothesis. Specifically, hyperthermic LBNP tolerance was moderately related to normothermic LBNP tolerance (Fig. 1), and a subset of observations deemed to have a high normothermic LBNP tolerance were also relatively tolerant to LBNP during hyperthermia (Fig. 3). These findings suggest that normothermic LBNP tolerance may be a predictor of hyperthermic tolerance and that the physiological mechanisms underlying variations in LBNP tolerance during normothermia may also be relevant during hyperthermia.

Relationships between normothermic and hyperthermic LBNP tolerance

Although hyperthermia decreases LBNP tolerance in every subject, LBNP tolerance while hyperthermic varies widely between individuals [3, 12, 19, 20]. Those individuals exhibiting relatively high hyperthermic LBNP tolerance do not have a greater cutaneous vasoconstrictor response [12], nor do they have attenuated hyperthermia-induced reductions in central venous pressure [3] or cerebral perfusion [20]. Thus, what makes someone more tolerant to LBNP during hyperthermia, relative to others, is unclear. In this regard, the present study identified that normothermic LBNP

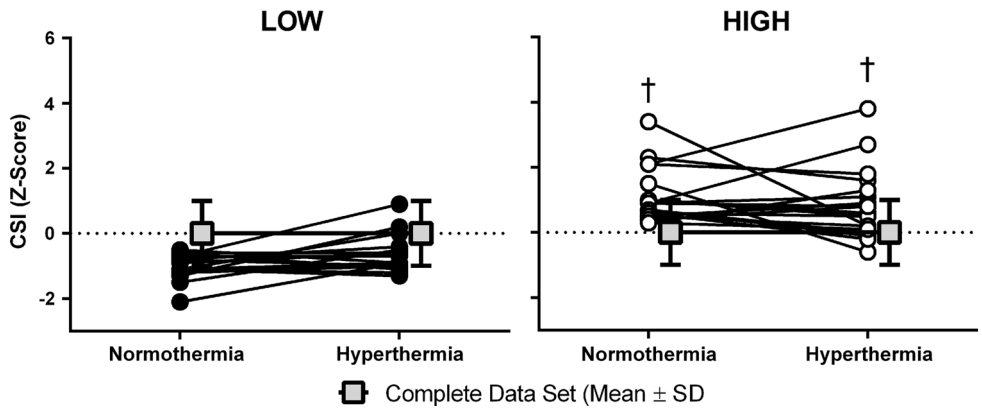

Fig. 3 Individual changes in standardized (i.e., *Z*-score) LBNP tolerance [indexed from the cumulative stress index (CSI)] from normothermia to hyperthermia in the 20 observations with the lowest (LOW) and highest (HIGH) normothermic tolerance. The mean data from the complete data set are also depicted ($n = 60$ pairs; *gray squares*). These data indicate that during hyperthermia the HIGH group remained relatively tolerant (mean value above 0), while the LOW group remained relatively intolerant (mean value below 0). *Dagger* indicates HIGH group is different from LOW group ($P < 0.001$). Mean (±SD) for each group within each condition is reported in text. An explanation of the *Z*-score, its interpretation, and how it was calculated is presented in "Methods"

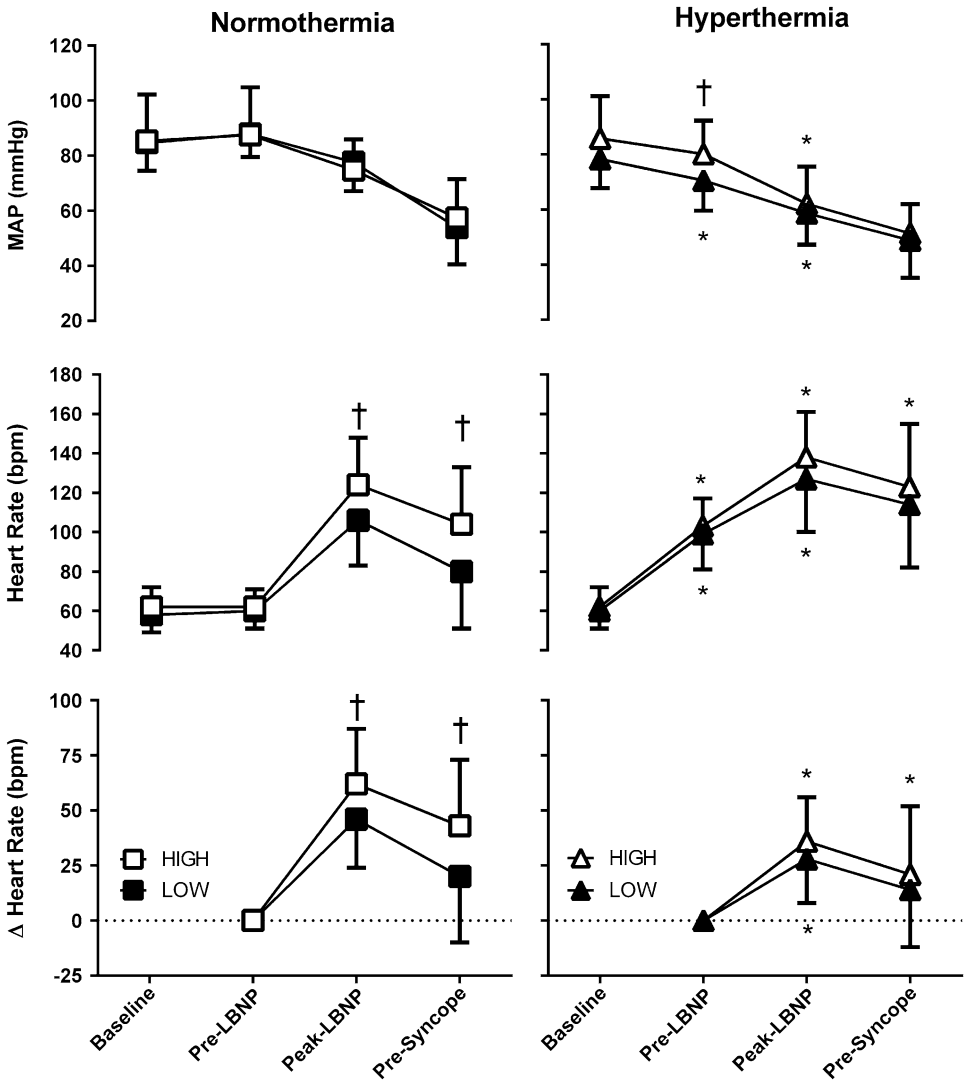

Fig. 4 Mean arterial pressure (MAP; *top*), heart rate (*middle*), and the change (Δ) in heart rate from Pre-LBNP (*bottom*) at Baseline, Pre-LBNP, Peak-LBNP, and immediately prior to LBNP termination (Pre-Syncope) in the 20 observations with the lowest (LOW) and highest (HIGH) normothermic tolerance during normothermia (*on left*) and hyperthermia (*on right*) (mean ± SD). *Dagger* indicates different from LOW ($P \leq 0.022$); *asterisks* indicate different from normothermia for the respective group ($P \leq 0.013$). Peak-LBNP is the period with the highest heart rate achieved during the final 2 min of LBNP (i.e., prior to any bradycardia associated with progressive LBNP)

tolerance accounts for ~38 % of the variance observed in hyperthermic LBNP tolerance (Fig. 1). Related to this observation, a group observed to be highly tolerant during normothermia was also found to have high tolerance during hyperthermia (Fig. 3). Thus, both approaches strongly suggest that a high hyperthermic LBNP tolerance is associated with a high normothermic LBNP tolerance.

Mechanisms underlying variations in hyperthermic LBNP tolerance

Mechanisms responsible for elevated LBNP tolerance while normothermic vary and include differences in the release of vasoactive hormones [9, 16], enhanced vasoconstriction and increases in heart rate [7, 8, 26], augmented protection of cardiac output and cerebral perfusion [21], greater oscillations in arterial pressure and cerebral perfusion [25], and a higher capacity to increase sympathetic nerve activity [7]. The present study confirms that high LBNP tolerance during normothermia is associated with an augmented heart rate response, despite similar mean arterial pressures (Fig. 4). By contrast, this study demonstrates that the heart rate response to LBNP is not enhanced in those who are relatively tolerant to LBNP while hyperthermic (Fig. 4). Thus, an enhanced increase in heart rate is associated with higher LBNP tolerance during normothermia, but not during hyperthermia.

Mechanisms of hyperthermic LBNP intolerance

The mechanisms by which hyperthermia impairs LBNP tolerance are numerous and include insufficient increases in peripheral resistance [12, 15, 24], hyperthermia-induced decreases in ventricular filling pressures [14, 29] (likely occurring subsequent to reductions in the central blood

volume [13, 14]), impaired arterial baroreflex control of blood pressure [11], and reductions in cerebral perfusion [4, 23, 28]. The present study indicates that, although heart rate is elevated by hyperthermia itself, there is an attenuated increase in heart rate during LBNP while in this thermal condition (Fig. 2). That is, the magnitude of the elevation in heart rate during LBNP is greater when individuals are normothermic relative to when hyperthermic. This observation is likely related to hyperthermia-induced tachycardia prior to LBNP (Fig. 2), which may limit the range by which heart rate can further increase during LBNP. Notably, however, it remains uncertain whether, and the extent to which, attenuated increases in heart rate potentially contribute to reductions in hyperthermic LBNP tolerance. Thus, the implications of this observation remain unclear.

Considerations

Critical to the conclusions drawn from these data is the test–retest reliability of LBNP tolerance. Notably, LBNP tolerance during normothermia elicits repeatable results [18]. Unfortunately, however, no such investigation has been undertaken with regard to hyperthermic LBNP tolerance. Nevertheless, given that hyperthermia unanimously reduces LBNP tolerance (by upwards to 60–70 %), it is likely that the observed magnitude of the effect of hyperthermia in reducing LBNP tolerance far outweighs any test–retest variability in LBNP tolerance while hyperthermic.

A limitation of the present study is the lack of 'mechanistic' insights that would help to further explain the present data. For instance, it would have been beneficial to compare how LBNP changed central blood volume, peripheral resistance, or baroreflex function in the high vs. the low tolerance groups during hyperthermia, as has been done previously during normothermia [7–9, 16, 21, 26]. However, given the retrospective nature of this study, which permitted the evaluation of a very large number of subjects ($n = 60$), such analyses were not possible. Nevertheless, the heart rate and blood pressure observations presented in the current study remain novel and clinically relevant.

Conclusions

The present study demonstrates that LBNP tolerance while hyperthermic is related to normothermic tolerance, and that those who have high normothermic tolerance are relatively tolerant during hyperthermia. These data suggest that normothermic LBNP tolerance may be a predictor of hyperthermic tolerance, and thus the physiological mechanisms underlying variations in LBNP tolerance during normothermia are likely relevant during hyperthermia.

Perspectives

The data presented have implications for conditions in which individuals (e.g., soldiers [5], miners [2], and firefighters [6]) are often hyperthermic and at an increased risk of central hypovolemia; as occurs during a hemorrhagic injury [10]. Specifically, these data suggest that, should an individual with high normothermic tolerance to central hypovolemia encounter a similar circumstance when hyperthermic, they will, theoretically, endure such an insult for a longer period of time prior to cardiovascular collapse, when compared to an individual with low normothermic tolerance. Further research is required to understand the mechanisms of these observations.

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Conflict of interest There are no known conflicts of interest.

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