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

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

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1. Introduction

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

2. Keywords

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

3. Overall Project Summary

The global objective of this project tests the hypothesis that environmental and physiological conditions, often experiences on the battlefield, alters hemodynamic and hemostatic function. The following are the items under the Statement of Work that we proposed would be accomplished during Year 3 of the project:

1) Submit and present work from specific aim 1b to a national meeting.
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.
3) Analyze and interpret data obtained during the experiments outlined in specific aim 2.
4) Write technical reports and/or scientific publications to disseminate information obtained from Specific Aims 1B and 2.

Report on accomplishment related to the Statement of Work.

1) Submit and present work from specific aim 1b to a national meeting.
The following presentations originating from the funded project have either been presented in 2014/2015 or will be presented this spring (2015) at national meetings.

- "Reductions in tolerance to central hypovolemia during passive heat stress are accurately tracked by the Compensatory Reserve Index." Daniel Gagnon, Zachary J. Schlader, Eric Rivas, Victor A. Convertino, Jane Mulligan, Greg Grudic, Craig G. Crandall.

- "Fluid restriction during exercise in the heat reduces tolerance to central hypovolemia." Zachary J. Schlader, Daniel Gagnon, Eric Rivas, Victor A. Convertino, Craig G. Crandall

- "Hemodynamic responses to mild warming during simulated mild hemorrhage." Paula Y.S. Poh, Steven A. Romero, Steven J. Petruzzello, Victor A. Convertion, Craig G. Crandall

- "Elevated core and skin temperatures independently attenuate simulated hemorrhage tolerance." James Pearson, Rebekah A.I. Lucas, Zachary J. Schlader, Daniel G. Gagnon, Craig G. Crandall

- "Whole-body warming during a simulated hemorrhagic insult compromises arterial blood pressure but not cerebral perfusion." Paula Y. S. Poh, Daniel Gagnon, Steven A. Romero, Steven J. Petruzzello, Victor A. Convertino and Craig G. Crandall (acceptance for presentation pending)

2) Accomplish objectives outlined in specific aim 2 and;
3) Analyze and interpret data obtained during the experiments outlined in specific aim 2

Below is a graphical depiction of the protocol for Aim 2 that was completed:

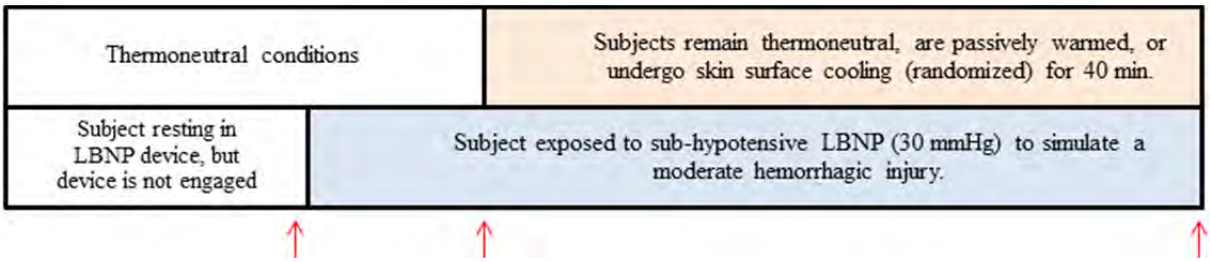

Figure 1: Graphical illustration of the procedures for Aim 2. Subjects will be supine within the lower-body negative pressure (LBNP) device under thermoneutral conditions but without LBNP turned on. Subjects will then be exposed to 10 min of sub-hypotensive LBNP while remaining in a thermoneutral condition. Next, for the ensuing 40 min and with LBNP continuing, subjects will either remain thermoneutral, will undergo mild passive warming, or will undergo skin surface cooling (each on a different day and randomized). The arrows indicate when blood will be drawn for evaluation of markers of hemostatic function. Thermal and hemodynamic variables will continuously be obtained.

Purpose: This project tested the hypothesis that warming a normothermic individual during a simulated hemorrhagic insult can be detrimental to the maintenance of arterial pressure and

cerebral perfusion, while skin surface cooling may be beneficial. We also tested the hypothesis that the various thermal provocations would alter markers of hemostasis.

Methods: Nine men (mean ± SD: age, 30 ± 9 y; weight, 79.4 ± 15.2 kg; height, 79.4 ± 15.2) 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 at the red arrow points illustrated in Figure 1.

Results: 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. Blood based markers of hemostasis were collected and shipped to the US Army Institute of Surgical Research but have not yet been analyzed.

Conclusion: Mild warming of the skin during simulated hemorrhage resulted in greater reductions in arterial blood pressure, which can be detrimental since hemorrhage itself will cause hypotension. Alternatively, blood pressure was elevated during skin surface cooling suggesting that cooling may be beneficial to a hemorrhaging individual who is not hypothermic. Although cerebral perfusion was unaffected by the thermal perturbations, prolonged exposure to the mild simulated hemorrhagic insult slightly reduced cerebral perfusion over time across all thermal perturbations.

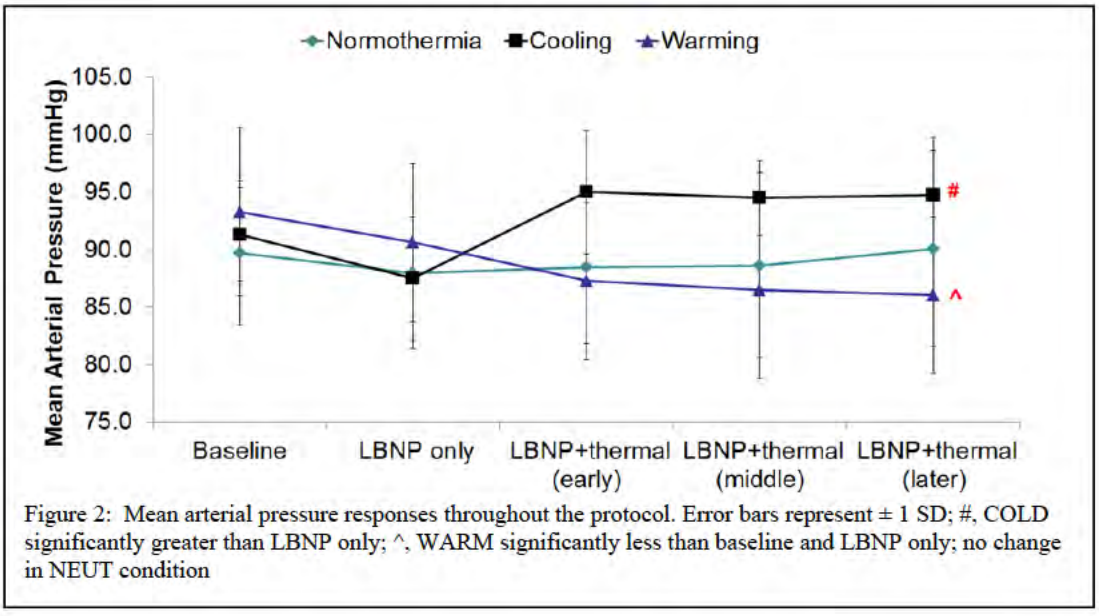

Figure 2: Mean arterial pressure responses throughout the protocol. Error bars represent ± 1 SD; #, COLD significantly greater than LBNP only; ^, WARM significantly less than baseline and LBNP only; no change in NEUT condition

4) Write technical reports and/or scientific publications to disseminate information obtained from Specific Aims 1B and 2.

We are unable to write the report for hemostatic markers for Aims 1B and 2 as the plasma based samples have yet to be analyzed by the US Army Institute of Surgical Research. We will continue to pressure this lab to run these samples, with the expectation that those samples will be assayed within the next 3 months. A colleague, Morten Zaar, who is currently working in Victor Convertino's laboratory at the US Army Institute of Surgical Research will write the manuscript from the combined hemostatic data obtained during Aims 1A and 1B. We anticipate hemostatic data from Aim 2 will be a separate publication.

As shown in point 1 above, we had numerous abstracts from this work presented at national meetings during the prior year. The following manuscripts were published during the prior year from the work originating directly or indirectly from these studies.

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

Schlader, Z.J., E. Rivas, B.R. Soller, V.A. Convertino, C.G. Crandall. Tissue oxygen saturation during hyperthermic progressive central hypovolemia. *Am J Physiol Reg Comp Physiol* 307:R731-736, 2014

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

Schlader, Z.J., D. Gagnon, E. Rivas, V.A. Convertino, C.G. Crandall. Fluid restriction during exercise in the heat reduces tolerance to progressive central hypovolemia. This paper has been written and is expected to be "in review" before May 1, 2015

4. 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 hemorrhage 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 insult. 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. Primary deliverable: Information that warming may not always be beneficial to the hemorrhaging soldier, and that skin surface cooling may be beneficial in a soldier who is not hypothermic.

5. Conclusion:

We were very enthusiastic regarding the outcome of the data collection and reduction over the prior 12 months. The obtained findings should lead to further reinforcement of the importance of hydration as a prophylactic against a possible hemorrhagic insult. We are also enthusiastic about the findings that warming a hemorrhaging victim, which is universally employed regardless of the thermal state of the injured soldier, may not always be beneficial, and may actually be detrimental for the management of arterial blood pressure. This latter observation may result in significant changes in standard military procedures for the treatment of a mildly hemorrhaging soldier. Our contention is that if a soldier is not hypothermic, then warming should not be applied to this victim and that mild cooling may actually be beneficial. A deficit that we have not been able to overcome is the analysis and interpretation of the Compensatory Reserve Index (CRI). During the next 3-6 months, we will continue to work with Flashback Technologies to have the obtained data reduced thereby we can analyze whether this device appropriately tracks CRI status during an exercise induced heat stress followed by a hemorrhagic insult, as well as whether hydration status affects the capabilities of this device. We will also evaluate whether mild warming and cooling of a mild hemorrhage victims alters CRI measures. Finally, upon obtaining the plasma based markers of hemostasis from the US Army Institute of Surgical Research, we will identify whether the aforementioned conditions alters hemostatic capabilities.

6. Publications, Abstracts, and Presentations:

1. Lay Press: None
2. Peer-Reviewed Scientific Journals
    - 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
    - Schlader, Z.J., E. Rivas, B.R. Soller, V.A. Convertino, C.G. Crandall. Tissue oxygen saturation during hyperthermic progressive central hypovolemia. *Am J Physiol Reg Comp Physiol* 307:R731-736, 2014
    - Schlader, Z.J. & **C.G.** Crandall. Normothermic central hypovolemia tolerance reflects hyperthermic tolerance. *Clin Auto Res* 24:119-126, 2014
    - Schlader, Z.J., D. Gagnon, E. Rivas, V.A. Convertino, C.G. Crandall. Fluid restriction during exercise in the heat reduces tolerance to progressive central hypovolemia. This paper has been written and is expected to be "in review" before May 1, 2015
3. Invited Articles: None
4. Abstracts
    - "Reductions in tolerance to central hypovolemia during passive heat stress are accurately tracked by the Compensatory Reserve Index." Daniel Gagnon, Zachary J. Schlader, Eric

Rivas, Victor A. Convertino, Jane Mulligan, Greg Grudic, Craig G. Crandall. *Medicine and Science in Sports & Exercise* (in press).

- "Fluid restriction during exercise in the heat reduces tolerance to central hypovolemia." Zachary J. Schlader, Daniel Gagnon, Eric Rivas, Victor A. Convertino, Craig G. Crandall. *The Faseb J* 29:823.2, 2015.

- "Hemodynamic responses to mild warming during simulated mild hemorrhage." Paula Y.S. Poh, Steven A. Romero, Steven J. Petruzzello, Victor A. Convertino, Craig G. Crandall. *The Faseb J* 29:LB714, 2015.

- "Elevated core and skin temperatures independently attenuate simulated hemorrhage tolerance." James Pearson, Rebekah A.I. Lucas, Zachary J. Schlader, Daniel G. Gagnon, Craig G. Crandall. *The Faseb J* 29:994.18, 2015

- "Whole-body warming during a simulated hemorrhagic insult compromises arterial blood pressure but not cerebral perfusion." Paula Y. S. Poh, Daniel Gagnon, Steven A. Romero, Steven J. Petruzzello, Victor A. Convertino and Craig G. Crandall (acceptance for presentation pending from the Military Health System Research Symposium)

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.

7. Inventions, patents, and licenses:

Nothing to Report

8. Reportable Outcomes:

See item 5 Conclusions above.

9. Other Achievements

Paula Poh is a graduate student from the University of Illinois Urbana-Champaign who has been working in my laboratory while we completed the objectives outlined in Aim 2. She has been 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, an under-representative minority student (Pacific Islander heritage) was able to receive outstanding training while completing the projects outlined above.

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

Dan Gagnon, Ph.D. (post-doctoral fellow)
Zachary Schlader, Ph.D. (post-doctoral fellow)

Steven Romero, Ph.D. (under-represented minority post-doctoral fellow)
Paula Poh, MS. (under-represented minority graduate student)
Hai Ngo, BS. (graduate student)

10. References

None

11. Appendices

The following manuscripts are included in the Appendix

- 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

- Schlader, Z.J., E. Rivas, B.R. Soller, V.A. Convertino, C.G. Crandall. Tissue oxygen saturation during hyperthermic progressive central hypovolemia. *Am J Physiol Reg Comp Physiol* 307:R731-736, 2014

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

Am J Physiol Regul Integr Comp Physiol 307: R822–R827, 2014.
First published July 30, 2014; doi:10.1152/ajpregu.00199.2014.

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

J. Pearson,[1,2] R. A. I. Lucas,[1,3] Z. J. Schlader,[1] J. Zhao,[4] D. Gagnon,[1] and C. G. Crandall[1]

[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 ($P \leq 0.05$).

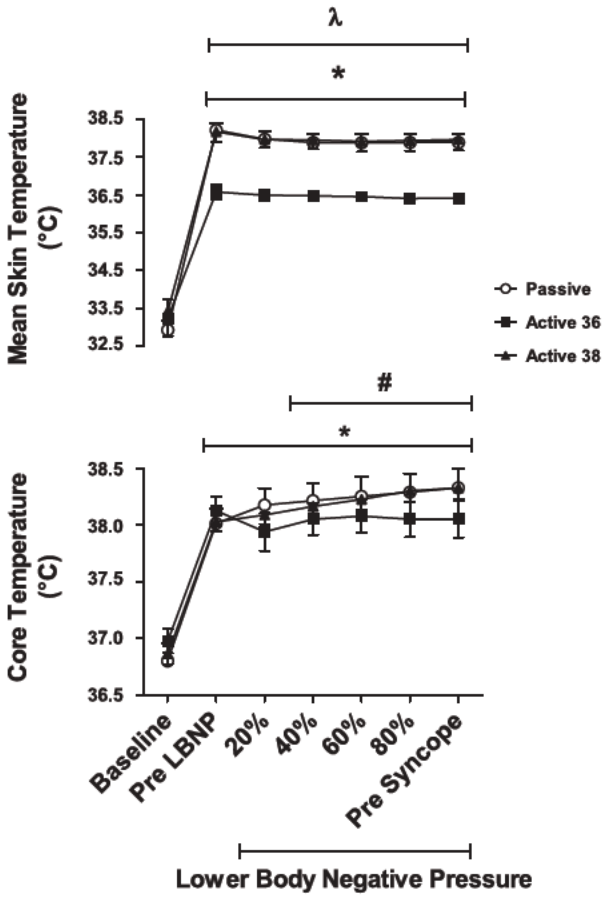

Fig. 1. Core and skin temperatures before and during lower body negative pressure (LBNP) to presyncope in all conditions. Mean skin and core body temperatures increased with all methods of heat stress before LBNP. However, by design, mean skin temperatures were higher after passive heat stress and *active 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,

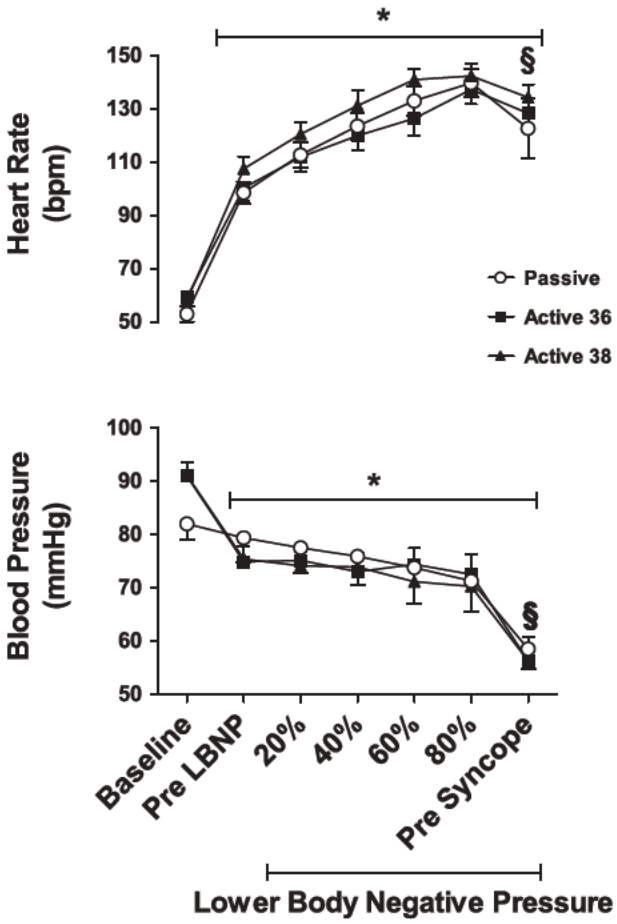

Fig. 2. Heart rate and blood pressure responses before and during LBNP to presyncope in all conditions. When expressed relative to a percentage of maximal CSI, heart rate and mean arterial pressure were not different between trials at any point. In all trials, blood pressure and heart rate decreased 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 ($P \leq 0.05$).

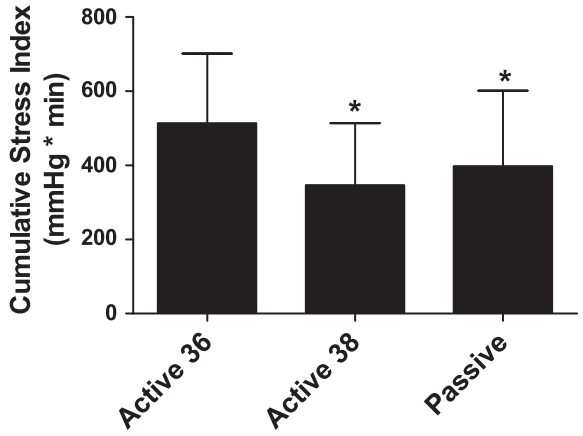

Fig. 3. Cumulative stress index in all trials. Tolerance to simulated hemorrhage (expressed as CSI) was similarly reduced in passive heat stress and *active 38* trials relative to *active 36* ($P \leq 0.05$). Data are means ± SD. *Different from *active 36* ($P \leq 0.05$).

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

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

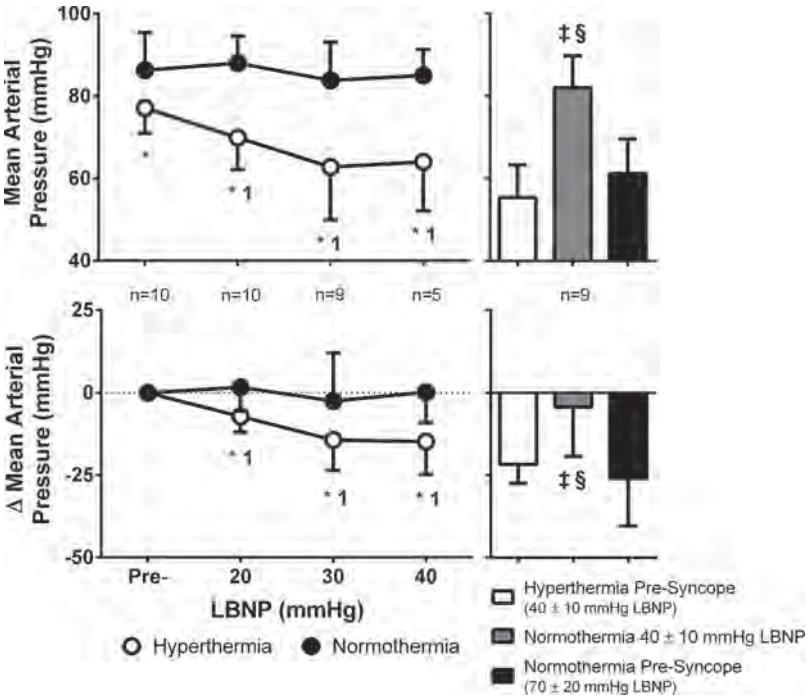

Fig. 1. Mean arterial pressure, expressed as absolute (*top*) and the change (Δ) from pre-lower body negative pressure (Pre-LBNP) (*bottom*), during normothermia and hyperthermia (means ± SD). On the left, data are presented from Pre-LBNP through 40 mmHg LBNP, while data on the right data are presented 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 ($P \leq 0.007$).

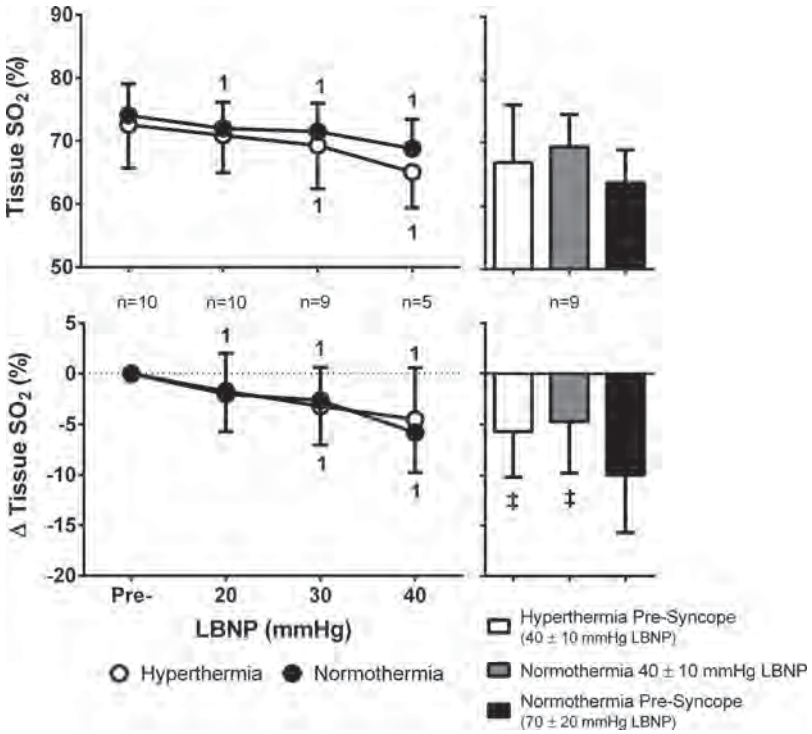

Fig. 2. Near infrared spectroscopy-derived tissue oxygen saturation (tissue So_2), expressed as absolute (*top*) and the change (Δ) from Pre-LBNP (*bottom*), during normothermia and hyperthermia (mean ± SD). On the left, data are presented from Pre-LBNP through 40 mmHg LBNP, while data on the right are 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,

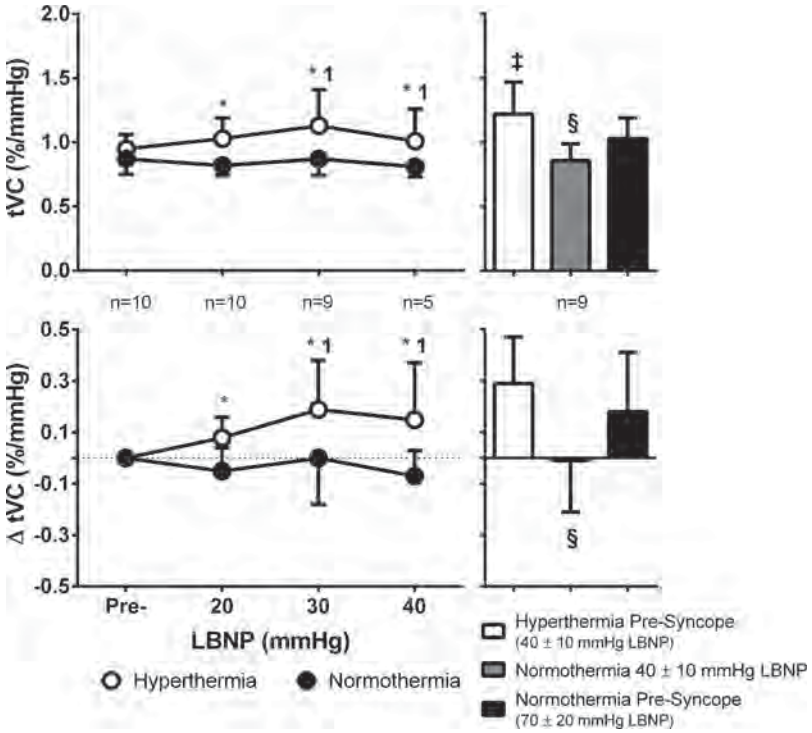

Fig. 3. Tissue vascular conductance (tVC), expressed as absolute (*top*) and the change (Δ) from Pre-LBNP (*bottom*), during normothermia and hyperthermia (means ± SD). On the left, data are presented from Pre-LBNP through 40 mmHg LBNP, while data on the right data are presented at presyncope during 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

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

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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

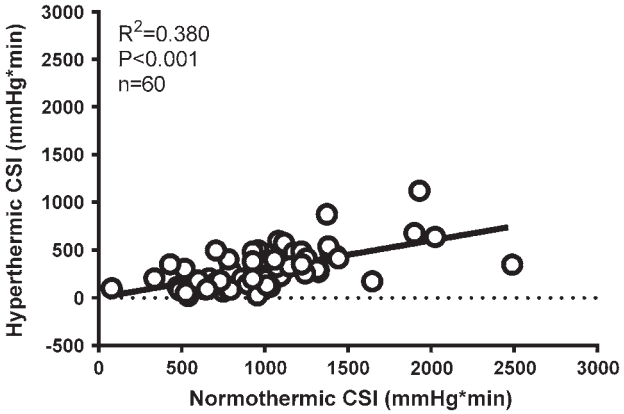

Fig. 1 Correlation between normothermic lower body negative pressure (LBNP) tolerance [i.e., the cumulative stress index (CSI)] and hyperthermic LBNP tolerance

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

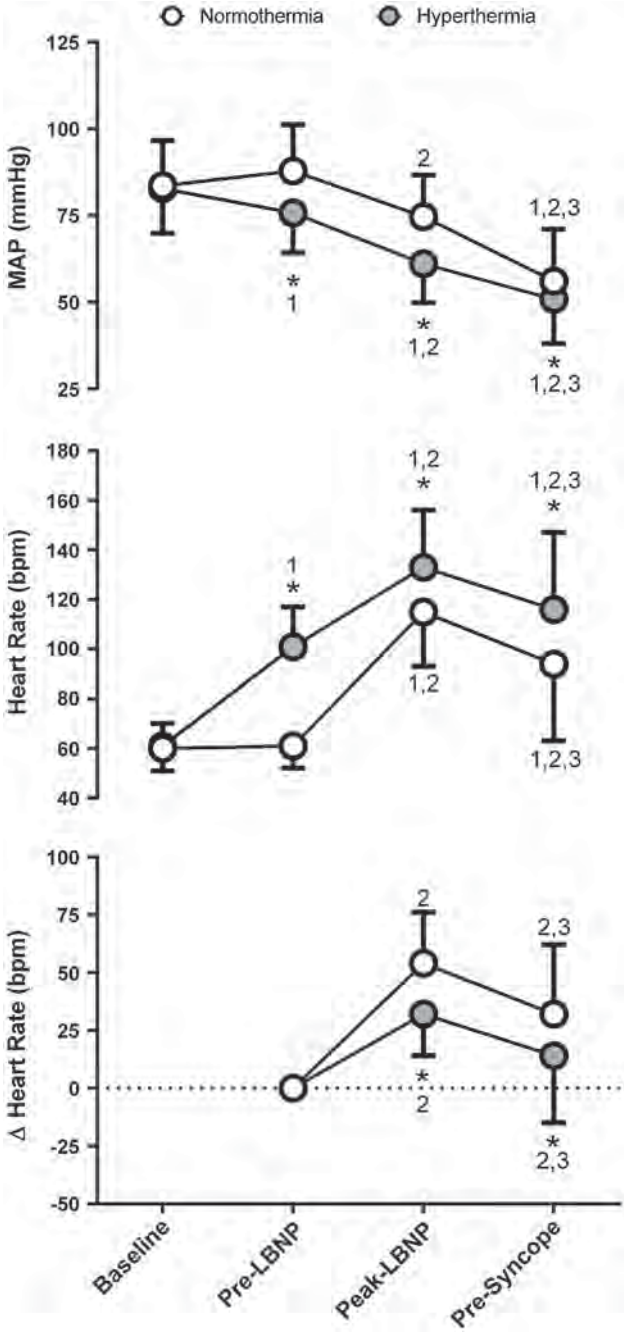

Fig. 2 Mean arterial pressure (MAP; *top*), heart rate (*middle*), and the change (Δ) in heart rate from Pre LBNP (*bottom*) during normothermic and hyperthermic LBNP at Baseline, Pre LBNP, Peak LBNP, and immediately prior to LBNP termination (Pre Syncope) (mean ± SD). *Asterisks* indicate different 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)

absolute reduction in LBNP tolerance from normothermia to hyperthermia (HIGH −992 ± 362 mmHg min, LOW −406 ± 193 mmHg min; $P < 0.001$).

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

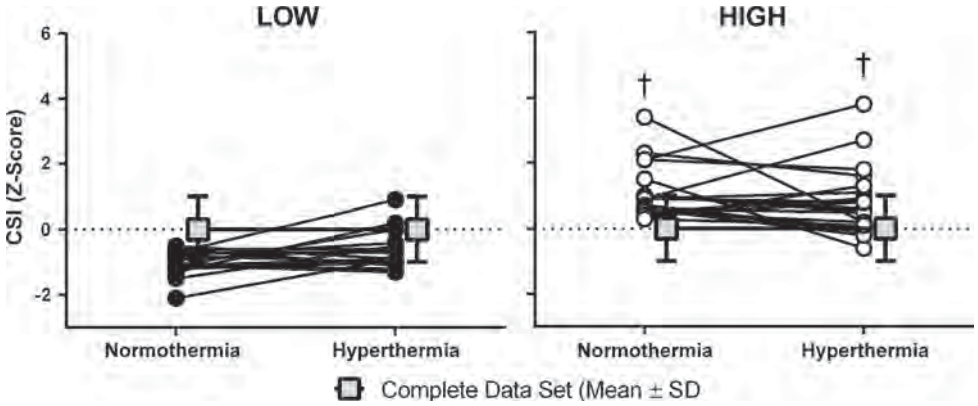

Fig. 3 Individual changes in standardized (i.e., *Z* score) LBNP tolerance [indexed from the cumulative stress index (CSI)] from normothermia to hyperthermia in the 20 observations with the lowest (LOW) and highest (HIGH) normothermic tolerance. The mean data from the complete data set are also depicted (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"

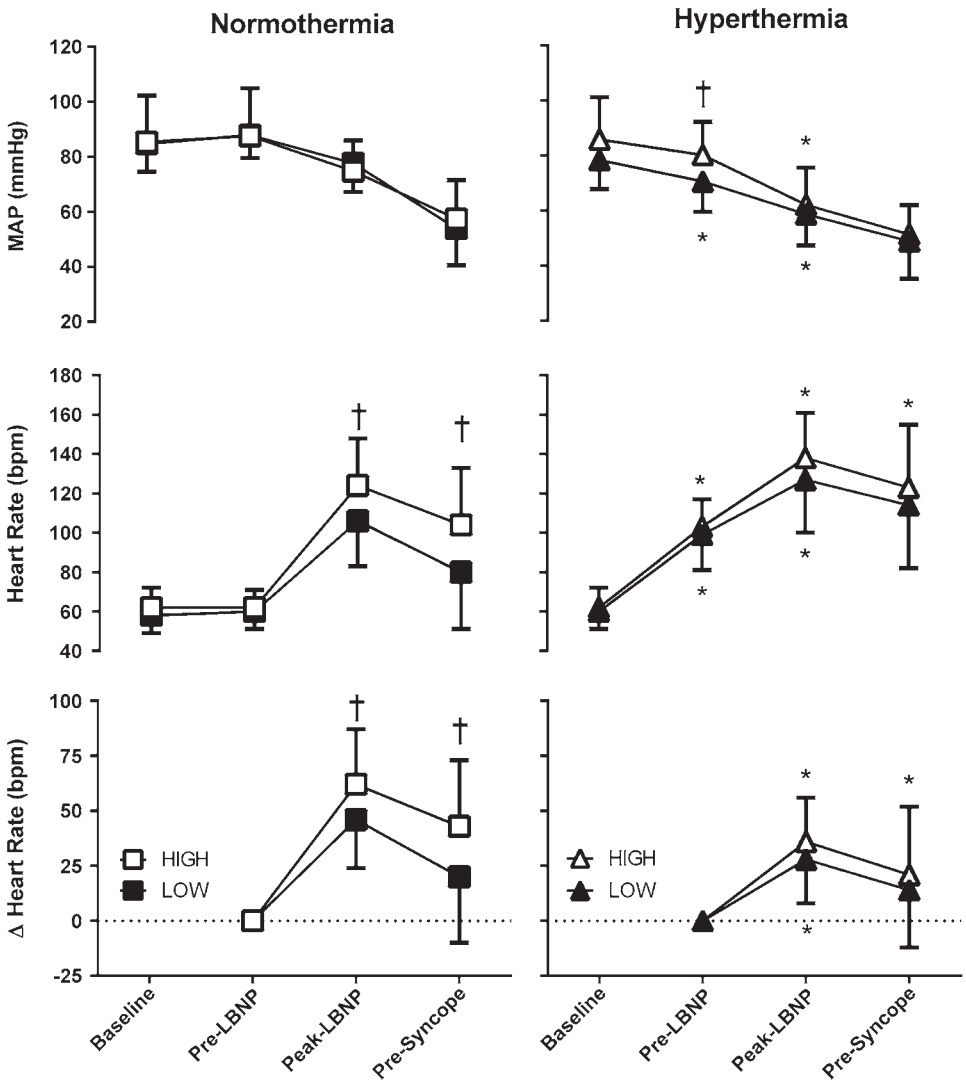

Fig. 4 Mean arterial pressure (MAP; *top*), heart rate (*middle*), and the change (Δ) in heart rate from Pre LBNP (*bottom*) at Baseline, Pre LBNP, Peak LBNP, and immediately prior to LBNP termination (Pre Syncope) in the 20 observations with the lowest (LOW) and highest (HIGH) normothermic tolerance 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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