Articles in PresS. J Appl Physiol (April 11, 2013). doi:10.1152/japplphysiol.00079.2013

ACUTE VOLUME EXPANSION ATTENUATES HYPERTHERMIA-INDUCED REDUCTIONS IN CEREBRAL PERFUSION DURING SIMULATED HEMORRHAGE

Zachary J. Schlader[1], Thomas Seifert[2], Thad E. Wilson[3], Morten Bundgaard-Nielsen[2], Niels H. Secher[2,4], and Craig G. Crandall[1]

[1] Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital of Dallas and the Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA

[2] Department of Anesthesiology, Rigshospitalet, University of Copenhagen, Denmark

[3] Ohio Musculoskeletal and Neurological Institute, Ohio University, and Departments of Biomedical Sciences and Speciality Medicine, Ohio University Heritage College of Osteopathic Medicine, Athens, OH, USA

[4] Copenhagen Muscle Research Center, Rigshospitalet, University of Copenhagen, Denmark

Corresponding Author:
Craig G. Crandall, PhD
Institute for Exercise and Environmental Medicine
Texas Health Presbyterian Hospital of Dallas
7232 Greenville Ave
Dallas, TX, 75231, USA
Email: CraigCrandall@texashealth.org
Phone: 214-345-4623

Running Head: Cerebral Perfusion and Volume Expansion during Hyperthermia

Word Count: 2827

ABSTRACT

Hyperthermia reduces the capacity to withstand a simulated hemorrhagic challenge, but volume loading preserves this capacity. This study tested the hypotheses that acute volume expansion during hyperthermia increases cerebral perfusion and attenuates reductions in cerebral perfusion during a simulated hemorrhagic challenge induced by lower body negative pressure (LBNP). Eight healthy young male subjects underwent a supine baseline period (pre-LBNP), followed by 15 and 30 mmHg LBNP while normothermic, hyperthermic (increased pulmonary artery blood temperature ~1.1°C), and following acute volume infusion while hyperthermic. Primary dependent variables were: mean middle cerebral artery blood velocity ($MCAv_{mean}$) - serving as an index of cerebral perfusion, mean arterial pressure (MAP), and cardiac output (thermodilution). During baseline, hyperthermia reduced $MCAv_{mean}$ ($P=0.001$) by 12 ± 9% relative to normothermia. Volume infusion while hyperthermic increased cardiac output by 2.8 ± 1.4 L/min ($P<0.001$), but did not alter $MCAv_{mean}$ ($P=0.99$) or MAP ($P=0.39$), when compared to hyperthermia alone. Relative to hyperthermia, at 30 mmHg LBNP acute volume infusion attenuated reductions ($P<0.001$) in cardiac output (by 2.5 ± 0.9 L/min; $P<0.001$), MAP (by 5 ± 6 mmHg; $P=0.004$), and $MCAv_{mean}$ (by 12 ± 13%; $P=0.002$). These data indicate that acute volume expansion does not reverse hyperthermia-induced reductions in cerebral perfusion pre-LBNP, but that it does attenuate reductions in cerebral perfusion during simulated hemorrhage in hyperthermic humans.

Abstract word count: 216

Keywords: Lower body negative pressure; heat stress; brain blood flow; volume infusion

INTRODUCTION

In humans, the ability to maintain arterial pressure, and ultimately cerebral perfusion, during an orthostatic (e.g., by standing up or upright tilt) or a simulated hemorrhagic challenge (e.g., by lower body negative pressure; LBNP (4)) is compromised during hyperthermia (17, 19, 30). This hyperthermia-induced impairment involves numerous mechanisms including, insufficient increases in peripheral resistance (8, 14, 25), decreases in ventricular filling pressures (10, 32) - which occur subsequent to reductions in the central blood volume (9, 10) - impaired arterial baroreflex control of blood pressure (5), and reductions in cerebral perfusion (2, 21, 30).

Various countermeasures are effective in maintaining arterial pressure and cerebral perfusion during a simulated hemorrhagic challenge in heat stressed individuals, such as rapid skin cooling (31) and acute plasma volume expansion (17). The mechanism(s) by which acute volume expansion preserves the capability to maintain cardiovascular integrity during hyperthermia remains unknown. Volume loading may attenuate the LBNP-induced reduction in stroke volume and cardiac output such that arterial pressure and therefore, presumably, cerebral perfusion is preserved (3, 10). Alternatively, acute volume expansion in heat stressed individuals may, independent of changes in arterial pressure, augment cerebral perfusion secondary to an increase in cardiac output, and subsequent arterial baroreflex mediated changes in cerebral vascular tone (22, 23), as shown in normothermic individuals (24). However, the effect of acute volume loading on cerebral perfusion in heat stressed individuals, either at rest or during a subsequent simulated hemorrhage, remains unknown. Therefore, the purpose of this study was to test the hypotheses that acute plasma volume expansion while hyperthermic will: 1) reverse hyperthermia-induced reductions in cerebral perfusion, and 2) attenuate reductions in cerebral perfusion during a simulated hemorrhagic challenge (i.e., LBNP). The results were considered to illuminate the mechanisms by which acute volume infusion preserves the capacity to withstand a subsequent hemorrhagic challenge during hyperthermia, which is of importance for the treatment of hyperthermic, hemorrhagic individuals.

METHODS

Eight healthy male volunteers participated in this study. The subject characteristics were (mean ± SD) - Age: 29 ± 5 y; Height: 180 ± 5 cm; Weight: 75 ± 4 kg; Body surface area: 1.5 ± 0.1 m^2. All subjects were non-smokers, not taking medications and were free of any known cardiovascular, metabolic, or neurological diseases. Each subject was fully informed of the experimental procedures and possible risks before giving informed written consent. This protocol was approved by the Ethics Committee of Copenhagen (H-KF-090/04) and was registered with the Danish data protection agency and ClinicalTrials.gov under the national library of medicine (NCT00714766). All procedures conformed to the standards set by the Declaration of Helsinki. Subjects arrived at the laboratory euhydrated (having ingested ~1.8 L of fluid during the prior 24 h) and having refrained from strenuous exercise, alcohol and caffeine for a period of 24 h. These data were collected concurrently with those presented in a published manuscript, which tested a unique research hypothesis (3).

Instrumentation and measurements

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, one forearm, and the feet. Heart rate was continually recorded from a five lead electrocardiogram. Mean arterial pressure (MAP) was measured via a catheter placed in the brachial artery of the non-dominant arm. Pulmonary artery blood temperature was measured via a flow-directed pulmonary arterial catheter (93A-831H-7.5F, Baxter Healthcare Corporation, Irvine, CA, USA) introduced through the basilica vein of the left arm and advanced to the pulmonary artery. Central venous pressure (CVP) was measured via an alternate port on the pulmonary arterial catheter. Vascular pressures were referenced to atmospheric pressure via uniflow pressure transducers (Baxter Healthcare Corporation) that were zeroed 5 cm below the sternal notch and connected to a pressure-monitoring system (Dialogue 2000, ICB-Danica, Copenhagen, Denmark). All catheters were flushed with isotonic saline at 3 ml/h. Arterial blood samples were analyzed for changes in arterial carbon dioxide tension (P_aCO_2), hemoglobin, and thus hematocrit (Radiometer ABL700, Brønshøj, Denmark) and corrected to pulmonary artery blood temperature. Cardiac output was measured in triplicate via the thermodilution method (15). Mean middle cerebral artery blood velocity ($MCAv_{mean}$) served as an index of cerebral perfusion and was measured by adjusting a 2 MHz Doppler probe (Multidop X, DWL, Sipplingen, Germany) over the temporal window (1, 29).

Experimental protocol

Following instrumentation, subjects rested quietly in the supine position while normothermic water (34°C) perfused the suit. After normothermic baseline data collection, LBNP commenced at 15 mmHg, which was immediately followed by 30 mmHg. 30 mmHg LBNP was the highest level applied as it was expected that all subjects could tolerate this LBNP during hyperthermia for a period sufficient to obtain the desired data prior to symptoms of syncope. The duration of each LBNP stage for all thermal conditions was ~15 min, which was required to obtain the data reported in the companion paper (3). Following normothermic LBNP, the subjects underwent whole-body passive heat stress by perfusing 46-48°C water through the suit. This heat stress continued until pulmonary artery blood temperature increased ~1.0°C (typically after 30-45 min), after which the water temperature was slightly reduced to attenuate further increases in body temperature during the ensuing data collection periods. The subjects were not allowed to drink at any time during the experimental procedures. Hyperthermic baseline data were then obtained, which was followed by 15 and 30 mmHg LBNP. Following a brief recovery after the cessation of LBNP and while remaining heat stressed, 500 ml of ~38°C colloid solution (HES 130/0.4, Voluven, Fresenius Kabi, Sweden) followed by warm saline was rapidly infused. The total infused volume was ~12 ml/kg and typically administered in less than 10 min. Baseline data were collected after the completion of the infusion, which was then followed by 15 and 30 mmHg LBNP.

Data analysis

Thermal, hemodynamic, and pressure data were sampled at 50 Hz via a data acquisition system (Biopac System, Santa Barbara, CA, USA). Data were reduced into 60 s averages during the baseline periods and following 5 min of each stage of LBNP. Stroke volume was calculated from cardiac output and heart rate, while systemic vascular resistance (SVR) was calculated as (MAP – CVP) divided by cardiac output. An index of cerebral vascular resistance (CVR) was calculated as the quotient of MAP and $MCAv_{mean}$. Percentage changes in plasma volume, occurring as a result of the volume infusion, were estimated from changes in hematocrit and hemoglobin (12). Data during LBNP are presented as absolute values and as a change (Δ) from pre-LBNP baseline for each respective condition.

Statistical analysis

Data at baseline for normothermia, hyperthermia, hyperthermia + infusion were analyzed using one-way repeated measures analysis of variance (ANOVA) (*hypothesis one*), while data during LBNP during the hyperthermia and hyperthermia + infusion conditions were analyzed using a two-way repeated measures ANOVA (2 x 3; condition x LBNP) (*hypothesis two*). Data during the normothermic condition were not included in the analysis for *hypothesis two* given that the inclusion of this data was

not necessary to test this hypothesis. Where appropriate, *post hoc,* pair-wise, 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 ± SD.

RESULTS

Normothermia, hyperthermia, and hyperthermia + volume infusion baselines (hypothesis one)

Thermal and hemodynamic variables during normothermia, hyperthermia, and following the volume infusion baseline (pre-LBNP) periods are presented in Table 1. Relative to normothermia, hyperthermia was characterized by ~1.1 and ~2.8°C increases ($P<0.001$) in pulmonary artery blood and mean skin temperatures, respectively, which were maintained ($P=0.084$) during the infusion. The volume infusion increased plasma volume by 18 ± 5%, and augmented ($P<0.001$) cardiac output, but did not affect ($P=0.999$) MAP. Notably, P_aCO_2 was well maintained ($P=0.351$) throughout these baseline periods. $MCAv_{mean}$ and CVR at baseline are presented in Figure 1. Relative to normothermia, $MCAv_{mean}$ was reduced ($P=0.001$) by 12 ± 9% during hyperthermia and was unaffected ($P=0.394$) by volume infusion. CVR was similar ($P=0.471$) throughout all baseline periods.

Lower body negative pressure (hypothesis two)

Pulmonary artery temperature was ~0.2°C higher ($P=0.006$) throughout LBNP following the volume infusion (38.0 ± 0.3°C), relative to during hyperthermia alone (37.8 ± 0.4°C). $MCAv_{mean}$ and CVR during LBNP are presented in Figure 2. CVR remained constant ($P=0.281$) as LBNP progressed, while $MCAv_{mean}$ decreased ($P<0.001$) in both conditions. However, relative to hyperthermia, volume infusion attenuated the reduction in $MCAv_{mean}$ during 15 ($P=0.049$) and 30 ($P=0.002$) mmHg LBNP. Hemodynamic variables during LBNP are presented in Figure 3. Cardiac output, stroke volume, MAP, and CVP all decreased ($P<0.001$) as LBNP progressed, while SVR increased ($P<0.001$). At both stages of LBNP, volume infusion augmented ($P\leq0.001$) cardiac output and stroke volume, while it attenuated ($P=0.004$) the reduction in MAP at 30 mmHg LBNP. During LBNP, P_aCO_2 was similar ($P=0.559$) between hyperthermia (33 ± 5 mmHg) and volume infusion (35 ± 5 mmHg) conditions.

DISCUSSION

The purpose of this study was to test the hypotheses that acute plasma volume expansion during hyperthermia elevates cerebral perfusion at rest and attenuates reductions in cerebral perfusion during a subsequent simulated hemorrhagic challenge. The novel findings of this study are that acute volume expansion: a) does not reverse hyperthermia-induced reductions in cerebral perfusion at rest, but that b) it attenuates reductions in cerebral perfusion during a subsequent simulated hemorrhage while hyperthermic. That is, an increase in pulmonary artery blood temperature of ~1.1°C (Table 1) reduced $MCAv_{mean}$ by ~12% (Fig. 1), but acute volume infusion sufficient to increase cardiac output by almost 3 L/min (Table 1) did not restore $MCAv_{mean}$ towards normothermic levels (Fig. 1). However, acute volume infusion during hyperthermia did attenuate LBNP-induced reductions in $MCAv_{mean}$ (Fig. 2). This was likely a function of the volume infusion's augmentation of cardiac output and stroke volume prior to LBNP (Fig. 3), which better maintained MAP at a given level of LBNP (Fig. 3), thereby forestalling LBNP-induced reductions in $MCAv_{mean}$ (Fig. 2). These data indicate that cardiac output is not capable of directly (i.e., independent of arterial pressure) modulating cerebral blood flow during hyperthermia. However, the data do support that the beneficial effects of acute volume loading prior to a hyperthermic simulated hemorrhage are, at least partially, mediated via the preservation of arterial pressure and by extension, the maintenance of cerebral perfusion.

Acute volume expansion and cerebral perfusion during hyperthermia

During normothermia, experimentally-induced fluctuations in cardiac output directly modulate brain blood flow in humans (24). Presumably, this effect is mediated via the arterial baroreflex and subsequent changes in cerebral vascular tone occurring secondary to changes in sympathetic nerve activity (22, 23). Accordingly, we hypothesized that the same phenomenon would occur during hyperthermia, such that the augmentation of cardiac output would (at least partially) restore hyperthermia-induced reductions in cerebral perfusion. However, this was not the case (Fig. 1). There are two potential reasons for this observation.

First, acute volume expansion during hyperthermia restores central blood volume to normothermic levels (9) and augments an already elevated skin blood flow (7). Thus, increases in blood flow as a consequence of volume infusion in those vascular beds with a low vascular resistance (e.g., the cutaneous vasculature) appear likely, whereas blood flow in vascular beds with a higher vascular resistance (e.g., the cerebral vasculature) would remain constant. As evidence for this hypothesis, in the present study systemic vascular resistance further decreased during volume infusion (Table 1), while $MCAv_{mean}$ and CVR were unchanged (Fig. 1).

Second, the effect of increasing cardiac output on the cerebral vasculature during normothermia may be mediated by the arterial baroreflex and subsequent changes in sympathetic nerve activity (22,

23). Hyperthermia impairs the responsiveness of some aspects of the baroreflex (5) and also shifts the baroreflex operating point (5, 11) in order to accommodate hyperthermia-induced hemodynamic changes (6). Thus, the sensitivity of the cerebral vasculature to modify blood flow to a given change in cardiac output may be reduced during hyperthermia. However, this remains speculative as there is no direct evidence in support of such an arrangement. It is also notable that this hypothesis requires that the sympathetic nervous system innervate cerebral vessels and modify cerebral blood flow in humans, which remains debated (28).

Acute volume expansion and cerebral perfusion during hyperthermic LBNP

The ability to withstand a hemorrhagic challenge is reduced during hyperthermia, while acute volume expansion reverses this intolerance (17). The mechanisms underlying this observation are not currently known. We hypothesized that, relative to hyperthermia alone, acute volume expansion during hyperthermia would attenuate reductions in cerebral perfusion at a given level of LBNP. The present data support this hypothesis. Specifically, following volume infusion, $MCAv_{mean}$ during LBNP was higher than during hyperthermia alone (Fig. 2). These data indicate that during simulated hemorrhage cerebral perfusion is better maintained during hyperthermia following volume expansion. From the testing of our first hypothesis, the mechanism for this observation is not via the direct influence of acute volume loading, and subsequent increases in cardiac output, on cerebral perfusion prior the simulated hemorrhagic challenge. Rather, these data support the proposal (see: 3, 10) that volume expansion results in a higher cardiac output and stroke volume at a given level of LBNP (Fig. 3), which better maintains arterial pressure (Fig. 3) and in turn maintains cerebral perfusion as LBNP progressed (Fig. 2). Thus, these data suggest that the preservation of simulated hemorrhagic tolerance following volume infusion during hyperthermia is, at least partially, mediated via attenuations in LBNP-induced reductions in arterial pressure and cerebral perfusion.

Methodological considerations

Given the invasive nature of this study, it was not feasible to randomize the three experimental conditions as this would have required three separate laboratory visits and thus, arterial and right heart catheterization on three occasions. Accordingly, pulmonary artery blood temperature was slightly higher (~0.2°C) during LBNP with volume infusion relative to LBNP during hyperthermia alone. Given these slight temperature differences and that cerebral perfusion progressively decreases as internal body temperature increases - albeit at larger increments than 0.2°C (13) - these findings suggest that we likely underestimated the magnitude of the beneficial effect of acute volume expansion on changes in cerebral perfusion during LBNP.

Given that these data were collected concurrently with another study (3), coupled with the aforementioned challenges resulting in it not being feasible to perform the experimental sessions on three separate days, in the present study we did not measure LBNP tolerance. Thus, these data have been carefully interpreted and the conclusions drawn only pertain to situations involving the administered levels of LBNP. Therefore, cerebral vascular responses during hyperthermia following acute volume expansion at the point of LBNP intolerance remain unknown. Nevertheless, the presented data provide insights into possible mechanisms underlying the beneficial effects of acute volume expansion on LBNP tolerance during hyperthermia.

It is also worth nothing that in the present study subjects were exposed to moderate levels of LBNP three times within a relatively short time period (~90 min). It remains unknown whether there are any physiological effects of repeated moderate LBNP over this period, and if so whether those effects would be sufficient to influence the profound changes observed during heat stress and heat stress plus volume infusion. That said, some changes do occur given that adaptation to daily LBNP exposures has been observed in as little as five consecutive days (18). Whether this influenced the conclusions drawn presently remains uncertain.

We utilized transcranial Doppler to quantify $MCAv_{mean}$, which served as an index of cerebral perfusion given that this artery supplies ~80% of the blood flow received by each cerebral hemisphere (20). However, it must be acknowledged that if the diameter of the insonated artery changes, then changes in blood velocity does not always reflect proportional changes in blood flow. Yet, the diameter of the middle cerebral artery is unaffected by moderate carbon dioxide and blood pressure perturbations (16, 26). Even still, the effect of hyperthermia on middle cerebral artery diameter remains uncertain.

Conclusions

The present study demonstrates that acute volume expansion does not reverse hyperthermia-induced reductions in cerebral perfusion at rest, but that it does attenuate the reduction in cerebral perfusion during a subsequent simulated hemorrhage while hyperthermic. Thus, cardiac output does not appear to directly (i.e., independent of arterial pressure) modulate cerebral blood flow during hyperthermia. However, acute volume loading prior to a simulated hemorrhagic event while hyperthermic attenuates the LBNP-induced reductions in arterial pressure and, thereby better maintains cerebral perfusion.

Perspectives

Testing of these hypotheses has furthered the understanding of the mechanisms by which acute volume infusion preserves the capacity to withstand a subsequent hemorrhagic challenge during

hyperthermia. The findings presented have implications for individuals at risk of a hemorrhagic injury under heat stress conditions (e.g., soldiers, firefighters, miners, etc.). The data suggest that plasma volume loading in a hyperthermic individual prior to a hemorrhagic injury will assist in the maintenance of cerebral perfusion through stabilizing arterial pressure, as opposed to a direct effect of an increase in cardiac output, independent of arterial pressure, as has been found in normothermic individuals (24).

Acknowledgements

This research was funded in part by grants from the National Heart, Lung, and Blood Institute (C.G.C.: HL-61388 and HL-67422; and Z.J.S.: T32HL007360), Aase & Ejnar Danielsens Foundation (N.H.S.) and Jeppe Juhl & Hustrus Foundation (M.B).

REFERENCES

1. **Aaslid R, Markwalder TM, and Nornes H**. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* 57: 769-774, 1982.
2. **Brothers RM, Wingo JE, Hubing KA, and Crandall CG**. The effects of reduced end-tidal carbon dioxide tension on cerebral blood flow during heat stress. *J Physiol-London* 587: 3921-3927, 2009.
3. **Bundgaard-Nielsen M, Wilson TE, Seifert T, Secher NH, and Crandall CG**. Effect of volume loading on the Frank-Starling relation during reductions in central blood volume in heat-stressed humans. *J Physiol* 588: 3333-3339, 2010.
4. **Cooke WH, Ryan KL, and Convertino VA**. Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *J Appl Physiol* 96: 1249-1261, 2004.
5. **Crandall CG**. Carotid baroreflex responsiveness in heat-stressed humans. *Am J Physiol Heart Circ Physiol* 279: H1955-1962, 2000.
6. **Crandall CG, Cui J, and Wilson TE**. Effects of heat stress on baroreflex function in humans. *Acta Physiol Scand* 177: 321-328, 2003.
7. **Crandall CG, Levine BD, and Etzel RA**. Effect of increasing central venous pressure during passive heating on skin blood flow. *J Appl Physiol* 86: 605-610, 1999.
8. **Crandall CG, Shibasaki M, and Wilson TE**. Insufficient cutaneous vasoconstriction leading up to and during syncopal symptoms in the heat stressed human. *Am J Physiol Heart Circ Physiol* 299: H1168-1173, 2010.
9. **Crandall CG, Wilson TE, Marving J, Bundgaard-Nielsen M, Seifert T, Klausen T, Andersen F, Secher N, and Hesse B**. Colloid volume loading does not mitigate decreases in central blood volume during simulated hemorrhage while heat stressed. *J Physiol* 590: 1287-1297, 2012.
10. **Crandall CG, Wilson TE, Marving J, Vogelsang TW, Kjaer A, Hesse B, and Secher NH**. Effects of passive heating on central blood volume and ventricular dimensions in humans. *J Physiol* 586: 293-301, 2008.
11. **Cui J, Wilson TE, and Crandall CG**. Baroreflex modulation of sympathetic nerve activity to muscle in heat-stressed humans. *Am J Physiol Regul Integr Comp Physiol* 282: R252-258, 2002.
12. **Dill DB, and Costill DL**. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247-248, 1974.
13. **Fan JL, Cotter JD, Lucas RA, Thomas K, Wilson L, and Ainslie PN**. Human cardiorespiratory and cerebrovascular function during severe passive hyperthermia: effects of mild hypohydration. *J Appl Physiol* 105: 433-445, 2008.
14. **Ganio MS, Overgaard M, Seifert T, Secher NH, Johansson P, Meyer M, and Crandall CG**. Effect of heat stress on cardiac output and systemic vascular conductance during simulated hemorrhage to pre-syncope in young men. *Am J Physiol Heart Circ Physiol* 302: H1756-H1761, 2012.
15. **Ganz W, Donoso R, Marcus HS, Forrester JS, and Swan HJ**. A new technique for measurement of cardiac output by thermodilution in man. *Am J Cardiol* 27: 392-396, 1971.
16. **Giller CA, Bowman G, Dyer H, Mootz L, and Krippner W**. Cerebral Arterial Diameters during Changes in Blood Pressure and Carbon Dioxide during Craniotomy. *Neurosurgery* 32: 737-742, 1993.
17. **Keller DM, Low DA, Wingo JE, Brothers RM, Hastings J, Davis SL, and Crandall CG**. Acute volume expansion preserves orthostatic tolerance during whole-body heat stress in humans. *J Physiol* 587: 1131-1139, 2009.
18. **Lightfoot JT, Febles S, and Fortney SM**. Adaptation to repeated presyncopal lower body negative pressure exposures. *Aviat Space Environ Med* 60: 17-22, 1989.
19. **Lind AR, Leithead CS, and McNicol GW**. Cardiovascular changes during syncope induced by tilting men in the heat. *J Appl Physiol* 25: 268-276, 1968.
20. **Lindegaard KF, Lundar T, Wiberg J, Sjoberg D, Aaslid R, and Nornes H**. Variations in middle cerebral artery blood flow investigated with noninvasive transcranial blood velocity measurements. *Stroke* 18: 1025-1030, 1987.

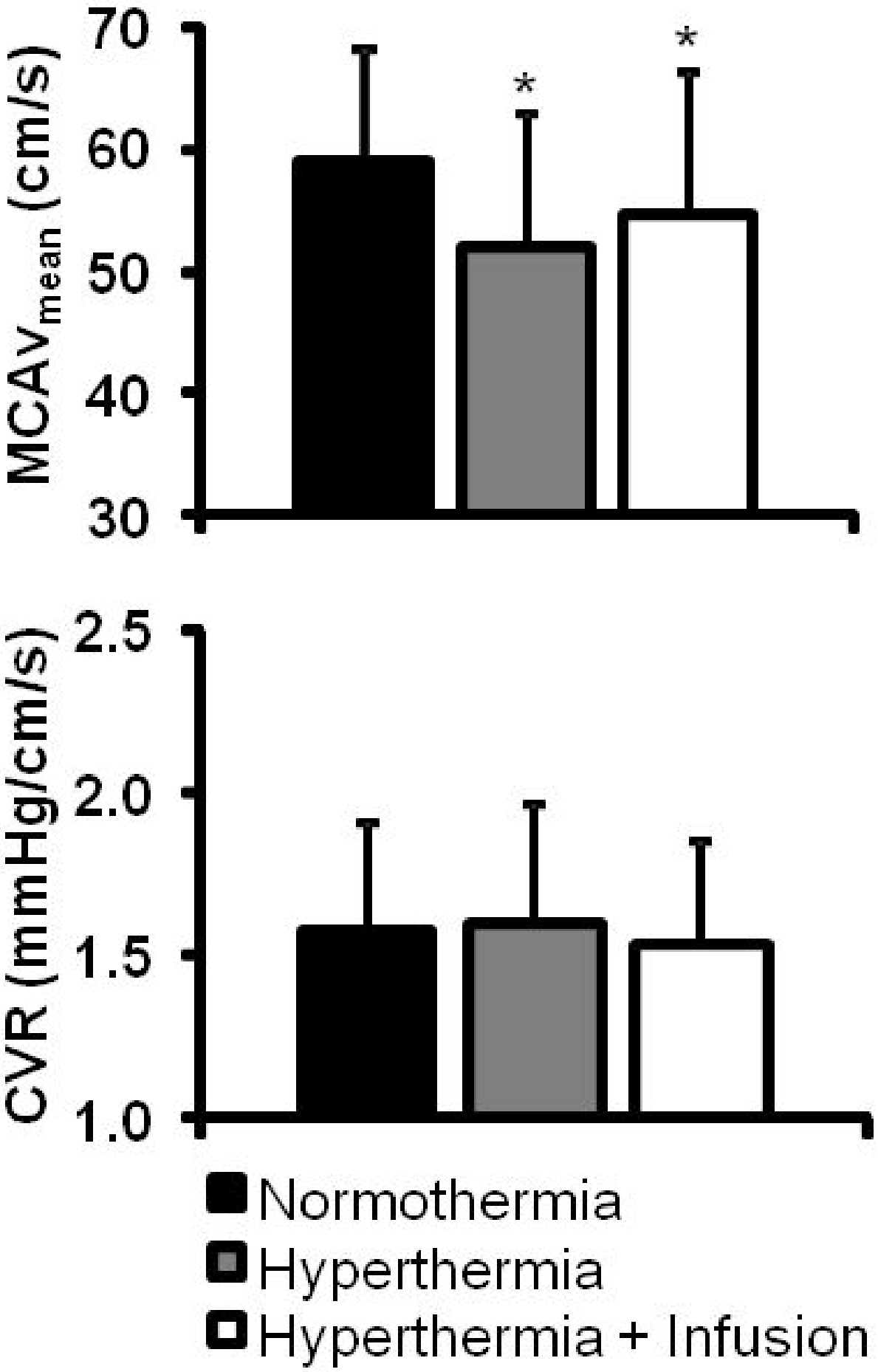
21. **Nelson MD, Haykowsky MJ, Stickland MK, Altamirano-Diaz LA, Willie CK, Smith KJ, Petersen SR, and Ainslie PN**. Reductions in cerebral blood flow during passive heat stress in humans: partitioning the mechanisms. *J Physiol-London* 2011.
22. **Ogoh S, and Ainslie PN**. Cerebral blood flow during exercise: mechanisms of regulation. *J Appl Physiol* 107: 1370-1380, 2009.
23. **Ogoh S, and Ainslie PN**. Regulatory mechanisms of cerebral blood flow during exercise: new concepts. *Exerc Sport Sci rev* 37: 123-129, 2009.
24. **Ogoh S, Brothers RM, Barnes Q, Eubank WL, Hawkins MN, Purkayastha S, A OY, and Raven PB**. The effect of changes in cardiac output on middle cerebral artery mean blood velocity at rest and during exercise. *J Physiol* 569: 697-704, 2005.
25. **Pearson J, Lucas RA, and Crandall CG**. Elevated local skin temperature impairs cutaneous vasoconstrictor responses to a simulated hemorrhagic challenge while heat stressed. *Exp Physiol* 2012.
26. **Serrador JM, Picot PA, Rutt BK, Shoemaker JK, and Bondar RL**. MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis. *Stroke* 31: 1672-1678, 2000.
27. **Taylor WF, Johnson JM, Kosiba WA, and Kwan CM**. Cutaneous vascular responses to isometric handgrip exercise. *J Appl Physiol* 66: 1586-1592, 1989.
28. **van Lieshout JJ, and Secher NH**. Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Point: Sympathetic activity does influence cerebral blood flow. *J Appl Physiol* 105: 1364-1366, 2008.
29. **Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK, and Ainslie PN**. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods* 196: 221-237, 2011.
30. **Wilson TE, Cui J, Zhang R, and Crandall CG**. Heat stress reduces cerebral blood velocity and markedly impairs orthostatic tolerance in humans. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* 291: R1443-1448, 2006.
31. **Wilson TE, Cui J, Zhang R, Witkowski S, and Crandall CG**. Skin cooling maintains cerebral blood flow velocity and orthostatic tolerance during tilting in heated humans. *J Appl Physiol* 93: 85-91, 2002.
32. **Wilson TE, Tollund C, Yoshiga CC, Dawson EA, Nissen P, Secher NH, and Crandall CG**. Effects of heat and cold stress on central vascular pressure relationships during orthostasis in humans. *J Physiol* 585: 279-285, 2007.

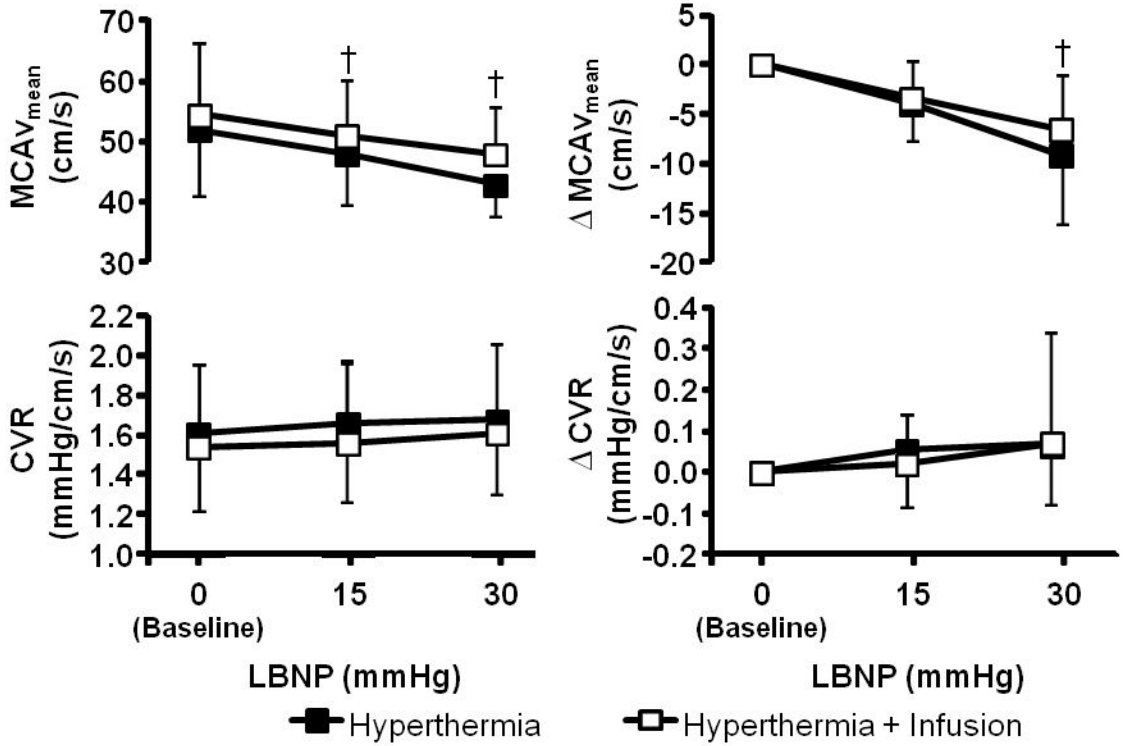
FIGURE LEGENDS

Figure 1: Indices of cerebral perfusion and cerebral vascular resistance during Normothermia, Hyperthermia, and during hyperthermia following acute volume infusion (Hyperthermia + Infusion) (mean ± SD). $MCAv_{mean}$, mean middle cerebral artery blood velocity; CVR, cerebral vascular resistance; *, indicates significantly different from Normothermia ($P \leq 0.03$).

Figure 2: Indices of cerebral perfusion and cerebral vascular resistance at baseline and during 15 and 30 mmHg lower body negative pressure (LBNP) during Hyperthermia and hyperthermia following an acute volume infusion (Hyperthermia + Infusion) (mean ± SD). $MCAv_{mean}$, mean middle cerebral artery blood velocity; CVR, cerebral vascular resistance; Δ, indicates change from pre-LBNP baseline for each condition; †, indicates significantly different from Hyperthermia ($P < 0.05$).

Figure 3: Hemodynamic variables at baseline and during 15 and 30 mmHg lower body negative pressure (LBNP) during Hyperthermia and hyperthermia following an acute volume infusion (Hyperthermia + Infusion) (mean ± SD). MAP, mean arterial pressure; CVP: central venous pressure; SVR, systemic vascular resistance; Δ, indicates change from pre-LBNP baseline for each condition; †, indicates significantly different from Hyperthermia ($P \leq 0.011$).


70
60
50
40
30
MCAV mean (cm/s)
*
*
2.5
2.0
1.5
1.0
CVR (mmHg/cm/s)
Normothermia
Hyperthermia
Hyperthermia + Infusion


MCAv$_{mean}$ (cm/s)
70
60
50
40
30
†
†
CVR (mmHg/cm/s)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
(Baseline)
15
30
LBNP (mmHg)
Hyperthermia
Δ MCAv$_{mean}$ (cm/s)
5
0
-5
-10
-15
-20
†
Δ CVR (mmHg/cm/s)
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
(Baseline)
15
30
LBNP (mmHg)
Hyperthermia + Infusion

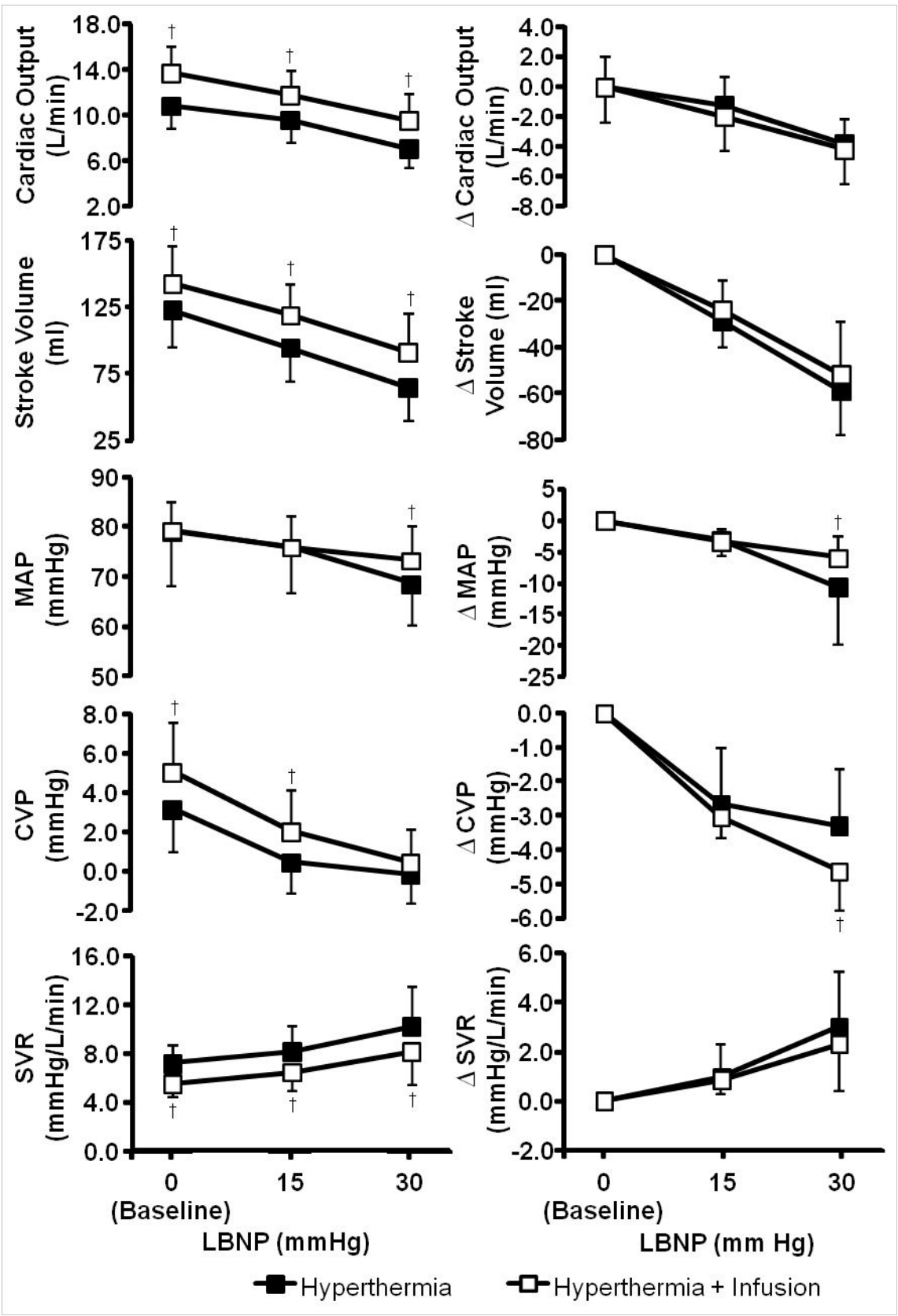

Cardiac Output (L/min)
18.0
14.0
10.0
6.0
2.0
Δ Cardiac Output (L/min)
4.0
2.0
0.0
-2.0
-4.0
-6.0
-8.0
Stroke Volume (ml)
175
125
75
25
Δ Stroke Volume (ml)
0
-20
-40
-60
-80
MAP (mmHg)
90
80
70
60
50
Δ MAP (mmHg)
5
0
-5
-10
-15
-20
-25
CVP (mmHg)
8.0
6.0
4.0
2.0
0.0
-2.0
Δ CVP (mmHg)
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
SVR (mmHg/L/min)
16.0
12.0
8.0
4.0
0.0
Δ SVR (mmHg/L/min)
6.0
4.0
2.0
0.0
-2.0
0
15
30
(Baseline)
LBNP (mmHg)
LBNP (mm Hg)
Hyperthermia
Hyperthermia + Infusion

Table 1: Baseline thermal and hemodynamic data (mean ± SD)

	Normothermia	Hyperthermia	Hyperthermia + Infusion
Pulmonary artery blood temperature (°C)	36.6 ± 0.2	37.7 ± 0.4 *	37.9 ± 0.3 *
Mean skin temperature (°C)	34.9 ± 0.2	37.7 ± 0.4 *	37.7 ± 0.4 *
Cardiac output (L/min)	6.4 ± 0.8	10.9 ± 2.0 *	13.8 ± 2.4 *†
Heart rate (bpm)	61 ± 11	90 ± 14 *	98 ± 11 *†
Stroke volume (ml)	108 ± 19	123 ± 28	143 ± 29 *
MAP (mmHg)	89 ± 8	79 ± 7 *	79 ± 6 *
CVP (mmHg)	5.8 ± 1.5	3.2 ± 2.1 *	5.1 ± 2.5 †
SVR (mmHg/L/min)	13.2 ± 2.3	7.2 ± 1.5 *	5.5 ± 1.0 *†
P_aCO_2 (mmHg)	39 ± 2	39 ± 4	37 ± 6

MAP, mean arterial blood pressure; CVP, central venous pressure; SVR, systemic vascular resistance; P_aCO_2, arterial carbon dioxide tension; * indicates significantly different than normothermia ($P \leq 0.01$); † indicates significantly different than hyperthermia ($P < 0.05$).