Hypercapnia-induced increases in cerebral blood flow do not improve lower body negative pressure tolerance during hyperthermia

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Lucas RA, Pearson J, Schlader ZJ, Crandall CG. Hypercapnia-induced increases in cerebral blood flow do not improve lower body negative pressure tolerance during hyperthermia. Am J Physiol Regul Integr Comp Physiol 305: R604–R609, 2013. First published July 17, 2013; doi:10.1152/ajpregu.00052.2013.—Heat-induced hyperventilation in humans (14, 16) is associated with high skin and body core temperatures (Tc in excess of +1.0°C) (6, 12, 17). In normothermic humans, hyperventilation can also occur during a hypotensive challenge, such as lower body negative pressure (LBNP) or head-up tilt (30). During a combined hyperthermic and hypotensive challenge there is a marked increase in ventilation that significantly reduces end-tidal and arterial carbon dioxide tensions (5, 31).

Cerebral perfusion is profoundly influenced by arterial carbon dioxide tension (3, 19), changing 2–5% per mmHg of carbon dioxide (32). Increases and decreases in arterial carbon dioxide tension influence cerebral perfusion via cerebral arteriolar vasodilation and vasoconstriction, respectively (1). Subsequently, hyperventilation and related reductions in arterial carbon dioxide tension significantly reduce cerebral perfusion. During steady-state head-up tilt, cerebral hypoperfusion can be reversed via carbon dioxide rebreathing, which elevates arterial carbon dioxide tension (30). Furthermore, inhaling a 5% CO2 gas mixture prevents hypocapnia and improves LBNP tolerance under normothermic conditions, presumably due to increases in cerebral perfusion (18). However, an index of cerebral perfusion was not measured in that study, and thus it remains unknown how cerebral perfusion responded to the hypercapnic stimulus at LBNP tolerance.

Heat stress significantly reduces an individual’s ability to withstand a hypotensive challenge (21, 28). While the mechanisms underlying this impaired tolerance are not fully elucidated, it is clear that hyperthermia reduces cerebral perfusion at rest (4, 10, 29, 40) and that these reductions are exacerbated during a hypotensive perturbation (i.e., tilt or stand) (25, 40). Hyperventilation and related reductions in end-tidal CO2 (PETCO2) are purported to contribute to at least 50% of said reductions in cerebral perfusion (4, 11, 29, 33). As such, inhaling a hypercapnic gas mixture and elevating cerebral perfusion should improve tolerance to a hypotensive challenge, given that cerebral hypoperfusion ultimately results in syncope (38). However, it is unknown if inhaling a hypercapnic gas mixture and elevating cerebral perfusion improves tolerance to a hypotensive challenge during heat stress. Such information could have important ramifications in the treatment of hemorrhagic, hyperthermic individuals, as it may extend treatment time. Therefore, the purpose of this study was to test the hypothesis that a hypercapnia-induced increase in cerebral perfusion improves LBNP tolerance in hyperthermic individuals.

METHODS

Participants. Eleven healthy individuals (8 males, 3 females; 31 ± 7 yr, 75 ± 12 kg, body mass index, 25 ± 3 kg/m²) participated in this study. Subjects were not taking medications and were free of any known cardiovascular, metabolic, or neurological diseases and were nonsmokers. Repeated testing was conducted at the same phase of each female subject’s menstrual cycle, although menstrual cycle phase was not controlled for between subjects as tolerance to a hyperthermic hypotensive challenge is unaffected by menstrual cycle phase (28).

Participants abstained from exercise and alcohol for 24 h before, as well as caffeine for 12 h before testing. Written informed consent was obtained before participation in this study, which was approved by the University of Texas Southwestern Medical Center at Dallas and Texas Health Presbyterian Hospital Dallas. All procedures conformed to the standards set by the Declaration of Helsinki.

Instrumentation. At the beginning of each experimental day, subjects voided their bladder before nude body mass was recorded. Urine specific gravity was measured using a digital refractometer. Subjects
were then dressed in a long-sleeved and legged, two-piece, tube-lined perfusion suit (Med-Eng, Ottawa, Canada) enabling the control of skin temperature and $T_e$ via the temperature of the water-perfusing the suit. Body core temperature was measured using a telemetry temperature pill swallowed ~2 h before the onset of data collection (HQ, Palmetto, FL). Whole body mean skin temperature ($T_m$) was measured from the electrical average of six thermocouples (37) fixed to the skin with porous adhesive tape. Beat-to-beat arterial blood pressure was measured and reconstructed to the brachial artery via finger cuff photoplethysmography (Finometer Pro, FMS, Amsterdam, The Netherlands or NexFin HD, BMEYE B.V, Amsterdam, The Netherlands). Arterial blood pressure was also measured by auscultation of the brachial artery (Tango, Suntech Medical Instruments, Raleigh, NC). Finger arterial pressure was used for data analysis while measures from the brachial artery and Finometer were used to aid the detection of ensuing syncope. Mean blood velocity in the right middle cerebral artery (MCAvmean) served as an index of cerebral perfusion and was measured using 2-MHz pulsed Doppler ultrasound (Multi-flow, DWL Elektronische Systeme, Singen, Germany). The Doppler probe was maintained in position using a commercially available headpiece. An index of cerebrovascular conductance (CBVC) was calculated as MCAvmean/mean arterial pressure (MAP). Expired air was sampled via a facemask attached to a two-way valve (Hans Rudolf, Shawnee, KS). Ventilatory parameters (ventilation, tidal volume, breathing rate) were measured (in BTPS) using an automated gas analysis system (TrueOne 2400, Parvo-Medics, Provo, UT), with values recorded over 15-s epochs. The partial pressure of $P$CO2 was measured from the mask and measured using a capnograph (9004 Capnocheck Plus, Smiths Medical International, Watford, Herts, UK). Heart rate (HR) was collected from an electrocardiogram signal (Agilent, Munich, Germany) interfaced with a cardiotachometer (1,000 Hz sampling rate, CWE, Ardmore, PA). Thermal and hemodynamic data were acquired continuously at 50 Hz throughout the experiment (Biopac, Santa Barbara, CA).

Experimental protocol. Subjects reported to the laboratory on two separate occasions. At each visit subjects underwent passive heat stress followed by LBNP to tolerance. Experimental trials were at least 3 days apart and were performed at the same time of day. After instrumentation, subjects were positioned in the LBPN box that was sealed at the level of the iliac crest. Subjects rested quietly while normothermic water (34°C) circulated through the suit for at least 30 min. After ~20 min of wearing a face mask connected to the gas analysis system (ensuring steady-state ventilatory responses), normothermic baseline measures were obtained for 6 min while the subject breathed room air. Subjects were then passively heated by circulating ~49°C water through the suit until $T_e$ increased by ~1.3°C, at which point water temperature was lowered to ~46°C. The face mask was reattached 5–10 min before pre-LBNP measures were obtained. Progressive LBNP to tolerance was initiated after $T_e$ was raised ~1.3°C. Beginning at 20 mmHg, 3-min stages of LBNP were applied at 10 mmHg per stage until the occurrence of syncopal symptoms. In both trials, subjects inhaled room air during the 20-mmHg LBNP stage. In the CO2 trial, subjects inhaled a hypercapnic gas mixture (5% CO2, 21% oxygen, balanced nitrogen) from the onset of the 30-mmHg stage through to LBNP tolerance. In the SHAM trial, subjects continued to breathe room air. Subjects were blinded to the gas mixture they were inhaling, which was administered in a randomized and counterbalance manner between trials. Criteria for LBNP test termination were the following: continued self reporting by the subject of feeling faint and/or sustained nausea; a rapid and progressive decrease in blood pressure resulting in sustained systolic blood pressure being less than 80 mmHg; and/or relative bradycardia accompanied by narrowing of pulse pressure. Typically, a combination of the aforementioned conditions was observed at the cessation of the tolerance test. The total time of each test was measured and used to determine a cumulative stress index (CSI), which was calculated by summing the product of the negative pressure and the duration at that negative pressure (e.g., 20 mmHg × 3 min + 30 mmHg × 3 min + 40 mmHg × 3 min, etc.) until test termination (23, 27).

Data analysis. In both the SHAM and CO2 trials, 60 s of data were averaged for normothermic baseline measures. Pre-LBNP heat stress values were averaged from 60 s of data before the onset of LBNP. During LBNP, data from the last 60 s at 20 mmHg and the highest common LBNP stage completed by each respective participant in both trials (classified as “severe”) were analyzed. Hemodynamic LBNP tolerance data were obtained by averaging responses during the last 10 s before cessation of the LBNP challenge due to syncopal symptoms (26). Ventilatory LBNP tolerance data were obtained by averaging responses during the last 30 s, thus allowing for inclusion of multiple breaths in this analysis.

A two-way repeated measures analysis of variance (ANOVA) with main factors of time (normothermia, heat stress, 20 mmHg LBNP, severe LBNP, LBNP tolerance) and experimental day (SHAM vs. CO2) was used to identify differences in thermal, hemodynamic, and respiratory measures between the SHAM and CO2 trials. Bonferroni-corrected post-hoc tests were used to determine differences when a significant interaction was identified from the ANOVA. Paired $t$-tests were used to identify differences in CSI, body mass, and urine specific gravity. The a priori α level for all analyses was set at 0.05. Results are reported as means ± SD.

RESULTS

Before instrumentation, subjects’ body mass (SHAM, 74.8 ± 12.4 kg vs. CO2, 74.5 ± 12.5 kg; $P = 0.15$) and urine specific gravity (SHAM, 1.014 ± 0.006 vs. CO2, 1.011 ± 0.005; $P = 0.39$) were similar between experimental days. Thermal, hemodynamic, and respiratory baseline measures, while subjects were normothermic, were not different between the two experimental trials ($P > 0.05$, Table 1). Before the onset of LBNP, passive heat stress caused similar ($P > 0.05$) increases in $T_e$ (−1.3°C) and $T_{sk}$ (−3.9°C), as well as similar hemodynamic and respiratory responses ($P > 0.05$; see Table 1). At the completion of testing, sweating-induced reductions in body mass were similar (P = 0.79) in both the SHAM and CO2 trials (−1.2 ± 0.5 and −1.1 ± 0.4 kg, respectively).

LBNP tolerance was similar between the two trials, with no difference in CSI (SHAM: 273 ± 158 mmHg × min vs. CO2: 339 ± 155 mmHg × min, $P = 0.26$), time to tolerance (SHAM: 514 ± 211 s vs. CO2: 604 ± 199 s, $P = 0.22$), or the final LBNP stage reached (SHAM: 50 ± 10 vs. CO2: 50 ± 10 mmHg, $P = 0.37$). At 20 mmHg LBNP, respiratory and hemodynamic variables were not different between trials (Figs. 1, 2, and 3). Under severe LBNP (−40 mmHg LBNP), inhaling hypercapnic gas increased $P$CO2 (by 16 ± 4 mmHg, $P < 0.01$), ventilation (by 5.2 ± 8.2 l/min, $P = 0.03$), MCAvmean (by 21 ± 12 cm/s, or 31 ± 3%, $P < 0.01$), CBVC (by 0.2 ± 0.2 cm/s·mmHg, $P < 0.01$), and MAP (by 10 ± 10 mmHg, $P < 0.01$), relative to the SHAM trial. At LBNP tolerance the following variables were higher in the CO2 trial relative to the SHAM trial: $P$CO2 (by 18 ± 5 mmHg, $P < 0.01$), ventilation (by 9.1 ± 12.0 l/min, $P < 0.01$), tidal volume (by 0.3 ± 0.4 liter, $P = 0.02$), and respiratory rate (by 3 ± 4 breaths/min, $P = 0.01$). Likewise, MCAvmean (by 18 ± 10 cm/s, or 25 ± 13%, $P < 0.01$) and CBVC (by 0.2 ± 0.2 cm/s·mmHg, $P < 0.01$) were higher in the CO2 trial at LBNP tolerance. Despite those findings, MAP and HR were not different ($P > 0.05$) between trials at LBNP tolerance. Both trials showed a similar ($P = 0.82$) relative bradycardia at LBNP tolerance, with HRs decreasing 23 ± 12 and 24 ± 19 beats/min from maximum
during the final LBNP stage in the SHAM and CO\textsubscript{2} trials, respectively.

**DISCUSSION**

This is the first study to examine whether elevating cerebral perfusion via inhalation of a hypercapnic gas mixture improves LBNP tolerance under heat stress conditions. The novel findings from this study are the following: 1) inhaling a hypercapnic gas mixture restores MCA\textsubscript{mean} to pre-LBNP values, resulting in cerebral perfusion being elevated at LBNP tolerance compared with the control trial (Fig. 2), but 2) this higher cerebral perfusion did not improve LBNP tolerance.

*Significantly different from normothermic baseline, *P* < 0.05.

Table 1. Baseline thermal, hemodynamic, and respiratory measures during normothermia and heat stress (immediately before lower body negative pressure) for both the control and CO\textsubscript{2} trials

<table>
<thead>
<tr>
<th></th>
<th>Baseline SHAM</th>
<th>Baseline CO\textsubscript{2}</th>
<th>Heat Stress (Pre-LBNP)</th>
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<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>T\textsubscript{c}, °C</strong></td>
<td>36.8 ± 0.3</td>
<td>36.7 ± 0.3</td>
<td>38.1 ± 0.3*</td>
</tr>
<tr>
<td><strong>T\textsubscript{sk}, °C</strong></td>
<td>34.5 ± 0.3</td>
<td>34.4 ± 0.5</td>
<td>38.4 ± 0.5*</td>
</tr>
<tr>
<td><strong>MCA\textsubscript{mean}, cm/s</strong></td>
<td>65 ± 17</td>
<td>66 ± 12</td>
<td>55 ± 15*</td>
</tr>
<tr>
<td><strong>CBVC, cm\textsuperscript{-1}·mmHg\textsuperscript{-1}</strong></td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td><strong>P\textsubscript{ETCO\textsubscript{2}}, mmHg</strong></td>
<td>41 ± 3</td>
<td>41 ± 3</td>
<td>36 ± 3*</td>
</tr>
<tr>
<td><strong>Ventilation BTPS, l/min</strong></td>
<td>8.1 ± 2.2</td>
<td>8.0 ± 3.7</td>
<td>11.0 ± 3.8*</td>
</tr>
<tr>
<td><strong>Tidal volume, liters</strong></td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.4*</td>
</tr>
<tr>
<td><strong>Respiratory rate, breaths/min</strong></td>
<td>14 ± 2</td>
<td>14 ± 4</td>
<td>15 ± 5</td>
</tr>
<tr>
<td><strong>MAP, mmHg</strong></td>
<td>86 ± 10</td>
<td>83 ± 10</td>
<td>80 ± 7</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td>57 ± 8</td>
<td>58 ± 8</td>
<td>96 ± 14*</td>
</tr>
</tbody>
</table>

Values are means ± SD. SHAM, control; Pre-LBNP, before lower body negative pressure; T\textsubscript{c}, body core temperature; T\textsubscript{sk}, mean skin temperature; MCA\textsubscript{mean}, middle cerebral artery velocity; CBVC, cerebrovascular conductance; P\textsubscript{ETCO\textsubscript{2}}, end-tidal carbon dioxide; MAP, mean arterial pressure; HR, heart rate.

Fig. 1. Ventilatory measures (body temperature and pressure saturated, BTPS) during both heat stress lower body negative pressure (LBNP) tests where subjects inhaled either a hypercapnic gas mixture or room air (SHAM), P\textsubscript{ETCO\textsubscript{2}}, end-tidal partial pressure of carbon dioxide.

†Significantly different from SHAM trial (*P* < 0.05); ‡Significantly different from 0 mmHg (*P* < 0.05); §Significantly different from 20 mmHg (*P* < 0.05); ¶Significantly different from severe LBNP (*P* < 0.05).
as reductions in perfusion pressure or an increased sympathetic activity, seemingly contribute to heat-related reductions in MCAvmean (3). In the current study, heat- and LBNP-induced reductions in MCAvmean were essentially ameliorated by inhaling a 5% CO2 gas mixture and elevating PETCO2. Similarly, clamping PETCO2 during hyperthermic head-up tilt restores cerebral perfusion to hyperthermic supine values (29). Furthermore, with severe heat stress (approximately 1.8°C Tc) hyperventilation hypocapnia appears the primary mechanism in reducing cerebral perfusion (29). Thus, in the presence of a strong hyperventilation stimulus, such as severe hyperthermia and/or a hypotensive challenge, elevating PETCO2 restores cerebral perfusion.

Cerebral perfusion, LBNP tolerance, and circulatory collapse. Under normothermic conditions, hypocapnia-related cerebral hypoperfusion can be reversed by rebreathing CO2 (30), and LBNP tolerance is improved by inhaling 5% CO2 (18). As shown in the present data, inhaling a 5% CO2 gas mixture during hyperthermia circumvents hyperventilatory hypocapnia and accompanying reductions in cerebral perfusion. However, this CO2 load did not improve LBNP tolerance, which is surprising given that hypocapnia significantly elevated cerebral perfusion and CBVC. Indeed, relative to pre-LBNP, in the SHAM trial LBNP tolerance was accompanied by a 27 ± 9% reduction in MCAvmean, whereas in the CO2 trial, LBNP tolerance was accompanied by just a 10 ± 10% reduction in MCAvmean. Despite these differences, similar decreases in MAP and HR occurred in both the CO2 and SHAM trials, confirming that LBNP-induced circulatory collapse was achieved under both conditions. These findings indicate that inhaling 5% CO2 dissociated cerebral perfusion from circulatory collapse during simulated hemorrhage in hyperthermic individuals, demonstrating that cerebral hypoperfusion is not requisite for cardiovascular collapse.

Cardiovascular (or circulatory) collapse occurs when cardiac output falls to critically low levels, often in concert with reduced sympathetic activity (8). This, accompanied by increases in cardiac parasympathetic activity, results in a sudden bradycardia and/or decrease in systolic blood pressure (7, 8). In the current study, there were similar decreases in MAP and HR in both the CO2 and SHAM trial at LBNP tolerance. Thus both trials exhibited typical hallmarks of cardiovascular collapse (sympathoinhibition and vagal activation) that resulted in a similar reduction in MAP without a corresponding physiologically relevant reduction in MCAvmean in the CO2 trial.

Fig. 2. Cerebrovascular measures during both heat stress LBNP tests where subjects inhaled either a hypercapnic gas mixture or room air (SHAM). MCAvmean, mean middle cerebral artery blood velocity; CBVC, cerebrovascular conductance. ‡Significantly different from SHAM trial (P < 0.05); 1Significantly different from 0 mmHg (P < 0.05); 2Significantly different from 20 mmHg (P < 0.05).

Fig. 3. Mean arterial pressure (MAP) and heart rate (HR) measures during both heat stress LBNP tests where subjects inhaled either a hypercapnic gas mixture or room air (SHAM). ‡Significantly different from SHAM trial (P < 0.05); 1Significantly different from 0 mmHg (P < 0.05); 2Significantly different from 20 mmHg (P < 0.05); 3Significantly different from severe LBNP (P < 0.05).
Interestingly, the current data indicate that cardiac vagal discharge and accompanying bradycardia typically preceding syncope may be unrelated to cerebral perfusion under hypertermic conditions. This is perhaps not surprising given that this bradycardia has been proposed to be mediated by reductions in ventricular volumes and subsequent activation of cardiac vagal afferents (9). Furthermore, other studies have shown a dissociation between cerebral perfusion and LBNP tolerance in normothermic individuals; that is, LBNP intolerance persisted despite elevated cerebral perfusion (20), while hyperventilation-induced reductions in cerebral perfusion during LBNP failed to initiate premature presyncope or hemodynamic collapse (23). These and the present findings support the hypothesis that cerebral perfusion may not always be the primary factor leading to intolerance to a hypertensive challenge. That cerebral perfusion can essentially be maintained in the face of profound central hypovolemia could have important ramifications for trauma and hemorrhage treatment; although, it is unclear whether CO2-induced increases in cerebral perfusion would have prolonged consciousness should the trial have continued to the point of unconsciousness. Indeed, in the current study cardiovascular measures were the primary objective criteria used to determine LBNP cessation.

Ventilation and hypertermic LBNP. Heat stress often causes hyperventilation and related hypocapnia, evidenced in the present investigation by elevating ventilation ~3.1 l/min and decreasing PetCO2 ~4 mmHg in both the SHAM and CO2 trials before LBNP. This heat-induced hyperventilation is similar (13) or lower (6, 12) than that reported in other studies. As anticipated (5, 31), ventilation continued to increase and PetCO2 to decrease with progressive LBNP in the SHAM trial.

Hypocapnia caused further increases in ventilation in the CO2 trial relative to that which occurred with the SHAM trial. Similar hypocapnic ventilatory responses have been shown during normothermic LBNP (40 mmHg) (22). Such increases in ventilation, and particularly tidal volume, may aid venous return and subsequently help maintain cardiac output via the respiratory pump (2, 36), though it is unknown whether this occurs during hyperthermia. Certainly, the maintenance of MAP during severe LBNP in the CO2 trial versus the gradual reduction in MAP during the SHAM trial suggests that larger increase in ventilation during the CO2 trial augments venous return. However, despite this, hypocapnia did not improve LBNP tolerance under heat stress conditions, thereby indicating that any increases in cardiac output due to increased tidal volume was insufficient to improve tolerance.

Technological considerations. Transcranial Doppler was used to measure blood velocity in the middle cerebral artery. This approach has been used as an index of cerebral blood flow, because this artery supplies ~80% of the blood flow received by each cerebral hemisphere (24), and its diameter is reported to not change during moderate CO2 and blood pressure perturbations (15, 35). However, recent studies have shown that the regulation of blood flow differs between the brain stem and cortex with the brain stem being less sensitive to hypocapnia (34, 39). Although speculative, it may be that hypocapnia-induced increases in MCAvmean during hypertermic LBNP do not reflect comparable increases in blood flow to other areas of the brain, namely, the brain stem. It is also important to consider potential differences in cerebral hemodynamics during an LBNP challenge versus the upright posture. Orthostatic-induced syncope is reported to occur upon an ~50% reduction in MCAvmean (38). However, the current study indicates that presyncope symptoms can occur without a meaningful reduction in MCAvmean during LBNP.

Implications. The current study demonstrates that the administration of 5% CO2 could be advantageous in the maintenance of cerebral perfusion during a hypertensive challenge, attenuating the reduction in cerebral perfusion even at circulatory collapse. These findings could have implications for the treatment of individuals suffering from a hemorrhagic injury, when maintenance of brain perfusion becomes paramount. Though, it should be noted that the tracking of the MCAvmean will not necessarily enable the prediction or identification of circulatory collapse or shock, at least in heat-stressed individuals.

Perspectives and Significance

During hyperthermia, inhaling a hypercapnic gas mixture and circumventing hyperventilation-induced hypocapnia does not improve LBNP tolerance, despite restoring cerebral perfusion. This disassociation between cerebral perfusion and systemic circulatory responses during central hypovolemia indicates that cerebral perfusion may be maintained in the face of a severe hypertensive challenge, even to the point of circulatory collapse.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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