The role of cerebral oxygenation and regional cerebral blood flow on tolerance to central hypovolemia

Victoria L. Kay and Caroline A. Rickards

Institute for Cardiovascular and Metabolic Diseases, University of North Texas Health Science Center, Fort Worth, Texas

Submitted 20 August 2015; accepted in final form 14 December 2015

Kay VL, Rickards CA. The role of cerebral oxygenation and regional cerebral blood flow on tolerance to central hypovolemia. Am J Physiol Regul Integr Comp Physiol 310: R375–R383, 2016. First published December 16, 2015; doi:10.1152/ajpregu.00367.2015.—Tolerance to central hypovolemia is highly variable, and accumulating evidence suggests that protection of anterior cerebral blood flow (CBF) is not an underlying mechanism. We hypothesized that individuals with high tolerance to central hypovolemia would exhibit protection of cerebral oxygenation (ScO2), and prolonged preservation of CBF in the posterior vs. anterior cerebral circulation. Eighteen subjects (7 male/11 female) completed a presyncpe-limited lower body negative pressure (LBNP) protocol (3 mmHg/min onset rate). ScO2 (via near-infrared spectroscopy), middle cerebral artery velocity (MCAv), posterior cerebral artery velocity (PCAv) (both via transcranial Doppler ultrasound), and arterial pressure (via finger photoplethysmography) were measured continuously. Subjects who completed ≥70 mmHg LBNP were classified as high tolerant (HT; n = 7) and low tolerant (LT; n = 11) if they completed ≤60 mmHg LBNP. The minimum difference in LBNP tolerance between groups was 193 s (LT = 1,243 ± 185 s vs. HT = 1,996 ± 212 s; P < 0.001; Cohen’s d = 3.8). Despite similar reductions in mean MCAv in both groups, ScO2 decreased in LT subjects from −15 mmHg LBNP (P = 0.002; Cohen’s d = 1.8), but was maintained at baseline values until −75 mmHg LBNP in HT subjects (P < 0.001; Cohen’s d = 2.2); ScO2 was lower at −30 and −45 mmHg LBNP in LT subjects (P ≤ 0.02; Cohen’s d ≥ 1.1). Similarly, mean PCAv decreased below baseline from −30 mmHg LBNP in LT subjects (P = 0.004; Cohen’s d = 1.0), but remained unchanged from baseline in HT subjects until −75 mmHg (P = 0.006; Cohen’s d = 2.0); PCAv was lower at −30 and −45 mmHg LBNP in LT subjects (P ≤ 0.01; Cohen’s d ≥ 0.94). Individuals with higher tolerance to central hypovolemia exhibit prolonged preservation of CBF in the posterior cerebral circulation and sustained cerebral tissue oxygenation, both associated with a delay in the onset of presyncope.

posterior cerebral artery; middle cerebral artery; lower body negative pressure

Hemorrhage due to trauma is one of the leading causes of morbidity and mortality worldwide in both the civilian and military settings (1, 2, 13, 22, 32). A major factor contributing to death and disability from severe blood loss is poor tissue perfusion and oxygenation of the vital organs (1, 13, 22). Prolonged cerebral hypoperfusion can lead to neuronal cell death, and if the patient survives, long-term cognitive impairment and physical disability (32). Understanding cerebral hemodynamic responses to blood loss is an essential target for improving survival to hemorrhagic injury, and developing effective therapeutic interventions (35). As there is consider-

able variability in survival time following hemorrhagic injuries (40), as well as tolerance to simulated hemorrhage (7, 15, 24, 26), it is crucial to determine the role of cerebral blood flow (CBF) and oxygenation on the ability to tolerate severe blood loss.

Lower body negative pressure (LBNP) has been extensively utilized as an experimental technique to induce physiologically significant central hypovolemia, and can be used to simulate preshock hemorrhage in humans (4, 9, 44, 51). Two recent studies reported comparable hemodynamic responses to LBNP and blood loss up to 1,000 ml in humans (21, 36) and 25% loss of total blood volume in baboons (17). It is well established that during the initial stages of progressive central hypovolemia, reflex cardiovascular responses are initiated (e.g., tachycardia, peripheral vasoconstriction) (5, 6, 15, 19, 24, 38), protecting the vital organs from hypoperfusion. Although traditionally protection of absolute CBF was thought to be essential in determining tolerance to central hypovolemia (24), recent studies have indicated a disconnect between tolerance to LBNP and protection of absolute flow [predominantly assessed by middle cerebral artery velocity (MCAv), an index of global cerebral blood flow] (20, 27, 28, 37). Most recently, Ogoh et al. (33) suggested that flow in the posterior cerebral circulation (indexed by flow in the vertebral arteries) feeding the brain stem (specifically, the medulla oblongata) is more likely associated with tolerance to central hypovolemia than flow in the anterior cerebral circulation (indexed by flow in the internal carotid arteries); responses between the two regions with central hypovolemia to presyncope, however, were not evaluated.

In addition to delivery of oxygen to the cerebral tissues, extraction of that oxygen from the blood may also play a crucial role in tolerance to central hypovolemia. Near-infrared spectroscopy (NIRS) is often used as a noninvasive method to measure cerebral oxygen saturation (ScO2) via assessment of oxy- (HbO2) and deoxy-hemoglobin (dHb) concentrations within the frontal cortex. NIRS measures oxygen saturation predominantly from venous blood (75%), with just 25% from arterial and capillary blood (30, 34). As such, following oxygen exchange within the capillaries, we and others (43) interpret a decrease in the HbO2 concentration and increase in the dHb concentration as an increase in cerebral oxygen extraction from the arterial blood supplying the tissue. Torella et al. (47) reported that HbO2 decreased, while dHb increased in proportion to mild blood loss in humans (~9–12% total blood volume). Although the magnitude of blood loss in this study was not sufficient to delineate the role of cerebral oxygen extraction on tolerance, Lewis et al. (25) recently suggested that decreases in CBF would have minimal impact on tolerance to central hypovolemia due to compensatory increases in cerebral oxygen extraction; quantification of oxygen extraction dur-

Address for reprint requests and other correspondence: C. A. Rickards, Inst. for Cardiovascular and Metabolic Diseases, Univ. of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107 USA (e-mail: caroline.rickards@unthsc.edu).

http://www.ajpregu.org

0363-6119/16 Copyright © 2016 the American Physiological Society

http://ajpregu.physiology.org/
ing LBNP, however, was not reported. Furthermore, in a study of fainters vs. nonfainters following withdrawal of 500 ml of blood plus head-up tilt (3), the fainters had decreased oxygen extraction in the cerebral tissues (positive oxygenation index, i.e., HbO2 - dHb), while the nonfainters had increased oxygen extraction (negative oxygenation index), suggesting that increased tolerance may be due to increased cerebral oxygen extraction. The onset of presyncope is thought to be due to a mismatch between oxygen supply and demand in the brain (3, 25), but this hypothesis has not been explored in relation to tolerance to maximal central hypovolemia.

By applying LBNP continuously (3 mmHg/min decompression rate) to induce significant central hypovolemia to presyncope in healthy, conscious humans, we assessed if 1) maintenance of ScO2 and/or increased oxygen extraction plays a role in determining tolerance to this stress, and, 2) differences between perfusion of the anterior and posterior regions of the brain were related to tolerance to central hypovolemia. We hypothesized that individuals with higher tolerance to central hypovolemia would have protection of ScO2, and prolonged preservation of CBF in the posterior vs. anterior cerebral circulation, thus delaying the onset of presyncope.

METHODS

Subjects. Thirty-four healthy, normotensive, nonsmoking subjects volunteered to participate in this study, conducted at the University of North Texas Health Science Center (UNTHSC) in Fort Worth, TX. The experimental protocol was reviewed and approved by the Institutional Review Board at UNTHSC. Prior to approval to participate in the study, each subject completed an orientation session, where a medical history was obtained and physical exam was performed, including seated and standing ECG and blood pressure measurements. Females underwent a urine pregnancy test and were excluded if pregnant; the pregnancy test was repeated immediately prior to experimentation. Subjects were given a verbal briefing and were made familiar with the laboratory, personnel, procedures, and monitoring equipment. Each subject gave written informed consent to participate in this study. Because of the potential effects on vascular volume and cerebrovascular and baroreflex function, subjects were asked to refrain from exercise, stimulants that might alter autonomic function (e.g., caffeine and cold medications, including ephedrine, diphenhydramine), alcohol, prescription or nonprescription drugs, and herbal medications for 24 h prior to the orientation and experimental sessions. Subjects were also instructed to remain hydrated (ad libitum water consumption) and maintain their normal sleep pattern. Experiments were conducted at the same time of day (morning) to avoid potential effects of circadian rhythm on the study outcomes, in a temperature-controlled laboratory (22°C–24°C).

Instrumentation. Subjects were placed in the supine position with their lower body inside a LBNP chamber (VUV Analytics, Austin, TX) and positioned on a bicycle seat to ensure they did not move during chamber decompression. Durable plastic and a neoprene band were wrapped around the subject’s waist to create an airtight seal with the LBNP chamber; the seal was in line with the subject’s iliac crest. All subjects were instrumented for the continuous measurement of heart rate (HR) via a standard lead II ECG (shielded leads, cable, and amplifier, AD Instruments, Bella Vista, NSW, Australia), and beat-to-beat arterial pressure and stroke volume (SV) via infrared finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). Respiration rate and end tidal CO2 (etCO2) were measured on a breath-by-breath basis through a face-mask via capnography (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW, Australia). Cerebral blood velocity was recorded from the middle cerebral artery (MCAv) and posterior cerebral artery (PCAv) via transcranial Doppler ultrasound (2-MHz probes; ST3, Spencer Technologies, Seattle, WA). HbO2, dHb, total hemoglobin concentration (THC; HbO2 + dHb), and ScO2 [(HbO2/THC)-100] were measured or calculated from the frontal cortex via NIRS (OxiplexTS, ISS, Champaign-Urbana, IL). Efforts were made to ensure both MCAv, and cerebral oxygenation measurements were made on same side of the head within each subject.

Protocol. Each subject was exposed to LBNP to the point of maximal tolerance (i.e., presyncope). The protocol consisted of a 5-min baseline followed by continuous application (ramp-profile) of negative pressure at a decompression rate of 3 mmHg/min until the onset of presyncope (23), determined by one or more of the following criteria: 1) instantaneous systolic arterial pressure (SAP) below 80 mmHg; 2) sudden relative bradycardia, and/or 3) voluntary subject termination due to subjective presyncope symptoms such as gray-out, nausea, sweating, dizziness, blurred vision, or general discomfort. The chamber pressure was released immediately at the onset of hemodynamic decompensation or upon reaching −100 mmHg LBNP. Release of the chamber pressure occurred within seconds, and presyncope symptoms generally resolved within 30–60 s. Following LBNP termination, subjects remained in the chamber for a 10-min recovery period.

Data analysis. All continuous waveform data (e.g., ECG, arterial blood pressure, SV, MCAv, PCAv, ScO2, THC, etCO2) were collected at 1,000 Hz (LabChart, AD Instruments, Bella Vista, NSW, Australia) and analyzed offline via specialized software (WinCPRS, Absolute Aliens, Turku, Finland). R-waves that were generated from the ECG signal were detected to determine the timing of each cardiac cycle. Beat-to-beat SAP and diastolic arterial pressures were then detected from the continuous arterial pressure tracing. Systolic and diastolic cerebral blood velocities were also detected and marked from the continuous MCAv and PCAv tracings. MAP, mean MCAv, and mean PCAv were automatically calculated as the area under the arterial pressure and cerebral blood velocity waveforms via the WinCPRS software.

Since there is variable tolerance to central hypovolemia (15, 24, 26), subjects became presyncopal at different levels of LBNP. As such, subjects were classified as high tolerant (HT) if they made it to −70 mmHg LBNP or greater, and low tolerant (LT) if they made it to −60 mmHg LBNP or less. If the LBNP protocol was terminated between −61 and −69 mmHg LBNP, these subjects were excluded from all subsequent analysis to ensure there was a definitive separation between groups.

Statistical analysis. Physiological responses were compared between the HT and LT groups at 15-mmHg intervals up to −45 mmHg LBNP, as this was the last common maximal level of LBNP for the majority of LT subjects. HT subject data are also presented up to −75 mmHg LBNP, since they were able to tolerate longer periods of LBNP. All variables were calculated from the final 4-min of each 15-mmHg interval of LBNP, yielding data points approximating responses at baseline (0 mmHg), −15 mmHg (range: −6 to −15 mmHg), −30 mmHg (range: −21 to −30 mmHg), −45 mmHg (range: −36 to −45 mmHg), −60 mmHg (range: −51 to −60), and −75 mmHg (−66 to −75 mmHg) LBNP. In addition, to compare physiological responses between the HT and LT subjects at presyncope, the final 1-min (PS-1) immediately prior to presyncope was assessed for each subject. Absolute and percentage change from baseline values are reported for the key variables of interest.

The physiological responses to LBNP up to −45 mmHg were analyzed using a two-way (LBNP level and tolerance) repeated-measures ANOVA followed by Tukey post hoc tests. For the HT group only, physiological responses up to −75 mmHg LBNP were also analyzed via a one-way ANOVA, followed by Tukey post hoc tests. The −75 mmHg LBNP level was used for this analysis as 6 of
the 7 HT subjects reached this level of LBNP. Unpaired t-tests were used to compare the HT vs. LT group responses at baseline and at the PS-1 time point or Mann-Whitney U-tests were run on data that was not normally distributed (dHb at PS-1 only). A Fisher exact test was used to compare the ratio of males to females between the HT and LT groups. All data are presented as means ± SE (unless otherwise stated). Effect size was calculated using Cohen’s d. A critical α level of 0.05 is used for all comparisons, and exact P values are also reported where appropriate.

**RESULTS**

**LBNP tolerance.** Of the 34 subjects who participated in this study, data were analyzed and included from 27 subjects who 1) reached the maximal LBNP pressure (~100 mmHg), or 2) had a minimum instantaneous SAP < 80 mmHg, or 3) exhibited subjective presyncope symptoms combined with mean SAP < 100 mmHg for the 1 min prior to presyncope and/or minimum SAP ≥ 90 mmHg within the 1 min prior to presyncope. Of the 27 subjects who reached these thresholds, seven subjects reached presyncope between ~61 and ~69 mmHg LBNP and, therefore, are not included in this particular data set [these subjects are included in other independent analyses conducted on this data set, including Kay and Rickards (23)]. Of the remaining 20 subjects, 2 were excluded from subsequent analyses, as they exhibited a resting SAP > 140 mmHg. The remaining 18 subjects (11 male, 7 female; age, 26 ± 3 yr; height, 172 ± 9 cm; weight, 74 ± 15 kg; means ± SD) were allocated to the HT (n = 7) or LT (n = 11) groups based on the level of LBNP that they reached prior to presyncope, as previously described (i.e., HT ≥ 70 mmHg LBNP; LT ≤ 60 mmHg LBNP). By design, the minimum difference in LBNP tolerance between the HT and LT groups was 193 s (LT = 1,243 ± 185 s vs. HT = 1,996 ± 212 s; P < 0.001; Cohen’s d = 3.8). Of the 18 subjects included in the final analysis, the LBNP protocol was terminated at or before ~45 mmHg LBNP for four subjects, between ~45 and ~60 mmHg LBNP for seven subjects, between ~70 and ~90 mmHg LBNP for five subjects, between ~90 and ~99 mmHg LBNP for one subject, and one subject completed the protocol to ~100 mmHg LBNP.

Data in Tables 1 and 2 compare the baseline characteristics of each group.

**Cardiovascular responses to LBNP.** Data are presented as means ± SE at 15-mmHg intervals of LBNP. Both groups experienced progressive reductions in SV from baseline, reaching 20–30% below baseline by ~45 mmHg of LBNP (P < 0.001, Cohen’s d ≥ 2.7; Fig. 1); the SV reduction in the LT group was greater at ~30 and ~45 mmHg LBNP (P ≤ 0.006; Cohen’s d ≥ 0.96) vs. the HT group. However, at the final 1 min prior to presyncope (PS-1), SV decreased by 63 ± 4% in the HT group compared with 42 ± 4% in the LT group (P = 0.004; Cohen’s d = 1.7). In response to these reductions in SV, compensatory increases in HR occurred in both the HT and LT groups (Fig. 2A), with the HT group exhibiting a greater maximal HR response at the final 1 min prior to presyncope (PS-1) compared with the LT group (HT: 132 ± 5 beats/min vs. LT: 92 ± 6 beats/min; P < 0.001, Cohen’s d = 2.3). MAP was maintained at baseline levels up to ~30 mmHg, then fell below baseline at ~45 mmHg LBNP in both the HT and LT groups (P ≤ 0.04, Cohen’s d = 0.67; Fig. 2B); by presyncope, MAP fell to similar levels in both groups (P = 0.92, Cohen’s d = 0.05).

Mean PCAv decreased below baseline from ~30 mmHg LBNP in the LT subjects (P = 0.013, Cohen’s d = 1.0), but remained unchanged in the HT subjects up to ~45 mmHg (P ≥ 0.86, Cohen’s d ≤ 0.32; Fig. 3B), only beginning to fall below baseline at 75 mmHg (P = 0.007, Cohen’s d = 1.98). By presyncope, mean PCAv had decreased by the same magnitude in both HT and LT groups (P = 0.77, Cohen’s d = 0.15) and to similar absolute values (P = 0.71, Cohen’s d = 0.23; Table 2). In comparison, a decrease (%Δ) in mean MCAv from baseline was observed in the LT group by ~30 mmHg and by ~45 mmHg in the HT group (P ≤ 0.05, Cohen’s d ≥ 0.89; Fig. 3A). Despite similar reductions in MCAv in both groups, however, there was an immediate and progressive decrease in ScO2 in the LT group only from ~15 mmHg LBNP (P = 0.004, Cohen’s d = 1.8), but no change in ScO2 for the HT group at any level of LBNP up to ~60 mmHg; ScO2 only began to fall below baseline from ~75 mmHg LBNP in HT subjects (P < 0.001,

---

**Table 1. Demographics for subjects with high tolerance and low tolerance to LBNP at baseline**

<table>
<thead>
<tr>
<th></th>
<th>HT</th>
<th>LT</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>LBNP tolerance, s</td>
<td>1996 ± 212</td>
<td>1243 ± 185</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>2/5</td>
<td>5/6</td>
<td>0.64</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26 ± 3</td>
<td>26 ± 3</td>
<td>0.80</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172 ± 10</td>
<td>171 ± 9</td>
<td>0.84</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70 ± 10</td>
<td>77 ± 16</td>
<td>0.34</td>
</tr>
<tr>
<td>Baseline HR, bpm</td>
<td>59.4 ± 2.8</td>
<td>60.4 ± 2.3</td>
<td>0.79</td>
</tr>
<tr>
<td>Baseline MAP, mmHg</td>
<td>96.5 ± 2.2</td>
<td>93.2 ± 2.3</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD for age, height, weight, and means ± SE for all other data. HT, high tolerance; LT, low tolerance; LBNP, lower body negative pressure; HR, heart rate; MAP, mean arterial pressure.
Cohen’s $d = 2.2$ (Fig. 4A). At presyncope, the HT group exhibited greater reductions in MCAv (HT: $-35 \pm 4\%$ vs. LT: $-23 \pm 4\%$, $P = 0.07$, Cohen’s $d = 1.0$), and lower absolute MCAv ($P = 0.06$, Cohen’s $d = 1.2$; Table 2).

Cerebral HbO$_2$ and dHb responses are presented in Fig. 4, B and C. HbO$_2$ was maintained throughout LBNP up to and including $-75$ mmHg LBNP in the HT group, while it started immediately decreasing at $-15$ mmHg LBNP for the LT group and continued to decrease until presyncope. At presyncope, HbO$_2$ had decreased by $\sim 10\%$ in both groups ($P = 0.86$, Cohen’s $d = 0.09$). Cerebral dHb progressively increased for both groups, from $-15$ mmHg LBNP for the LT group ($P = 0.02$, Cohen’s $d = 1.4$), and from $-60$ mmHg LBNP for the HT group ($P \leq 0.001$, Cohen’s $d = 1.9$). At presyncope, the increase in dHb in the HT group was greater than the LT group ($P = 0.03$, Cohen’s $d = 1.5$).

Fig. 3. Middle cerebral artery velocity (MCAv; A) decreased in the high-tolerant (HT, solid line, •) and low-tolerant (LT, dashed line, ○) groups during LBNP. Posterior cerebral artery velocity (PCAv; B) was lower in the LT group vs. HT group at $-30$ and $-45$ mmHg LBNP and did not change from baseline until $-75$ mmHg in the HT group. Over the 1 min prior to presyncope (PS-1), the % change in MCA was greater in the HT (•) vs. LT (○) group, but there was no difference in PCAv at this time point. *$P \leq 0.05$ compared with baseline. †$P = 0.03$, between groups. A two-way repeated-measures ANOVA (within and between HT and LT groups up to $-45$ mmHg LBNP), one-way repeated measures ANOVA (within HT group), and unpaired $t$-test (between HT and LT groups at PS-1) were used for analysis. The $P$ values for each main effect and interaction for the two-way ANOVA are presented.
CEREBRAL BLOOD FLOW AND OXYGENATION AND LBNP TOLERANCE

Table 2. Hemodynamic responses during progressive lower body negative pressure (LBNP) to presyncope in high tolerant (HT) and low tolerant (LT) groups

<table>
<thead>
<tr>
<th>LBNP Level, mmHg</th>
<th>ANOVA P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LBNP HT vs. LT Interaction PS-1 P Value</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SAP, mmHg</th>
<th>0.01</th>
<th>0.04</th>
<th>0.78</th>
<th>95.4 ± 2.6</th>
<th>0.16</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT</td>
<td>129.9 ± 2.9</td>
<td>130.0 ± 2.6</td>
<td>126.4 ± 3.1</td>
<td>122.0 ± 4.3*</td>
<td>116.2 ± 3.3*</td>
</tr>
<tr>
<td>LT</td>
<td>123.7 ± 2.0</td>
<td>124.5 ± 2.6</td>
<td>118.1 ± 1.7†</td>
<td>113.3 ± 2.7*</td>
<td>110.2 ± 2.8</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>74.1 ± 2.1</td>
<td>74.0 ± 2.0</td>
<td>74.0 ± 2.2</td>
<td>73.7 ± 2.1</td>
<td>73.6 ± 2.1</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>73.0 ± 2.0</td>
<td>73.7 ± 1.8</td>
<td>74.2 ± 2.0</td>
<td>72.4 ± 2.8</td>
<td>72.4 ± 2.8</td>
</tr>
<tr>
<td>dHb, %</td>
<td>96.5 ± 2.2</td>
<td>95.8 ± 2.0</td>
<td>94.6 ± 2.2</td>
<td>92.2 ± 2.3*</td>
<td>89.6 ± 2.1*</td>
</tr>
<tr>
<td>MCAv, cm/s</td>
<td>93.3 ± 2.1</td>
<td>93.8 ± 2.1</td>
<td>91.4 ± 1.9</td>
<td>87.9 ± 3.1*</td>
<td>87.9 ± 3.1*</td>
</tr>
<tr>
<td>etCO2, mmHg</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
</tr>
<tr>
<td>CO2, %Δ</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
<td>– – – – –</td>
</tr>
<tr>
<td>HbO2, μM</td>
<td>32.7 ± 3.2</td>
<td>32.0 ± 3.2</td>
<td>32.1 ± 3.1</td>
<td>32.1 ± 2.9</td>
<td>31.9 ± 2.7</td>
</tr>
<tr>
<td>dHb, μM</td>
<td>35.2 ± 2.2</td>
<td>33.5 ± 1.8*</td>
<td>33.6 ± 1.8*</td>
<td>33.2 ± 1.8*</td>
<td>33.2 ± 1.8*</td>
</tr>
<tr>
<td>THC, μM</td>
<td>16.5 ± 1.8</td>
<td>16.5 ± 1.8</td>
<td>16.7 ± 1.7</td>
<td>16.8 ± 1.7</td>
<td>17.2 ± 1.7*</td>
</tr>
<tr>
<td>etCO2, mmHg</td>
<td>49.2 ± 3.7</td>
<td>48.6 ± 3.6</td>
<td>48.7 ± 3.6</td>
<td>48.9 ± 3.5</td>
<td>49.1 ± 3.5</td>
</tr>
<tr>
<td>MCAv, cm/s</td>
<td>52.4 ± 2.9</td>
<td>51.1 ± 2.5*</td>
<td>51.1 ± 2.8*</td>
<td>51.4 ± 3.1*</td>
<td>51.4 ± 3.1*</td>
</tr>
</tbody>
</table>

Data are presented as absolute means ± SE. CO, carbon dioxide; HbO2, oxygenated hemoglobin concentration; etCO2, end tidal carbon dioxide; HbO2, oxygenated hemoglobin concentration; HT, high tolerance; HT, low tolerance; SAP, systolic arterial pressure; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; PCav, posterior cerebral artery velocity; ScO2, cerebral oxygen saturation; SV, stroke volume; THC, total hemoglobin concentration; TPR, total peripheral resistance. The PS-1 time point refers to the 1 min prior to presyncope. *P ≤ 0.05 compared to baseline within a group. †P ≤ 0.04 between HT and LT groups.

Absolute data for the measured variables at each level of LBNP and at presyncope are presented in Table 2.

The respiratory responses to LBNP are presented in Table 2. Respiration rate for both HT and LT subjects was maintained at baseline levels within groups throughout LBNP, but was higher overall in the LT group throughout LBNP up to −45 mmHg (Group main effect, P = 0.01). etCO2 progressively decreased from baseline in both groups, with a difference between groups only evident at −45 mmHg LBNP (P = 0.01, Cohen’s d = 1.2). There were no differences at presyncope between the HT and LT groups for either respiratory rate or etCO2.

DISCUSSION

In this study, we examined the role of regional cerebral blood flow and oxygenation on tolerance to central hypovolemia elicited by continuous application of LBNP to presyncope. The key findings of this study demonstrate that individuals with high tolerance to central hypovolemia 1) exhibit prolonged protection of cerebral tissue oxygen saturation in the frontal lobe despite early reductions in cerebral blood flow (i.e., delivery), 2) show similar reductions in anterior CBF (indexed by mean MCAv) as low tolerant subjects up to −45 mmHg LBNP, but a greater reduction in anterior CBF at presyncope, and 3) have protection of posterior CBF (indexed by mean PCav) at submaximal levels of LBNP. Our data support the hypothesis that individuals with higher tolerance to central hypovolemia appear to have prolonged preservation of CBF in the posterior vs. anterior cerebral circulation and a delayed mismatch in oxygen delivery-demand, resulting in sustained cerebral tissue oxygenation. Combined, these two responses were associated with a delay in the onset of presyncope.

Regional cerebral blood flow. To date, studies assessing CBF responses to maximal central hypovolemia to presyncope have focused primarily on the MCA as a marker of global cerebral blood flow. There is growing evidence, however, that protection of cerebral blood flow through the MCA is not necessarily associated with tolerance to central hypovolemia (20, 27, 28, 37); the findings from the current study support this concept as MCAv responses between HT and LT subjects were...
similar up to the last common level of LBNP, despite LT subjects reaching presyncope at this time point. The present study is one of very few to report CBF responses within the posterior cerebral circulation during central hypovolemia elicited by LBNP, including the PCA or vertebral arteries (VA) (12, 33). Autonomic and respiratory control centers are located within the medulla oblongata in the brain stem, which receives blood and oxygen supply through these posterior cerebral arteries (45). As such, disruption of posterior cerebral flow may be related to the symptoms and hemodynamic dysfunction associated with presyncope during central hypovolemia, such as LBNP or hemorrhage (33). Deegan et al. (12) reported similar responses between mean MCAv and blood flow in the VA during head-up tilt plus LBNP to presyncope, although they combined all 18 subjects into a single group and did not assess potential differences between individuals with varying tolerance to this stress. Most recently, Ogoh et al. (33) examined blood flow responses in the VA feeding the posterior cerebral circulation and internal carotid arteries (ICA) feeding the anterior cerebral circulation up to submaximal LBNP of $-50$ mmHg. On the basis of the significant, although weak, association between the fall in ICA flow and the magnitude of LBNP ($r = 0.29; P = 0.029$) vs. no change in VA flow with LBNP ($r = 0.167; P = 0.22$), these investigators postulated that cerebral perfusion of the posterior regions of the brain would only decrease with severe orthostatic stress, and may be associated with tolerance to central hypovolemia. We were able to explicitly test this hypothesis by exposing all of our subjects to maximal levels of presyncope-limited LBNP, and found that mean PCAv was protected in HT subjects up to $-60$ mmHg LBNP, but decreased progressively in LT subjects. At presyncope, the reduction in mean PCAv was similar between both groups and was coincident with subjective presyncopal symptoms (e.g., dizziness, lightheadedness, blurred vision, and nausea) in most subjects, indicating that hypoperfusion of the posterior regions of the brain is, indeed, associated with tolerance to maximal central hypovolemia.

Cerebral oxygen saturation and extraction. NIRS is often used to measure oxygen saturation in the cerebral tissues, measuring a mixed sample volume of arterial (20%), capillary (5%), and venous blood (75%) (30, 48). At the last common level of LBNP between groups (i.e., $-45$ mmHg LBNP), the LT subjects had a lower ScO2 than the HT group. At presyncope, the HT group had an 8% (range: 3.5–14.8%) reduction in ScO2 compared with 6% (range: 0.9–14.3%) reduction in the LT group. While previous studies have shown that a 10–15% reduction in ScO2 is associated with presyncope (3, 16, 29), we have demonstrated that much smaller reductions in ScO2 can be associated with presyncope.

The brain compensates for reductions in cerebral blood flow by increasing oxygen extraction (determined via assessment of cerebral arterial-venous oxygen difference) (25, 31). Reducing the cerebral blood flow reserve (by indomethacin) prior to exposure to maximal LBNP does not change tolerance, sug-

---

Fig. 4. Percent change from baseline for cerebral oxygen saturation (ScO2; A), oxygenated hemoglobin (Oxy Hb; B), and deoxygenated hemoglobin (Deoxy Hb; C) in the HT (solid line, •) vs. LT (dashed line, ○) group throughout LBNP. The reductions in ScO2 and Oxy Hb were greater in the LT vs. HT group at $-30$ and $-45$ mmHg LBNP, but similar between groups for the final 1 min prior to presyncope (PS-1), represented by solid (HT) and open (LT) triangles. Deoxy Hb only increased from early levels of LBNP in the LT group, and was higher at presyncope (PS-1) in the HT group. *$P \leq 0.02$, compared with baseline. †$P \leq 0.05$ between groups. A two-way repeated-measures ANOVA (within and between HT and LT groups up to $-45$ mmHg LBNP), one-way repeated-measures ANOVA (within HT group), and unpaired $t$-test or Mann-Whitney U-test (for deoxy Hb) (between HT and LT groups at PS-1) were used for analysis. The $P$ values for each main effect and interaction for the two-way ANOVA are presented.
suggested that increases in oxygen extraction would compensate for the decrease in oxygen delivery (25); oxygen extraction was not assessed during LBNP to directly test this hypothesis. While Glaister and Miller (14) reported a decrease in HbO2 and an increase in dHb during LBNP to presyncope, our data are the first, to our knowledge, to demonstrate differential responses of HbO2 and dHb within HT and LT subjects. With a decrease in HbO2 and an increase in dHb on the venous side of the circulation (i.e., NIRS sample volume is 75% venous blood), we interpret this as an increase in extraction of oxygen from the blood into the tissues. Our data show that the LT group had an immediate and progressive increase in oxygen extraction, evidenced by an immediate decrease in HbO2, and an increase in dHb beginning at −15 mmHg LBNP. This increased oxygen extraction was accompanied by a reduction in oxygen delivery (i.e., decreased mean MCAv). In contrast, despite comparable reductions in oxygen delivery as the HT subjects (evidenced by similar decreases in mean MCAv), the HT group exhibited maintenance of HbO2 throughout LBNP, suggesting constant oxygen extraction. Interestingly, despite greater reductions in SV (P = 0.004) and mean MCAv (P = 0.07) at presyncope in the HT group, and the small and highly variable reductions in ScO2, both the HT and LT groups experienced the same maximal reduction in HbO2 (∼10%).

This finding indicates that reductions in cerebral tissue HbO2 concentration may be a more accurate indicator of impending presyncope than cerebral oxygen saturation or CBF through the anterior circulation (i.e., mean MCAv).

There are a number of reasons that may account for the apparent increase in oxygen extraction, including a reduction in oxygen delivery via decreases in CBF and/or hypoxia, and/or increased metabolic demand. A reduction in CBF would increase oxygen extraction (25); with less oxygen available to the tissues, extraction would need to increase to compensate for decreased delivery and to meet metabolic demand. Interestingly, in our study, despite both groups experiencing the same magnitude of mean MCAv reductions up to ∼45 mmHg LBNP, the HT subjects were able to maintain stable tissue oxygen saturation and oxygen extraction (evidenced by constant HbO2). The LT group started to increase oxygen extraction with as little as a 4% reduction in MCAv (at ∼15 mmHg), while the HT group had constant oxygen extraction until their MCAv decreased from baseline by ∼35% (at presyncope). This finding suggests that HT subjects may have more efficient utilization of oxygen until a critical threshold of delivery (i.e., CBF) is reached, at which time, increases in extraction occurred with further reductions in flow.

A reduction in the partial pressure of oxygen and/or oxygen saturation, either due to hypoxia or impaired gas exchange, would also reduce oxygen delivery, eliciting an increase in oxygen extraction to meet metabolic demand. However, arterial oxygen content should be similar between groups as 1) experiments were conducted in a normoxic testing environment, 2) we assume hemoglobin concentration would increase in both groups on the basis of similar reductions in central blood volume and subsequent fluid extravasation (17, 21), and 3) central hypovolemic elicited by LBNP stress does not induce any changes in arterial oxygen saturation or PaO2 (21, 50).

Finally, psychological stress/anxiety and increased neuronal activity may also play a role in the increased oxygen extraction observed in the LT subjects, as well as subsequent oxygen demand and supply mismatch at earlier levels of LBNP. We did not systematically assess anxiety or psychological stress in our subjects; measurements of subjective stress levels and/or stress hormones such as cortisol could further elucidate this effect on tolerance to LBNP. Overall, the HT group only increased oxygen extraction once CBF decreased 35% from baseline, thus contributing to the capacity to tolerate a greater magnitude of central hypovolemia and delay the onset of presyncopal symptoms.

Methodological considerations. While application of LBNP does not mimic all of the responses observed in traumatic hemorrhage (e.g., tissue trauma, pain, and metabolic responses, such as acidosis), this technique allows us to isolate the physiological responses of central hypovolemia without these confounding factors. In addition, although LBNP does not elicit blood cell loss, as seen in actual hemorrhage, it does mimic some of the cardiovascular and cerebral blood flow responses elicited by hemorrhage (17, 21, 36).

We interpret the reduction in NIRS-derived HbO2 and the increase in dHb with LBNP as an increase in oxygen extraction at the level of the cerebral tissues within the frontal lobe. This interpretation should be experimentally quantified during LBNP with direct measures of oxygen extraction via arterial-jugular venous oxygen measurements across the brain, and direct assessment of CBF [e.g., via duplex Doppler ultrasound of the extracranial vessels (46)]. Additionally, NIRS is a noninvasive method to obtain measurements of ScO2, and concentrations of HbO2 and dHb within the cerebral tissue, but it also may be contaminated by changes in oxygenation of the skin (11, 18, 41, 42). For this study, we used a spatially resolved NIRS device with four emitters that were 2.0, 2.5, 3.0, and 3.5 cm from the detector, compared with only two emitters on many other NIRS devices. Theoretically, as each emitter distance samples from a different depth, via mathematical correction, measurements from the extracranial sample volume (i.e., skin, muscle, and fat) can be removed from the final oxygen saturation measurements of the intracranial cerebral tissues. The sensor was also covered with black cloth to reduce contamination from room light, and all sinus cavities were avoided during sensor placement. Furthermore, while preparation of this sensor for use includes a calibration step, this is simply to ensure that the known absorption and scattering coefficients of the calibration block have been detected by the sensor. Systematic calibration against known oxy- and deoxy-hemoglobin concentrations and/or known oxygen saturations is not performed with this device, or any cerebral NIRS device, as far as these authors are aware, and there is not a “gold standard” device for this purpose.

Finally, transcranial Doppler ultrasound is used to assess CBF with the assumption that the diameter of the insonated vessel remains constant. MCA diameter does not change with mild sympathetic activation induced by LBNP up to −40 mmHg (39), which may be comparable to our LT group, but not the HT group, as they reached levels of LBNP ≥ 70 mmHg. During greater levels of LBNP, there is increased sympathetic nerve activity in the periphery (8), but it is unknown whether cerebral sympathetic activity also increases, which could constrict the cerebral vessels. If this did occur, however, CBF would be further decreased relative to the observed reduction in velocity (i.e., we would be understi-
mating the reduction in flow). Recent studies (10, 49) using high-resolution MRI, also suggest that increases in etCO2 ≥ 9 mmHg above baseline elicited MCA vasodilation, while decreases in etCO2 ≥ 13 mmHg below baseline elicited MCA vasoconstriction. In the current study, etCO2 in both groups only fell by ~9 mmHg below baseline, so MCA caliber should be constant. If, however, there was a mild vasoconstrictive effect from this magnitude of hypocapnia, the observed decrease in velocity of blood flowing through the artery of interest would be attenuated, and the subsequent measure of velocity would underestimate the actual reduction in flow. The role of sympathetic activity and variations in arterial CO2 on PCA caliber, however, is unknown.

Conclusions. The novel findings of this study indicate that subjects with increased tolerance to central hypovolemia have prolonged maintenance of cerebral tissue oxygenation (despite reductions in blood flow within the anterior cerebral circulation), and protection of CBF within the posterior cerebral circulation. We postulate that HT subjects maintain relatively constant oxygen metabolism in the brain, thus delaying the onset of presyncope. These findings suggest that the measurement of NIRS-derived oxy-hemoglobin and/or posterior cerebral blood flow may be more sensitive indicators for tracking the onset of presyncope than oxygen saturation or measures of anterior cerebral blood flow and are targets for potential interventions to prolong tolerance to central hypovolemia in the clinical setting.

Perspectives and Significance

Reduced metabolic demand and/or increased oxygen efficiency in the HT group contributes to the delay in the onset of presyncope. This may have important clinical implications for the monitoring of cerebral perfusion and oxygenation in patients, where increased metabolic demand elicited by anxiety, stress, and/or pain may increase cerebral oxygen extraction and reduce tolerance to hypovolemic stress, such as traumatic hemorrhage and orthostasis. Under these conditions, the magnitude of hypovolemia that may induce unconsciousness will depend on individual cerebral metabolic demand; at presyncope, HT subjects had a 63% reduction in SV, compared with a 42% reduction in the LT group. In addition, these data demonstrate the important role of HbO2 on ScO2 calculations, the measurement most often reported when using cerebral NIRS monitoring devices. The HT group had stable ScO2 due to stable HbO2, whereas the LT group had progressively decreasing HbO2, which decreased ScO2. Despite differences in the onset of increasing oxygen extraction during LBNP, we can conclude that an overall 10–11% reduction in HbO2 is associated with an oxygen supply and demand mismatch, and the subsequent appearance of presyncopal symptoms, regardless of whether subjects were HT or LT. Therefore, it may be more applicable to use the measure of HbO2 as a reference point for impending presyncope as opposed to ScO2. Similarly, the finding that reduced PCAv distinguished LT from HT subjects from an early level of central hypovolemia suggests that investigation of the posterior cerebral circulation may provide important insight into the mechanisms of tolerance to hemorrhage, which could be a target for interventions to increase tolerance to this stress. These findings from healthy subjects in the current study require further investigation in clinical populations with orthostatic intolerance.

ACKNOWLEDGMENTS

The authors thank our subjects for their time and cheerful cooperation, Hannah Colby and Justin Sprick for their assistance with data collection and analysis on this project, and Drs. Albert Yurvati and Levi Rice for their assistance with subject medical examinations. The content is solely the responsibility of the authors and does not necessarily represent the official views of the U.S. Department of Defense.

GRANTS

Funding for this study was provided by the U.S. Army MRMC Combat Casualty Care Research Program Grant no. W81XWH-11-2-0137 (C. A. Rickards).

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


