Cerebral oxygenation and regional cerebral perfusion responses with resistance breathing during central hypovolemia

Victoria L. Kay, Justin D. Sprick, and Caroline A. Rickards
Institute for Cardiovascular and Metabolic Diseases, University of North Texas Health Science Center, Fort Worth, Texas

Submitted 9 September 2016; accepted in final form 11 May 2017

Kay VL, Sprick JD, Rickards CA. Cerebral oxygenation and regional cerebral perfusion responses with resistance breathing during central hypovolemia. Am J Physiol Regul Integr Comp Physiol 313: R132–R139, 2017. First published May 24, 2017; doi:10.1152/ajpregu.00385.2016.—Resistance breathing improves tolerance to central hypovolemia induced by lower body negative pressure (LBNP), but this is not related to protection of anterior cerebral blood flow [indexed by mean middle cerebral artery velocity (MCAv)]. We hypothesized that inspiratory resistance breathing improves tolerance to central hypovolemia by maintaining cerebral oxygenation (ScO2), and protecting cerebral blood flow in the posterior cerebral circulation [indexed by posterior cerebral artery velocity (PCAv)]. Eight subjects (4 male/4 female) completed two experimental sessions of a presyncopal-limited LBNP protocol (3 mmHg/min onset rate) with and without (Control) resistance breathing via an impedance threshold device (ITD). ScO2 (via near-infrared spectroscopy), MCAv and PCAv (both via transcranial Doppler ultrasound), and arterial pressure (via finger photoplethysmography) were measured continuously. Hemodynamic responses were analyzed between the Control and ITD condition at baseline (T1) and the time representing 10 s before presyncope in the Control condition (T2). While breathing on the ITD increased LBNP tolerance from 1,506 ± 75 s to 1,704 ± 88 s (P = 0.003), both mean MCAv and mean PCAv were similar between conditions at T2 (P ≥ 0.46), and decreased by the same magnitude with and without ITD breathing (P ≥ 0.53). ScO2 also decreased by ~9% with or without ITD breathing at T2 (P = 0.97), and there were also no differences in deoxygenated (dHb) or oxygenated hemoglobin (HbO2) between conditions at T2 (P ≥ 0.43). There was no evidence that protection of regional cerebral blood velocity (i.e., anterior or posterior cerebral circulation) nor cerebral oxygen extraction played a key role in the determination of tolerance to central hypovolemia with resistance breathing.

Address for reprint requests and other correspondence: C. A. Rickards, Institute for Cardiovascular and Metabolic Diseases, Univ. of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107 (e-mail: caroline.rickards@unthsc.edu).
protect the posterior cerebral circulation feeding the brain stem, the location of autonomic and respiratory control centers (17, 24). Recently, we demonstrated that, indeed, tolerance to central hypovolemia (via LBNP but without resistance breathing) was associated with protection of blood velocity in the posterior cerebral artery (PCAv) and sustained cerebral tissue oxygenation in the frontal cortex (17).

The effect of resistance breathing on regional cerebral perfusion and cerebral oxygenation has not been investigated under any condition. We hypothesized that respiratory resistance breathing would maintain cerebral oxygenation, compensating for reductions in anterior cerebral blood flow (indexed by mean MCAv) and would also protect cerebral perfusion in the posterior cerebral circulation (indexed by PCAv), thus improving tolerance to central hypovolemia.

**METHODS**

**Subjects**

Twenty-seven healthy, normotensive, nonsmoking subjects volunteered to participate in this study, conducted at the University of North Texas Health Science Center in Fort Worth, TX. The experimental protocol was reviewed and approved by the Institutional Review Board at the University of North Texas Health Science Center. Before 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 before each experiment. All female subjects were tested in the early follicular phase of their menstrual cycle (immediately before each experiment. All female subjects were tested in the early follicular phase of their menstrual cycle (immediately before each experiment).

**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. A durable plastic sleeve and neoprene band were wrapped around the subject’s waist to create an airtight seal with the decompression. A durable plastic sleeve and neoprene band were wrapped around the subject’s waist to create an airtight seal with the decompression chamber. The chamber pressure was released rapidly immediately after the experiment 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 and diphenhydramine), alcohol, prescription or nonprescription drugs, and herbal medications for 24 h before the orientation and experimental sessions. Subjects were also instructed to remain hydrated (ad libitum water consumption) and maintain their normal sleep pattern the day before each experiment. 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 (22–24°C).

**Data Analysis**

All continuous waveform data (ECG, arterial pressure, SV, MCAv, PCAv, ScO2, THC, and ETCO2) were collected at 1,000 Hz (PowerLab and LabChart; AD Instruments) 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 pressure were detected from the ECG signal were detected to determine the timing of each cardiac cycle. Beat-to-beat SAP and diastolic arterial pressure were detected from the ECG signal and were used to assess the subject’s baseline tolerance to a presyncopal-limited LBNP protocol. The protocol consisted of a 5-min rest period followed by continuous application of negative pressure at a decompression rate of 3 mmHg/min until the onset of presyncope, 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 presyncopal symptoms generally resolved within 30–60 s. Following LBNP termination, subjects remained in the chamber for a 10-min recovery period. Only the time to presyncope data from the Baseline experiments are reported for the current study; all hemodynamic data are included in separate publications (16, 17).

When subjects returned to the laboratory for their second LBNP exposure, they participated in either the “Control” or “ITD” protocols (randomized, crossover design to account for the possible order effect of the ITD intervention protocol always following the Control protocol) as described in the following sections.

**Control LBNP protocol.** The Control protocol was identical to the Baseline protocol described previously. Subjects were exposed to progressively decreasing LBNP at a rate of 3 mmHg/min until the onset of presyncope symptoms.

**ITD LBNP protocol.** Subjects were exposed to progressively decreasing LBNP at a rate of 3 mmHg/min, but 5 min before the predetermined time of presyncope (determined from the Baseline protocol on day 1), or with a 30% reduction in SV from baseline (whichever came first), the ITD was placed on the facemask, and subjects were instructed to breathe spontaneously at a rate and depth most comfortable to them. Application of LBNP continued at the same rate (3 mmHg/min) until the onset of presyncope symptoms.
CO. Cerebrovascular resistance (CVR) was calculated as MAP divided by MCAv and MAP divided by PCAv.

All data were analyzed from the final 4 min of the rest period before initiation of LBNP during both the Control and ITD experiments and was designated as time “T1.” To examine the hemodynamic effects of resistance breathing via the ITD during LBNP, the time point of presyncope during the Control trial was first identified for each subject (“T2”). Data were then analyzed for both the Control and ITD experiments at this T2 reference time point. All time domain variables were calculated from the 10 s before T2 to capture the dynamic responses of cardiovascular collapse and to examine the potential protective effects of resistance breathing at this time. Respiratory rate was calculated for the 60 s before T2, as 10 s is not sufficient to accurately assess this variable. Data were also compared for the last 60 s before presyncope between the two experiments (PS-1).

Statistical Analysis

A one-way repeated-measures ANOVA was used to compare LBNP tolerance times between the Baseline, Control, and ITD experiments, followed by Tukey post hoc tests. Two-way [factor 1: time (T1 vs. T2); factor 2: experiment (Control vs. ITD)] repeated-measures ANOVAs, followed by Tukey post hoc tests were used for comparison of all hemodynamic variables. Paired t-tests (two-tailed) were used to compare Δ and %Δ responses at T2 and all PS-1 data from the ITD vs. Control experiments. All data are presented as means ± SE (unless otherwise stated), and exact P values are reported for all comparisons.

RESULTS

Subject Selection

Of the 27 subjects who participated in this study, data were analyzed and included from 8 subjects (4 male, 4 female; age: 26 ± 4 yr; height: 170 ± 11 cm; weight: 74 ± 11 kg; means ± SD). Subjects were only included in the final analysis if 1) they reached true presyncope during both experimental conditions (i.e., Control and ITD); 2) adequate “cracking” pressures were reached during ITD breathing (i.e., at least −7 cmH2O); and 3) the difference in tolerance between the two experiments was at least 60 s. First, true presyncope was defined as average SAP <100 mmHg for the 1 min before presyncope and/or minimum SAP ≤90 mmHg within the 1 min before presyncope, as we have previously reported (16, 17); subjects who reported subjective symptoms only without reaching this objective arterial pressure threshold for both experiments were not included (n = 4). Second, as we were investigating the role of resistance breathing on cerebral blood flow and oxygen regulation, we had to ensure that subjects breathing on the ITD consistently reached cracking pressures of at least −7 cmH2O; four subjects did not reach this criterion, likely reflecting a leak in the mask setup. Third, as the ITD was being investigated as an intervention for improving tolerance to central hypovolemia, subjects were only included in the final analysis if tolerance was different between experiments by at least 60 s, as less than 60 s was not considered clinically significant; seven subjects were excluded based on this criterion. Two subjects also exhibited reduced tolerance to LBNP when using the ITD (−95 and −164 s) and were excluded from analysis; unfortunately, this small sample size does not facilitate statistical comparison of these two subjects with the eight subjects who exhibited improved tolerance with ITD breathing. Finally, two subjects were also excluded from analysis as they were not breathing on the ITD during the T2 time, as determined from the Control trial. As ITD placement during the ITD trial was determined by each subject’s Baseline LBNP tolerance, in these two cases, their Control LBNP tolerance was much lower than Baseline, so presyncope occurred before the time that the ITD was placed during the ITD trial. MCAv measurements were obtained on all eight subjects across both experimental conditions, but reliable PCAv measurements were only obtained in four of the eight subjects under both conditions at all time points of interest.

LBNP Tolerance

There was no difference in time to presyncope between the Baseline and Control experiments (P = 0.89). Breathing on the ITD increased LBNP tolerance from the Control experiment (without ITD) from 1,506 ± 75 to 1,704 ± 88 s (P = 0.009), an average of 3 min and 18 s. Tolerance was also increased from the Baseline experiment from 1,480 ± 119 to 1,704 ± 88 s (P = 0.004) when subjects were breathing on the ITD.

Hemodynamic Responses to Resistance Breathing During LBNP (T2 Comparisons)

All of the time domain variables at rest (T1) were similar between the Control and ITD experiments (P ≥ 0.29), except for ETCO2, which was higher for the ITD condition (P = 0.02; Table 1). During the Control experiment, SV, CO, and arterial pressure (SAP, diastolic arterial pressure, and MAP) all decreased during LBNP until the point of presyncope (T2); these decreases were attenuated with resistance breathing (Figs. 1 and 2 and Table 1). Given that HR and SV are both contributing factors to CO, the protection of CO (P = 0.05) is primarily due to SV (P = 0.01) as HR increased to the same degree in both experiments at T2 (Control: 96 ± 11 beats/min vs. ITD: 102 ± 7 beats/min; P = 0.29) (Fig. 2). TPR did not increase with LBMP under either condition (P ≥ 0.28), and there was no difference in TPR between conditions at T2 (P = 0.11; Table 1).

Both absolute mean MCAv and mean PCAv were similar between conditions at T2 (P ≥ 0.46; Fig. 3) and decreased by the same magnitude with and without ITD breathing (P ≥ 0.53) (Table 1). Resistance breathing increased MCAv CVR from baseline to T2 (1.5 ± 0.1 vs. 2.0 ± 0.3 mmHg·cm−1·s−1; P = 0.003), but MCAv CVR did not change from baseline in the Control condition (1.5 ± 0.1 vs. 1.5 ± 0.1 mmHg·cm−1·s−1; P = 0.53); at T2 MCAv CVR was higher in the ITD condition (2.0 ± 0.3 mmHg·cm−1·s−1) compared with the Control condition (1.5 ± 0.1 mmHg·cm−1·s−1; P = 0.003). PCAv CVR did not differ between conditions at T2 (Control: 2.7 ± 0.5 vs. ITD: 3.2 ± 0.1 mmHg·cm−1·s−1; P = 0.23) but did increase from baseline with resistance breathing (T1: 2.5 ± 0.3 vs. T2: 3.2 ± 0.1 mmHg·cm−1·s−1; P = 0.07). ScO2, HbO2, and THC all decreased, and dHb increased for both experiments (P ≤ 0.013; Fig. 4 and Table 1) but were also similar between the Control and ITD conditions at T2 (P ≥ 0.35). ScO2 decreased by −9% from baseline with or without ITD breathing at T2 (P = 0.97). HbO2 decreased by 13 ± 3% in the Control condition at T2 and by 14 ± 4% in the ITD condition (P = 0.69), while dHb increased by 12 ± 2% in the Control condition and by 9 ± 3% in the ITD condition (P = 0.16). While there was a reduction in respiration rate at T2 with ITD breathing...
compared with the Control condition (Control: 15.0 ± 3.0 breaths/min vs. ITD: 11.0 ± 1.7 breaths/min; P = 0.002), the fall in ETCO₂ was not protected with resistance breathing (P = 0.37; Δ from baseline, Table 1).

Responses to Resistance Breathing at Presyncope

When comparing the 60 s before presyncope for both the ITD and Control conditions, MAP, PCAv, and ETCO₂ all fell to similar levels between groups (P ≥ 0.36; Table 2). However, with resistance breathing, there were greater increases in HR (P = 0.01) and greater reductions in SV (P = 0.04), ScO₂ (P = 0.01), HbO₂ (P = 0.09), and MCAv (P = 0.03) compared with the Control condition.

DISCUSSION

We examined the role of cerebral oxygenation and regional cerebral blood flow velocity on tolerance to central hypovolemia with inspiratory resistance breathing. The key findings of this study demonstrate that increased tolerance to central hypovolemia via resistance breathing is associated with maintenance of central blood volume and cerebral perfusion pressure, but there was no evidence for the protection of regional cerebral blood velocity (i.e., anterior or posterior cerebral circulation) nor cerebral oxygen saturation or extraction.

Both hemorrhage and LBNP result in central hypovolemia due to decreased venous return, which leads to reduced SV and CO and a subsequent reduction in arterial pressure (7, 11, 14). The ITD was specifically designed to augment the physiological responses of inspiration by further decreasing ITP thereby causing an increase in venous return, SV, and CO (5, 22). Resistance breathing following severe hemorrhage in pigs has been shown to decrease ICP and right atrial pressure, resulting in increased cerebral perfusion pressure, coronary perfusion pressure, and MAP (31, 34, 35). With increased perfusion to vital organs such as the heart and brain, both acute and 24-h survival were increased following severe hemorrhage in pigs (31). In studies of resistance breathing in healthy, conscious humans, ITD breathing protected SV, CO, and MAP during progressive LBNP, resulting in delayed presyncopal symptoms, and increased tolerance (28, 29). Convertino et al. (3) also demonstrated increased systolic and diastolic blood pressures with inspiratory resistance in patients with hypotension secondary to blood loss or trauma.

The present study is the first to report cerebral blood velocity responses within both the anterior and posterior cerebral circulations and cerebral oxygenation responses with inspiratory resistance breathing during central hypovolemia to presyncope. Consistent with previous studies, we found that resistance breathing during progressive LBNP protects SV, CO, and MAP thus delaying presyncope, while having no effect on HR or cerebral blood velocity in the anterior circulation (indexed by mean MCAv) (28, 29). Moreover, resistance breathing did not protect the fall in cerebral oxygenation in the frontal cortex nor did it protect the reduction in cerebral blood velocity in the posterior circulation (indexed by mean PCAv). In a previous study from our laboratory comparing high versus low tolerant

---

**Table 1. Hemodynamic responses during progressive LBNP to presyncope with (ITD) and without inspiratory resistance breathing (Control)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>P value</th>
<th>T1</th>
<th>T2</th>
<th>P value</th>
<th>T2 vs. T2 P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP, mmHg</td>
<td>128.5 ± 3.2</td>
<td>85.6 ± 3.5</td>
<td>&lt;0.001</td>
<td>131.5 ± 4.7</td>
<td>108.3 ± 3.1</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>74.3 ± 2.5</td>
<td>55.6 ± 4.1</td>
<td>&lt;0.001</td>
<td>74.0 ± 1.8</td>
<td>72.2 ± 2.3</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TPR, mmHg·l⁻¹·min⁻¹</td>
<td>17.5 ± 1.6</td>
<td>16.6 ± 1.7</td>
<td>0.47</td>
<td>17.0 ± 1.1</td>
<td>18.5 ± 2.0</td>
<td>0.28</td>
<td>0.11</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>12.9 ± 1.4</td>
<td>15.0 ± 3.0</td>
<td>0.31</td>
<td>12.5 ± 1.2</td>
<td>11.0 ± 1.7</td>
<td>0.47</td>
<td>0.002</td>
</tr>
<tr>
<td>ETCO₂, mmHg</td>
<td>40.1 ± 2.5</td>
<td>25.7 ± 1.5</td>
<td>0.002</td>
<td>46.2 ± 1.0*</td>
<td>30.0 ± 3.6</td>
<td>&lt;0.001</td>
<td>0.09</td>
</tr>
<tr>
<td>ETCO₂, % from T1</td>
<td>−14.4 ± 3.1</td>
<td>0.002</td>
<td></td>
<td>−16.2 ± 3.5</td>
<td>&lt;0.001</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Mean MCAv, % from T1</td>
<td>−34.2 ± 5.0</td>
<td>&lt;0.001</td>
<td></td>
<td>−30.5 ± 4.4</td>
<td>&lt;0.001</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Mean PCAv, % from T1</td>
<td>−32.3 ± 4.6</td>
<td>0.001</td>
<td></td>
<td>−31.6 ± 4.8</td>
<td>0.002</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Total Hb, μM</td>
<td>52.4 ± 5.8</td>
<td>49.7 ± 5.5</td>
<td>0.01</td>
<td>50.6 ± 5.5</td>
<td>48.0 ± 6.1</td>
<td>0.01</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 8 subjects, except for PCAv where n = 4. LBNP, lower body negative pressure; ITD, impedance threshold device; T1, baseline; T2, absolute time point of presyncope during Control trial; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; TPR, total peripheral resistance; ETCO₂, end-tidal carbon dioxide; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; Total Hb, total hemoglobin. Two-way [time (T1 vs. T2); experiment (Control vs. ITD)] repeated-measures ANOVAs were used for comparison of all hemodynamic variables, followed by Tukey post hoc tests. Paired t-tests were performed for T2 comparisons between trials. *P = 0.02 for T1 vs. T1 time points.
The increase in amplitude of low-frequency MCAv oscillations replicated in the current study. Instead, it was suggested that is not associated with protection of absolute MCAv, a finding breathing-induced increase in tolerance to central hypovolemia representation of cerebral blood flow dynamics. As it is possible that the reduction in velocity is not an accurate MAP and MCAv or PCAv) should be interpreted with caution with this prior investigation as subjects reached a greater magnitude of central hypovolemia while breathing on the ITD. While cerebral blood velocity within both the MCA and PCA were not protected, a number of findings from this study provide indirect evidence that cerebral vessel diameter may be increasing with resistance breathing, challenging the assumption of constant vessel caliber and subsequent equivalence of cerebral blood velocity to cerebral blood flow. Resistance breathing ameliorated the reduction in MAP in the current study and has also been shown to decrease ICP in a number of animal studies (31, 34, 35). Together, protection of MAP and a reduction in ICP should result in protection of cerebral perfusion pressure. If cerebral perfusion pressure is maintained, but cerebral blood velocity is not protected in either the MCA or PCA, this indirectly suggests that the vessel diameter may be increasing, resulting in increased flow but, subsequently, decreased velocity. Alternatively, disparity between responses of cerebral perfusion pressure and cerebral blood flow could indicate increased cerebrovascular resistance; while this would result in a decrease in flow, it would increase velocity, which did not occur. The observed increase in cerebrovascular resistance with ITD breathing (calculated from MAP and MCAv or PCAv) should be interpreted with caution as it is possible that the reduction in velocity is not an accurate representation of cerebral blood flow dynamics.

Rickards et al. (28) previously demonstrated that the ITD breathing-induced increase in tolerance to central hypovolemia is not associated with protection of absolute MCAv, a finding replicated in the current study. Instead, it was suggested that the increase in amplitude of low-frequency MCAv oscillations may elicit a shear-stress mediated vasodilation, with a subsequent increase in flow and oxygen delivery. Unfortunately, the continuous nature of the LBNP stimulus in the present study does not facilitate the accurate assessment of the oscillatory characteristics of cerebral blood velocity via frequency domain analysis (i.e., nonstationarity of the signals). However, ITD breathing was associated with a lower (P = 0.001) respiration rate at T2 (11.0 ± 1.7 breaths/min) compared with the Control condition (15.3 ± 3.0 breaths/min), and findings from a previous study demonstrate an increase in tidal volume with ITD breathing at rest (0.86 vs. 0.67 liters) (5). In addition, Lucas et al. (20) compared the effect of controlled breathing (6 breaths/min; 0.1 Hz) vs. spontaneous breathing (16–20 breaths/min) on tolerance to central hypovolemia (combined head-up tilt and LBNP). Subjects breathing at 0.1 Hz had increased tolerance to central hypovolemia and also exhibited much larger tidal volumes compared with the spontaneous breathing condition (2.2 vs. 1.1 liters) (20). The greater tidal volumes likely represent greater reductions in ITP and ICP, which may further augment the decreases in ITP and ICP elicited by ITD breathing, culminating in further increases in cerebral perfusion pressure, which could play a major role in the observed increase in tolerance to central hypovolemia. It is possible that increasing the depth of breathing has the same physiological effect as increasing the resistance to breathing. Studies assessing tolerance to central hypovolemia with controlled variations in both the depth and frequency of breathing, in addition to direct measures of cerebral blood flow and measurement of vasoactive mediators (e.g., nitric oxide and prostaglandins), could further elucidate this mechanism.

In the current study, we also measured cerebral oxygenation in the frontal cortex using NIRS, a measurement that is not dependent on the assumption of constant vessel caliber. Under both ITD and Control conditions, however, ScO2, THC, HbO2,
and dHb all decreased from baseline to T2, and there were no differences in these responses between the two conditions. By presyncope with ITD breathing, however, HbO2 and ScO2 were both less than in the Control condition, suggesting an increase in oxygen extraction due to greater decreases in cerebral blood flow, likely reflecting the greater magnitude of central hypovolemia (Table 2). These data suggest that ITD breathing did not modify oxygen extraction within the cerebral tissues at T2, reflecting similar metabolic demand. Direct measurement of cerebral blood flow (i.e., oxygen delivery) and cerebral oxygen extraction (via cerebral arterial to venous oxygen difference), however, is necessary to confirm this speculation.

**Methodological Considerations**

Although LBNP does not simulate all the responses observed in traumatic hemorrhage such as pain, tissue trauma, and whole red blood cell loss, this model of inducing central hypovolemia has recently been validated against actual hemorrhage (11, 14, 26) and allows for the isolation of hemodynamic and cerebrovascular responses without the many confounding factors associated with traumatic hemorrhage.

For the measurements of ScO2, HbO2, and dHb within the cerebral tissue, we used the noninvasive NIRS technique. It has been suggested that this method of measurement could be contaminated by flow and oxygenation within the skin (9, 12, 32). To reduce the likelihood of skin contamination in the current study, we used a spatially resolved NIRS sensor with four light emitters (2.0, 2.5, 3.0, and 3.5 cm from the detector). Since multiple emitter distances enable the measurement of HbO2 and dHb from different depths, mathematical corrections can be applied to remove extracranial sample volume contamination (i.e., skin, muscle, and fat). We also speculate that posterior cerebral oxygenation could play a key role in tolerance to central hypovolemia, but we are limited by the capabilities of our NIRS device to only measure frontal lobe oxygenation.

We are also limited by the fact that transcranial Doppler is used to measure cerebral blood velocity only and not actual flow. While Serrador et al. (30) demonstrated with magnetic resonance imaging (3T MRI) that MCA diameter remains unchanged with application of LBNP to −40 mmHg, it is unknown if cerebral vasoconstriction occurs at higher levels of LBNP to the point of tolerance. Other recent studies utilizing higher resolution MRI have indicated that hypercapnia ≥9 mmHg above baseline elicits MCA vasodilation, while hypocapnia to ≥13 mmHg below baseline elicits MCA vasoconstriction.

**Table 2. Hemodynamic responses at the point of presyncope during LBNP in the Control vs. ITD condition**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ITD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>99.0 ± 8.1</td>
<td>121.6 ± 7.6</td>
<td>0.01</td>
</tr>
<tr>
<td>SV, %Δ from T1</td>
<td>−46.1 ± 5.2</td>
<td>−56.4 ± 3.7</td>
<td>0.04</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>77.1 ± 2.7</td>
<td>79.4 ± 2.2</td>
<td>0.40</td>
</tr>
<tr>
<td>TPR, mmHg·l−1·min−1</td>
<td>16.8 ± 1.3</td>
<td>17.3 ± 1.7</td>
<td>0.64</td>
</tr>
<tr>
<td>ScO2, %</td>
<td>61.6 ± 2.6</td>
<td>57.2 ± 3.0</td>
<td>0.01</td>
</tr>
<tr>
<td>HbO2, μM</td>
<td>31.1 ± 3.9</td>
<td>27.3 ± 4.3</td>
<td>0.09</td>
</tr>
<tr>
<td>dHb, μM</td>
<td>19.2 ± 2.3</td>
<td>20.0 ± 2.7</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean MCAv, cm/s</td>
<td>48.9 ± 3.5</td>
<td>41.2 ± 4.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean MCAv, %Δ from T1</td>
<td>−27.8 ± 4.2</td>
<td>−38.4 ± 3.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean PCAv, cm/s</td>
<td>27.4 ± 3.5</td>
<td>24.3 ± 0.9</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean PCAv, %Δ from T1</td>
<td>−27.7 ± 6.5</td>
<td>−39.3 ± 3.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>15.0 ± 3.0</td>
<td>13.5 ± 2.0</td>
<td>0.37</td>
</tr>
<tr>
<td>ETCO2, mmHg</td>
<td>27.7 ± 2.1</td>
<td>25.8 ± 3.3</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 8 subjects, except for PCAv where n = 4. T1, baseline; T2, absolute time point of presyncope during Control trial; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; ScO2, cerebral blood saturation; HbO2, oxygenated hemoglobin concentration; dHb, deoxygenated hemoglobin concentration; ETCO2, end-tidal carbon dioxide. Paired t-tests were performed for this analysis.

---

Fig. 4. Cerebral oxygen saturation (ScO2; A), and oxygenated hemoglobin (HbO2; B) decreased from baseline (T1) to T2 (time point of presyncope in the control condition). Deoxygenated hemoglobin (dHb; C) increased from T1 to T2 within each condition. Two-way repeated-measures ANOVAs (within and between control and ITD conditions) were used for analysis. *P ≤ 0.005 compared with baseline within groups.
estimating actual cerebral blood flow. The magnitude change in cerebral blood velocity via transcranial Doppler may be overestimated actual cerebral blood flow. The magnitude change in ETCO₂ decreased by ≥14 mmHg, suggesting that cerebral vasoconstriction may be taking place and measurements of cerebral blood velocity via transcranial Doppler may be overestimating actual cerebral blood flow. The magnitude change in ETCO₂ was similar between ITD and Control conditions, however, so this potential confounding factor affected both conditions equally.

Finally, while the small number of subjects with PCAv data may limit interpretation of these findings, the homogeneity of these responses within this subset of subjects and the robust statistical comparison provide confidence in these responses, despite the small sample size.

In conclusion, these findings suggest that inspiratory resistance breathing does not improve tolerance to central hypovolemia by protecting cerebral oxygenation to compensate for reductions in anterior cerebral blood velocity (indexed by mean MCAv), nor does resistance breathing protect cerebral blood velocity in posterior cerebral circulation (indexed by PCAv). However, we speculate that improved tolerance to central hypovolemia via resistance breathing may be due, in part, to ITD breathing causing a decrease in respiration rate and an increase in depth of breathing, subsequently decreasing ICP and increasing cerebral perfusion pressure, thus delaying the onset of presyncope.

Perspectives and Significance

The mechanisms through which inspiratory resistance breathing promotes improved tolerance to central hypovolemia remain to be fully elucidated. Findings from this investigation indicate that these mechanisms are not related to a protection of frontal lobe cerebral oxygenation nor the ability to maintain posterior cerebral blood velocity. Based on these findings, future work should seek to examine the absolute cerebral blood flow responses with resistance breathing by direct measurements of extracranial blood flow (i.e., through the internal carotid and vertebral artery) and/or intracranial blood flow and oxygenation via advanced imaging techniques (e.g., functional MRI). Additionally, based on the limitations of cerebral NIRS, which can only be used to indirectly assess frontal lobe oxygenation, valuable insights would be gained by performing arterial and cerebral venous (jugular vein) blood sampling for calculation of oxygen extraction across the whole brain. These techniques would provide a more mechanistic insight into the potential factors responsible for the improved tolerance to central hypovolemia observed with ITD breathing. Importantly, these approaches could detect the protection of cerebral blood flow that results from cerebral vasodilatation, which cannot be captured by the velocity measurements made in this study. Future work should also aim to investigate the effects of resistance breathing on respiratory mechanics (including tidal volume) during central hypovolemia, as an increased tidal volume may improve cerebral perfusion pressure. While the hypotheses proposed in this investigation were not supported, the reported findings provide important insight into future directions assessing mechanisms through which inspiratory resistance breathing manifests its positive effects in the protection of vital organ perfusion. As hemorrhage accounts for 30–40% of traumatic deaths (15), understanding these mechanisms may promote the development of this novel therapy to improve survival.

ACKNOWLEDGMENTS

We thank our subjects for time and cheerful cooperation, Hannah Colby for valuable assistance with data collection and analysis on this project, and Drs. Albert Yurvati and Levi Rice for assistance with subject medical examinations. In addition, we thank Drs. Keith Lurie and Anja Metzger from Advanced Circulatory Systems, Inc. (acquired by Zoll Medical Corp. in 2014) for their in-kind donation of the ITDs used in this study.

GRANTS

Funding for this study was provided by the US Army Medical Research and Material Command Combat Casualty Care Research Program Grant W81XWH-11-2-0137. The content is solely the responsibility of the authors and does not necessarily represent the official views the US Department of Defense.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


