Cerebral blood velocity regulation during progressive blood loss compared with lower body negative pressure in humans

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1Department of Integrative Physiology and Anatomy and Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, Texas; 2Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota; 3US Army Institute of Surgical Research, Fort Sam Houston, Texas; and 4Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota

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Rickards CA, Johnson BD, Harvey RE, Convertino VA, Joyner MJ, Barnes JN. Cerebral blood velocity regulation during progressive blood loss compared with lower body negative pressure in humans. J Appl Physiol 119: 677–685, 2015. First published July 2, 2015; doi:10.1152/japplphysiol.00127.2015.—Lower body negative pressure (LBNP) is often used to simulate blood loss in humans. It is unknown if cerebral blood flow responses to actual blood loss are analogous to simulated blood loss during LBNP. Nine healthy men were studied at baseline, during three levels of LBNP (5 min at −15, −30, and −45 mmHg), and during three levels of blood loss (333, 667, and 1,000 ml). LBNP and blood loss conditions were randomized. Intra-arterial mean arterial pressure (MAP) during LBNP was similar to that during blood loss (P ≥ 0.42). Central venous pressure (2.8 ± 0.7 vs. 4.0 ± 0.8, 1.2 ± 0.6 vs. 3.5 ± 0.8, and 0.2 ± 0.9 vs. 2.1 ± 0.9 mmHg for levels 1, 2, and 3, respectively, P ≤ 0.003) and stroke volume (71 ± 4 vs. 80 ± 3, 60 ± 3 vs. 74 ± 3, and 51 ± 2 vs. 68 ± 4 ml for levels 1, 2, and 3, respectively, P ≤ 0.002) were lower during LBNP than blood loss. Despite differences in central venous pressure, middle cerebral artery velocity (MCAv) and cerebrovascular conductance were similar between LBNP and blood loss at each level (MCAv at level 3: 62 ± 6 vs. 66 ± 5 cm·s⁻¹, P = 0.37; cerebrovascular conductance at level 3: 0.72 ± 0.05 vs. 0.73 ± 0.05 cm·s⁻¹·mmHg⁻¹, P = 0.53). While the slope of the MAP-MCAv relationship was slightly different between LBNP and blood loss (0.41 ± 0.03 and 0.66 ± 0.04 cm·s⁻¹·mmHg⁻¹, respectively, P = 0.05), time domain gain between MAP and MCAv at maximal LBNP/blood loss (P = 0.23) and low-frequency MAP-mean MCAv transfer function coherence, gain, and phase were similar (P ≥ 0.10). Our results suggest that cerebral hemodynamic responses to LBNP to −45 mmHg and blood loss up to 1,000 ml follow a similar trajectory, and the arterial pressure-cerebral blood velocity relationship is not altered from baseline under these conditions.

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METHODS

Subjects

Nine healthy men (age 31 ± 6 yr, height 183 ± 7 cm, weight 89 ± 9 kg, body mass index 26.7 ± 1.8 kg/m²) were recruited for the study. These subjects were a subset of the 12 subjects who participated in another study focused on hemodynamic and hormonal responses to this protocol (25). All subjects reported that they were free of cardiovascular, respiratory, neurological, or metabolic disease. Subjects were nonobese (body mass index <30 kg/m²), were nonsmokers, and were not taking medication. Prior to the study day, all subjects provided written informed consent after all procedures and risks of the study were fully explained; the study was approved by the Mayo Clinic Institutional Review Board. Subjects reported to the Clinical Research Unit at the Mayo Clinic at 0700 following an overnight fast. At this time, each subject consumed a small breakfast bar (Clif Bar, Shelton, CT; 240 kcal) and drank 250 ml of water. Subjects were studied in the supine position in a temperature-controlled (20–22°C) room. To ensure subject safety, a board-certified anesthesiologist was present throughout the study day, and a member of the Mayo Clinic autologous transfusion team was in attendance during the protocol.

Experimental Design

LBNP and blood loss protocols were performed on the same day in a counterbalanced order. Figure 1 illustrates the study protocol. The goal of the experimental design was to elicit a wide range of CVP in both protocols. On the basis of approximations for comparing LBNP levels with blood loss (13), we chose the initial stages of the US Army Institute for Surgical Research LBNP protocol (−15, −30, and −45 mmHg chamber pressure) and stepwise reductions in blood volume that would closely mirror CVP at each stage (three 333-ml aliquots of blood). Because the order of the protocols was mixed, we were unable to closely match CVP values between LBNP and blood loss, as described by Hinojosa-Laborde et al. in their baboon study, where LBNP always followed blood loss (21). Either protocol was terminated early if J) MAP fell by 30% compared with baseline MAP, 2) systolic blood pressure dropped below 80 mmHg, or 3) the subject began to experience symptoms of presyncope or syncope. Hematocrit was measured from arterial blood samples collected during the baseline period and at the termination of each experimental protocol.

Measurements and Procedures

Hemodynamic monitoring. Subjects were positioned in the supine posture on an adjustable bed. A three-lead electrocardiogram was used to continuously record HR (Cardiocap/5, Datex-Ohmeda, Louisville, CO). Arterial oxygen saturation was monitored using a finger pulse oximeter, and end-tidal CO₂ (ETCO₂) was collected from a nasal cannula (Cardiocap/5). A 20-gauge, 5-cm catheter was placed into the brachial artery under local anesthesia (2% lidocaine) using aseptic techniques and ultrasound guidance. The catheter was attached to a high-resolution transducer positioned at heart level to obtain continuous brachial arterial pressure waveforms. Continuous hemodynamic, oxygen saturation, and ETCO₂ traces were interfaced with a data acquisition system for offline analysis (WinDaq, DATAQ Instruments, Akron, OH).

Cerebral blood velocity. Subjects were imaged using a 2-MHz transcranial Doppler (TCD) probe (Neurovision System, Multigon, Yonkers, NY) to estimate MCAv. The basal portion of the left MCA was insonated by placement of the probe over the temporal bone just above the zygomatic arch in front of the ear. The Doppler signal was optimized by variation of the sample volume depth in incremental steps and variation of the angle of insonation to obtain the best-quality signal. Once the optimal signal was determined, the probe was secured with a headband device to maintain a constant angle throughout the protocol.

Central venous pressure. A 16-gauge central catheter was introduced into an antecubital vein under local anesthesia (2% lidocaine) using aseptic techniques and advanced to the superior vena cava prior to its junction with the right atrium. This catheter was connected to a high-resolution transducer (FloTrac, Edwards Lifesciences, Irvine, CA) positioned at heart level and interfaced with a personal computer for continuous measurement of CVP. Correct placement of the peripherally inserted central catheter was visually confirmed by two anesthesiologists using the CVP waveform.

Blood removal. A 14-gauge catheter was placed in an antecubital vein to facilitate blood removal for the blood loss protocol. The catheter was placed under local anesthesia (2% lidocaine) using aseptic techniques. Bags containing preservative/anticoagulant (63 ml of anticoagulant citrate phosphate dextrose solution) were placed below the level of the bed to allow transfer of blood from the subject to the blood collection bags via gravity. In two subjects, a blood pressure cuff was inflated around the upper arm to 40 mmHg to enhance the rate of blood removal; this cuff pressure was released during all subsequent hemodynamic measurements. As blood was being collected, it was weighed to determine the volume of blood removed by multiplying the weight of the blood by a factor of 1.06 ml/g. The removed blood was kept in the study room (20–22°C), the temperature of the blood was allowed to fluctuate, and the collection bags were periodically agitated to prevent clotting.

Blood loss protocol. After a 5-min baseline period, three 333-ml aliquots of blood were removed as described above. Measurements were made for 5 min after each aliquot. Subjects were not allowed to cross their legs and were instructed to refrain from contracting lower body muscles throughout the protocol. At the end of the protocol, all shed blood was reinfused at a rate of 20 ml/min into the antecubital vein. Subjects rested quietly in the supine position for 45–75 min between protocols.

LBNP protocol. Subjects were supine in an airtight LBNP chamber that was sealed at the iliac crest and covered the lower body. The LBNP protocol was based on the first three stages of a commonly used

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**Fig. 1.** Study protocol. Lower body negative pressure (LBNP) and blood loss conditions were counterbalanced. Duration of the rest period between LBNP and blood loss depended on which protocol was performed first, with more time required after the blood loss protocol.

**Table:**

<table>
<thead>
<tr>
<th>LBNP Protocol</th>
<th>Blood Loss Protocol</th>
</tr>
</thead>
</table>
| Baseline | 45 min
| 15 | 75 min rest | 333 |
| -30 | -667 |
| -45 | -1000 |

**Level (mmHg)**

**Level (mL)**

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protocol (8–10, 20, 41, 42) consisting of a 5-min baseline period followed by 5 min at −15, −30, and −45 mmHg of chamber decompression. Subjects were not allowed to cross their legs and were instructed to refrain from contracting lower body muscles throughout the protocol.

Data and Statistical Analysis

Data were collected at 500 Hz (WinDaq) and stored on a laboratory computer for offline analysis with signal-processing software (WinCPRS, Absolute Aliens, Turku, Finland). All variables of interest (HR, blood pressure, CVP, ETCO2, and MCAv) were continuously monitored throughout both protocols, and data were analyzed and averaged over the last 3 min of each stage for statistical analysis. MAP and mean MCAv were calculated as the area under the arterial pressure and MCAv curves. SV was calculated using specialized analysis software (WinCPRS) based on the brachial arterial pressure waveform (23). CO was derived using the calculated SV and HR obtained by electrocardiogram. The HR, MAP, SV, CO, and CVP responses for 12 subjects are presented elsewhere (25). Cerebrovascular conductance (CVC) was calculated as MCAv/MAP. The gain between changes in mean MCAv and MAP was calculated to assess arterial pressure-cerebral blood velocity relationships in the time domain at the maximal level of LBNP/blood loss for each subject. Arterial pressure-cerebral blood velocity relationships were also explored via transfer function analysis. Beat-to-beat time domain MAP and mean MCAv waveforms were processed with a fast Fourier transform. Data were made equidistant by linear interpolation and resampled at 5 Hz. Data were then passed through a low-pass filter with a cutoff frequency of 0.5 Hz. Three-minute data sets were fast Fourier-transformed with a Hanning window to obtain power spectra. Spectral power was expressed as the integrated area within the very-low-frequency (VLF) range of 0.004–0.04 Hz and low-frequency (LF) range of 0.04–0.15 Hz. We calculated the coherence between MAP and mean MCAv by dividing the squared cross-spectral densities of the two signals by the product of the individual autospectra. Transfer function gain and phase between MAP and mean MCAv represent a frequency dependence and can be used to assess dynamic cerebral blood flow-pressure relationships (17, 54). Transfer function gain and phase were considered valid and averaged in the VLF and LF ranges only when coherence values were ≥0.5.

To explore the relationships between the physiological responses from the two protocols, the amalgamated $r^2$ value was calculated using linear regression analysis for each variable of interest (SV and CVP) for blood loss vs. LBNP, as described by Johnson et al. (25). Linear mixed-effect model analysis with repeated measures was used to assess the mean MCAv-MAP relationship across LBNP and blood loss, as described by Johnson et al. (25). The interaction effect was detected, Tukey’s post hoc analyses were performed to determine where differences existed. Paired $t$-tests were used to compare hematocrit responses within the LBNP or hemorrhage protocols and maximal MAP-mean MCAv gain responses between conditions. Group data are presented as means ± SE, unless otherwise stated. Exact $P$ values are reported.

RESULTS

All nine subjects performed both trials. Due to presyncopal symptoms, one subject did not complete the last level of LBNP, one subject did not complete the last level of blood loss, and one subject did not complete the last level of either trial.

Table 1. Physiological responses to LBNP and blood loss

<table>
<thead>
<tr>
<th>Hypovolemic Stress</th>
<th>Baseline</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBNP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood loss, ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>94 ± 3</td>
<td>91 ± 3</td>
<td>87 ± 5</td>
<td>86 ± 4†</td>
</tr>
<tr>
<td>Blood loss</td>
<td>93 ± 3</td>
<td>92 ± 3</td>
<td>90 ± 3</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>7.4 ± 0.9</td>
<td>2.8 ± 0.7†</td>
<td>1.2 ± 0.6†</td>
<td>0.2 ± 0.9†</td>
</tr>
<tr>
<td>Blood loss</td>
<td>6.5 ± 0.8</td>
<td>4.0 ± 0.8†</td>
<td>3.5 ± 0.8†</td>
<td>2.1 ± 0.9†</td>
</tr>
<tr>
<td>SV, ml</td>
<td>81 ± 4</td>
<td>71 ± 4†</td>
<td>60 ± 3†</td>
<td>51 ± 2†</td>
</tr>
<tr>
<td>Blood loss</td>
<td>85 ± 5</td>
<td>80 ± 3*</td>
<td>74 ± 3*</td>
<td>68 ± 4*†</td>
</tr>
<tr>
<td>LBNP</td>
<td>57 ± 3</td>
<td>60 ± 2</td>
<td>67 ± 3†</td>
<td>76 ± 4†</td>
</tr>
<tr>
<td>Blood loss</td>
<td>57 ± 3</td>
<td>58 ± 2</td>
<td>61 ± 2*</td>
<td>65 ± 3*†</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.6 ± 0.3</td>
<td>4.2 ± 0.2†</td>
<td>3.9 ± 0.2†</td>
<td>3.8 ± 0.2†</td>
</tr>
<tr>
<td>Blood loss</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.3*</td>
<td>4.5 ± 0.2*</td>
<td>4.4 ± 0.3*</td>
</tr>
<tr>
<td>Mean MCAv, cm/s</td>
<td>70.0 ± 4.2</td>
<td>69.3 ± 4.3</td>
<td>65.2 ± 4.3</td>
<td>61.5 ± 5.8†</td>
</tr>
<tr>
<td>Blood loss</td>
<td>69.5 ± 5.1</td>
<td>69.6 ± 5.3</td>
<td>67.7 ± 5.0</td>
<td>66.5 ± 5.2</td>
</tr>
<tr>
<td>CVC, cm·s⁻¹·mmHg⁻¹</td>
<td>0.75 ± 0.04</td>
<td>0.77 ± 0.05</td>
<td>0.76 ± 0.05</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Blood loss</td>
<td>0.75 ± 0.04</td>
<td>0.75 ± 0.05</td>
<td>0.75 ± 0.04</td>
<td>0.73 ± 0.05</td>
</tr>
<tr>
<td>ETCO₂, mmHg</td>
<td>40 ± 2</td>
<td>40 ± 2</td>
<td>39 ± 2</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Blood loss</td>
<td>41 ± 2</td>
<td>40 ± 2</td>
<td>39 ± 2</td>
<td>38 ± 3†</td>
</tr>
<tr>
<td>Respiration rate, $n$</td>
<td>15 ± 1</td>
<td>13 ± 1†</td>
<td>13 ± 1†</td>
<td>14 ± 1†</td>
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<tr>
<td>Blood loss</td>
<td>13 ± 1*</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data are calculated from the final 3-min of each level. LBNP, lower body negative pressure; MAP, mean arterial pressure; CVP, central venous pressure; SV, stroke volume; HR, heart rate; CO, cardiac output; MCAv, middle cerebral artery velocity; CVC, cerebral vascular conductance; ETCO₂, end-tidal CO₂. *$P < 0.05$ vs. LBNP at the same level. †$P < 0.05$ vs. baseline of the same protocol.

The mean time for blood removal was 563 ± 49 s for the first 333-ml aliquot, 489 ± 56 s for the second 333-ml aliquot, and 467 ± 73 s for the final 333-ml aliquot ($P = 0.195$). Hematocrit increased with LBNP (from 40.6 ± 0.9% at baseline to 41.9 ± 0.9% at termination, $P = 0.020$) and decreased with hemorrhage (from 40.8 ± 0.9% at baseline to 39.7 ± 0.9% at termination, $P = 0.001$). Hemodynamic responses are shown in Table 1. MAP decreased between baseline and level 3 only during the LBNP trial ($P = 0.001$). There were no differences in MAP between LBNP and blood loss trials at any level ($P ≥ 0.42$). At each level, CVP decreased below baseline in both LBNP and blood loss protocols, but values were consistently higher during blood loss than LBNP ($P ≤ 0.003$). During the LBNP trial, SV and CO were lower than baseline at every level, but for the blood loss trial SV was reduced during levels 2 and 3 only and CO did not decrease below baseline values. Consistent with the CVP responses, SV and CO were higher during the blood loss than the LBNP trial at each level of the protocol except baseline. HR was higher than baseline for levels 2 and 3 of LBNP and during level 3 of blood loss; in response to the greater reduction in central blood volume, HR was higher during levels 2 and 3 of the LBNP trial compared with the blood loss trial. The CVP and SV responses during LBNP and blood loss trials are presented in Fig. 2; both amalgamated $r^2$ values were ≥0.80, but the slopes were <0.6.
reflecting the differences in central blood volume reduction between conditions.

Cerebral blood velocity and CVC responses to LBNP and blood loss are shown in Table 1. Mean MCAv decreased by 11 ± 3% and 3 ± 4% for the LBNP and blood loss protocols (P = 0.44) but was statistically distinguishable from baseline at the final level of the LBNP protocol only (P = 0.002). CVC did not change, and responses were similar between LBNP and blood loss trials (P ≥ 0.47). ETCO₂ decreased at level 3 for the blood loss trial only, and respiration rate decreased for the LBNP trial only.

Individual mean MCAv and MAP responses are presented in Fig. 3. There was intersubject variability in these responses, and as a group, the slope of the line between MCAv and MAP was lower with LBNP than blood loss (0.41 ± 0.03 vs. 0.66 ± 0.04 cm·s⁻¹·mmHg⁻¹, P = 0.05). The time domain gain between maximal changes in mean MCAv and MAP was similar between LBNP and blood loss (1.2 ± 0.2 vs. 4.3 ± 2.4 cm·s⁻¹·mmHg⁻¹, P = 0.23). LF and VLF power spectral density for MAP and mean MCAv are shown in Fig. 4. There were no differences from baseline (P ≥ 0.13) in power spectral density for MAP LF and VLF or for MCAv LF and VLF in either trial or in these responses between the LBNP and blood loss conditions (P ≥ 0.23). Similarly, there was no effect of condition or level for MAP-MCAv LF coherence, gain, or phase (P ≥ 0.10; Fig. 5). Because VLF coherence was consistently <0.5 for both conditions across all levels, phase and gain are not reported.

**DISCUSSION**

This is the first study to systematically compare cerebral blood velocity responses during LBNP and actual hemorrhage in healthy human subjects. The key findings from this investigation are that 1) LBNP up to −45 mmHg elicited greater reductions in central blood volume than hemorrhage up to −1,000 ml (as indicated by comparisons of SV, CO, and CVP); 2) the subsequent cerebral blood velocity responses reflected these differences in central blood volume, but the trajectories of the cerebral blood velocity and CVC responses were similar during LBNP and blood loss; and 3) neither the LBNP nor the blood loss protocol induced changes in the MAP-mean MCAv relationship as determined by gain calculations in the time domain and via transfer function analysis.

In 1940, Ebert and Stead (16) reported the sequestration of ~15% of total blood volume into the extremities (2 legs and 1 arm) following rapid application of tourniquets as a potential alternative to phlebotomy for treatment of congestive heart failure. Over 20 years later, a number of investigators introduced LBNP as a method to further decrease central blood volume to simulate the cardiovascular effects of hemorrhage and orthostasis (6, 46). Direct comparison of the hemodynamic responses to LBNP and removal of 450 ml of blood (i.e., 1 unit) from human volunteers suggested equivalency between 1 unit of blood loss and −10 to −20 mmHg LBNP determined by reductions in CVP (39) and SV (19) and subsequent reflex increases in sympathetic nerve activity (39). Recently, studies comparing the cardiovascular and neurohumoral responses to LBNP and blood loss >1 unit (i.e., >500 ml) were performed in baboons (21) and humans (25). Based on the results reported by Hinojosa-Laborde et al. (21), LBNP elicits a reduction in central blood volume (indexed by SV) of −0.25 ml·kg⁻¹·mmHg LBNP⁻¹, equating to blood loss of −450, 1,000, and 1,600 ml with LBNP of −30, −60, and −90 mmHg in a 70-kg human.

While protection of cerebral perfusion and oxygenation is essential for maintaining consciousness under hypotensive conditions of actual or simulated hemorrhage, few studies have measured these responses to actual blood loss, and none have compared responses between blood loss and LBNP. In studies assessing cerebral oxygen saturation responses (via NIRS) to blood loss of ≤500 ml, Colier et al. (7) and Torella et al. (48) reported increases in deoxyhemoglobin concentration and decreases in oxyhemoglobin concentration and cerebral oxygen saturation. As NIRS measures a sample volume consisting of ~25% arterial and 75% venous blood (33, 38), decreases in oxyhemoglobin and increases in deoxyhemoglobin suggest an increase in oxygen extraction, most likely to compensate for reduced blood flow supplying the cerebral tissues; measures of cerebral blood flow (or velocity), however, were not reported in either of these investigations. The current study is the first, to our knowledge, to report cerebral blood velocity responses to actual hemorrhage (up to −1,000 ml) in humans and to compare these responses with LBNP. As reported for a larger group of subjects (n = 12) (25), greater reductions in central blood volume are elicited by LBNP to −45 mmHg than by 1,000 ml of blood loss. As a consequence, mean MCAv was reduced by −11% with LBNP compared with −3% with blood loss, MAP decreased by −8% with LBNP and −2% with blood loss, and the gain between mean MCAv and MAP was lower for LBNP compared with blood loss (Fig. 3). We speculate that continued blood loss would eventually elicit similar cerebral blood velocity responses between conditions. Based on the cerebral blood velocity data presented in Table 1
and Fig. 3 and the hemodynamic data presented by Johnson et al. (25), blood loss of 1,000 ml implemented in the present protocol appears to be equivalent to LBNP of \( \frac{-15}{-30} \) mmHg. This is in contrast to estimations using SV responses from baboons exposed to both LBNP and hemorrhage (0.25 ml·kg\(^{-1}\)·mmHg\(^{-1}\)) (21), where \(-45\) mmHg LBNP would be equivalent to 1,000 ml of blood loss in the subjects used in the present investigation (i.e., \( \sim 90\) kg body wt). Prospective matching of both CVP responses and the time course of blood withdrawal and LBNP exposure between the two protocols, as described by Hinojosa-Laborde et al. (21), may address these differences in central hypovolemia observed in the current investigation and allow more accurate calculations of equivalency.

LF oscillatory power for both MAP and mean MCAv did not change from baseline during LBNP or blood loss. The stability of MAP LF was unexpected based on previously observed increases in MAP LF with LBNP of similar magnitude and duration (4, 5, 41, 55). LF oscillations in arterial pressure are primarily modulated by the baroreflex, evidenced by a strong association with LF power in muscle sympathetic nerve activity (MSNA), which in turn, is related to higher absolute MSNA (12, 27). As such, baroreflex-mediated sympathoexcitation with LBNP-induced hypotension increases MSNA and LF power in both MSNA and arterial pressure (12). The very mild reductions in MAP (\(-8\) and \(-2\) mmHg) by the final level of LBNP and blood loss in the current study may not have been sufficient to

\[
\begin{align*}
#1 & : y = 1.42x - 51.4 \\
R^2 & = 0.66 \\
#2 & : y = 0.39x + 36.4 \\
R^2 & = 0.40 \\
#3 & : y = 0.69x + 22.2 \\
R^2 & = 0.99 \\
#4 & : y = 0.94x - 34.7 \\
R^2 & = 0.79 \\
#5 & : y = 1.66x - 67.1 \\
R^2 & = 0.98 \\
#6 & : y = 0.64x + 11.9 \\
R^2 & = 0.96 \\
#7 & : y = 0.89x - 15.1 \\
R^2 & = 0.97 \\
#8 & : y = 0.85x + 0.32 \\
R^2 & = 0.65 \\
#9 & : y = 0.19x + 27.6 \\
R^2 & = 0.97
\end{align*}
\]
elicit increases in MSNA; hence, there was no increase in MSNA LF or, subsequently, MAP LF. This speculation is supported, in part, by an increase in circulating norepinephrine with LBNP, but not with blood loss, as reported by Johnson et al. (25). The small subject number, combined with high intersubject variability in MAP LF responses under both protocols, also contributes to this finding. As oscillations in arterial pressure are the primary factor driv-

Fig. 4. Low-frequency (LF) and very-low-frequency (VLF) power spectral density for MAP (A and C) and mean MCAv (B and D) during LBNP and blood loss. Values are means ± SE.

Fig. 5. LF coherence, phase, and gain between MAP and mean MCAv during LBNP and blood loss. Values are means ± SE.
Assessment of the arterial pressure-cerebral blood velocity relationship via transfer function analysis in the VLF and LF ranges has been interpreted as an index of cerebral autoregulation (54). The low coherence between MAP and mean MCAv in the VLF range (<0.5) across time and condition indicates an independence of flow from pressure within this frequency range (54). While coherence between MAP and mean MCAv was consistently >0.5 in the LF range, transfer function gain and phase did not change with LBNP or blood loss and were not different between conditions. In contrast, other studies show a reduction (41) or an increase (55) in MAP-mean MCAv gain during LBNP of similar magnitude. In particular, Zhang et al. (55) suggested that simultaneous increases in the magnitude of oscillations in arterial pressure and cerebral blood velocity and the subsequent increase in MAP-mean MCAv gain represented attenuated cerebral autoregulation that may, in turn, predispose individuals to presyncope. The stability of MAP-mean MCAv gain and phase reported in the current investigation is most likely associated with the stability of MAP and mean MCAv LF oscillations and the high intersubject variability inherent in transfer function estimates of cerebral pressureflow relationships, further compounded by the small sample size utilized in this study. In the time domain, cerebral autoregulation can also be assessed as the gain between changes in arterial pressure and cerebral blood velocity (36, 40); in the present study this relationship was not altered under either condition and was not statistically distinguishable between conditions. Together, these data suggest that cerebral pressureflow relationships across multiple time scales (fast component via transfer function analysis and slow component via time domain analysis) were not affected by the magnitude of central hypovolemia induced by LBNP or blood loss. Other factors, including small reductions in arterial CO2 and increased sympathetic drive, may also contribute to the small decrease in MCAv with LBNP and blood loss.

Methodological Considerations

Many of the key methodological considerations associated with the design of this study have been addressed by Johnson et al. (25), including removal of absolute blood volumes (i.e., 333, 666, and 1,000 ml) rather than a percentage of total blood volume, the inability to match CVP responses due to the random order of the protocols, restricting exposure to LBNP and blood loss to submaximal levels, differences in the time course of blood removal vs. LBNP exposure, and inclusion of only male subjects. There are some additional issues specific to the data included in this study that should be considered.

As we used TCD for assessment of cerebral blood velocity within the MCA, we assume that the measurement of velocity is equivalent to flow as long as the caliper of the MCA does not change over the course of the intervention. While recent studies have indicated changes in MCA cross-sectional area with both increases (ETCO2 ≥9 mmHg above baseline) and decreases (ETCO2 ≤13 mmHg below baseline) in arterial CO2 (14, 49), the magnitude of hypocapnia induced with both LBNP and blood loss in the current investigation (2–3 mmHg below baseline) was well above these limits. Additionally, sympathoexcitation with the hypotensive stimuli of both LBNP and blood loss could result in cerebral vasoconstriction, which may also invalidate the assumption of constant arterial diameter. MCA diameter is constant, however, with LBNP up to −40 mmHg (44), and the mild hypotensive stimulus elicited with both LBNP and blood loss in the current study renders this limitation unlikely. Future assessment of cerebral blood flow of the extracranial feeding arteries (e.g., internal carotid artery and vertebral artery) (22, 37, 43, 52) and/or use of transcranial color-coded Doppler ultrasound (34, 51) during this type of investigation would allow for direct assessment of cerebral blood flow without reliance on the assumption of constant arterial caliper. Furthermore, recent investigations have revealed potential regional differences in cerebral blood flow regulation, where the posterior circulation may be more sensitive to hypotension and hypocapnia than the anterior circulation (31), indicating inclusion of these measurements in future studies.

While maintenance of cerebral blood flow is crucial for the delivery of oxygen to the cerebral tissues, the ability of the brain to extract and utilize this oxygen may be of greater importance. This issue has been highlighted by a number of studies demonstrating that protection of absolute cerebral blood flow (or velocity) does not necessarily provide insight into tolerance to central hypovolemia (24, 30, 32, 41). Cerebral oxygenation, oxygen extraction, and/or cerebral oxygen metabolism measurements would be valuable additions to comparisons of LBNP and hemorrhage to address this important issue.

Conclusion

The findings from the present investigation indicate that cerebral blood velocity responses to central hypovolemia induced by LBNP to −45 mmHg and actual blood loss up to 1,000 ml follow a similar trajectory, and the arterial pressure-cerebral blood velocity relationship is not altered under these conditions. Careful matching of the magnitude of central hypovolemia (e.g., via CVP) and the time course of blood loss vs. LBNP exposure and inclusion of additional cerebral blood flow and oxygenation measurements in future studies will facilitate a more comprehensive understanding of these responses. This study represents an important step in understanding cerebral blood flow responses to hemorrhage and provides evidence for the continued use of LBNP as a model of hemorrhage in healthy, conscious volunteer subjects.

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AUTHOR CONTRIBUTIONS

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