AWARD NUMBER: W81XWH-13-2-0038

TITLE: Effects of Simulated Pathophysiology on the Performance of a Decision Support Medical Monitoring System for Early Detection of Hemodynamic Decompensation in Humans

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REPORT DATE: February 2017

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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### 14. ABSTRACT

A high fraction of both battlefield and civilian trauma deaths are caused by hemorrhage and subsequent cardiovascular collapse. It is estimated that ~85% of such deaths are potentially preventable with adequate detection and intervention. However, early detection of hemorrhage and proper intervention is confounded by physiological compensatory mechanisms that can keep blood pressure and heart rate in or near normal range during blood loss of up to 30% of total blood volume. These mechanisms limit the ability of care providers to detect the imminent risk of life threatening cardiovascular collapse with traditional vital signs. In this context, machine learning algorithms developed by the U.S. Army Institute of Surgical Research, using hemorrhage simulated by lower body negative pressure, have shown significant promise in detecting subtle changes in vital signs and estimating changes in cardiac output and blood volume. These tools are currently being validated via collaborative research between the Mayo Clinic Department of Anesthesiology and the U.S. Army Institute of Surgical Research along with several industry partners. In this context, the goal of this application is to extend the pre-clinical validation of the U.S. Army Institute of Surgical Research decision support algorithm for blood loss to incorporate simulated pathophysiological conditions likely to be encountered during combat casualty care. These conditions include: 1) mild hypoxia to simulate altitude or pulmonary/chest wall injury, 2) epinephrine infusions to simulate hyperdynamic circulatory response to stress, 3) blood loss and resuscitation with hypertonic saline, and 4) endotoxin administration to simulate the onset of sepsis. Using these approaches, and leveraging the skills of the strong collaborative team, we will be in a position to further refine and validate the decision support algorithm for blood loss during concurrent pathophysiological conditions likely to be encountered on the battlefield.

### 15. SUBJECT TERMS

- Blood Loss; decision support; resuscitation
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Introduction

This report details results over the entire research period of a study entitled "Effects of Simulated Pathophysiology on the Performance of a Decision Support Medical Monitoring System for Early Detection of Hemodynamic Decompensation in Humans." This monitoring technology was developed based on a simulated hemorrhage model using LBNP. Over the last three years we have published four papers related to the use of LBNP as a non-invasive surrogate to study the hemodynamic effects of actual blood loss and the physiological responses to these maneuvers.


These papers were published in Experimental Physiology, Journal of Applied Physiology, and American Journal of Physiology-Heart and Circulatory Physiology and are included as an additional attachment to this report.
Keywords

Trauma, coagulation, central venous pressure, stroke volume, pulse pressure, catecholamines, heart rate, mean arterial pressure, cerebral blood velocity, leukocytes.

Body, Key research accomplishments & reportable outcomes

The abstracts from these papers summarize key accomplishments and outcomes.

The purpose of this study was to compare hemodynamic and blood analyte responses to reduced central venous pressure (CVP) and pulse pressure (PP) elicited during graded lower body negative pressure (LBNP) to those observed during graded blood loss (BL) in conscious humans. We hypothesized that the stimulus-response relationships of CVP and PP to hemodynamic responses during LBNP would mimic those observed during BL. We assessed CVP, PP, heart rate, mean arterial pressure (MAP), and other hemodynamic markers in 12 men during LBNP and BL. Blood samples were obtained for analysis of catecholamines, hematocrit, hemoglobin, arginine vasopressin, and blood gases. LBNP consisted of 5-min stages at 0, 15, 30, and 45 mmHg of suction. BL consisted of 5 min at baseline and following three stages of 333 ml of hemorrhage (1,000 ml total). Individual r(2) values and linear regression slopes were calculated to determine whether the stimulus (CVP and PP)-hemodynamic response trajectories were similar between protocols. The CVP-MAP trajectory was the only CVP-response slope that was statistically different during LBNP compared with BL (0.93 ± 0.27 vs. 0.13 ± 0.26; P = 0.037). The PP-heart rate trajectory was the only PP-response slope that was statistically different during LBNP compared with BL (-1.85 ± 0.45 vs. -0.46 ± 0.27; P = 0.024). Norepinephrine, hematocrit, and hemoglobin were all lower at termination in the BL protocol compared with LBNP (P < 0.05). Consistent with our hypothesis, LBNP mimics the hemodynamic stimulus-response trajectories observed during BL across a significant range of CVP in humans.

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 3 levels of LBNP (5-min at -15, -30, -45 mmHg), and during 3 levels of blood loss (333, 667, 1000 ml). LBNP and blood loss conditions were randomized. Intra-arterial mean arterial pressure (MAP) was similar during LBNP compared with blood loss (p≥0.42). Central venous pressure (CVP; 2.8±0.7 vs. 4.0±0.8, 1.2±0.6 vs. 3.5±0.8, 0.2±0.9 vs. 2.1±0.9 mmHg for level 1, 2, and 3; p≤0.003) and stroke volume (71±4 vs. 80±3, 60±3 vs. 74±3, 51±2 vs. 68±4 ml for level 1, 2, and 3; p≤0.002) were lower during LBNP compared with blood loss. Despite differences in CVP, middle cerebral artery velocity (MCAv) and cerebrovascular conductance (CVC) were similar between LBNP and blood loss at each level (MCAv at level 3: 62±6 vs. 66±5 cm/s; p=0.37; CVC at level 3: 0.72±0.05 vs. 0.73±0.05 cm/s/mmHg; p=0.53). While the slope of the relationship between MAP and MCAv was slightly different between LBNP and blood loss (LBNP: 0.41 ±0.03 cm/s/mmHg vs. Blood Loss: 0.66 ± 0.04 cm/s/mmHg; 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 1000 ml follow a similar trajectory, and the relationship between arterial pressure and cerebral blood velocity are not altered from baseline under these conditions.

We tested the hypothesis that markers of coagulation activation are greater during lower body negative pressure (LBNP) than those obtained during blood loss (BL). We assessed coagulation using both standard clinical tests and thrombelastography in 12 men who performed a LBNP and BL protocol in a randomized order. LBNP consisted of 5-minute stages at 0, -15, -30, and -45 mmHg of suction. BL included 5 minutes at baseline and following three stages of 333 mL of blood removal.
Arterial blood draws were performed at baseline and after the last stage of each protocol. We found that LBNP to -45mmHg is a greater central hypovolemic stimulus vs. BL, therefore the coagulation markers were plotted against central venous pressure (CVP) to obtain stimulus- response relationships using the linear regression line slopes for both protocols. Paired t-tests were used to determine if the slopes of these regression lines fell on similar trajectories for each protocol.

Mean regression line slopes for coagulation markers vs. CVP fell on similar trajectories during both protocols, except for TEG α° angle (-0.42 ± 0.96 during LBNP vs. -2.41 ± 1.13 °/mmHg during BL, p<0.05). During both LBNP and BL coagulation was accelerated as evidenced by shortened R-times (LBNP 9.9 ± 2.4 to 6.2 ± 1.1 BL 8.7 ± 1.3 to 6.4 ± 0.4, both p<0.05). Our results indicate that LBNP models the general changes in coagulation markers observed during BL.

Hypovolaemia has been associated with an immune response that might be secondary to sympathoexcitation. We tested the hypothesis that simulated hypovolaemia using lower body negative pressure (LBNP) and real hypovolaemia induced via experimental blood loss (BL) cause similar increases in the white blood cell concentration ([WBC]). We measured [WBC] and catecholamine concentrations in 12 men who underwent an LBNP and a BL protocol in a randomized order. We compared 45 mmHg of LBNP with 1000 ml of BL; therefore, [WBC] and catecholamine concentrations were plotted against central venous pressure to obtain stimulus-response relationships using the linear regression line slopes for both protocols. Mean regression line slopes were similar for total [WBC] (LBNP 183 ± 4 μl-1 mmHg-1 versus BL 155 ± 109 μl-1 mmHg-1 , P = 0.15), neutrophils (LBNP 110 ± 2 μl-1 mmHg-1 versus BL 96 ± 72 μl-1 mmHg-1 , P = 0.15) and lymphocytes (LBNP 65 ± 21 μl-1 mmHg-1 versus BL 59 ± 38 μl-1 mmHg-1 , P = 0.90). Mean regression line slopes for adrenaline were similar (LBNP 15 ± 5 pg ml-1 mmHg-1 versus BL 16 ± 4 pg ml-1 mmHg-1 , P = 0.84) and were steeper during LBNP for noradrenaline (LBNP 28 ± 6 pg ml-1 mmHg-1 versus BL 9 ± 6 pg ml-1 mmHg-1 , P = 0.01). These data indicate that central hypovolaemia elicits a relative leucocytosis with a predominantly neutrophil-based response. Additionally, our results indicate that LBNP models the stimulus-response relationship between central venous pressure and [WBC] observed during BL.
Next Steps

Additional data analysis and manuscript preparation/submission is in process related to our second and third studies on the effects of hypoxia and systemic epinephrine infusion respectively on responses to simulated blood loss is underway. Data collection for both studies has been completed. We are currently preparing/submitting manuscripts related to the hemodynamics, cerebral blood flow, cardiac baroreflex sensitivity, and hormonal responses associated with our study on the effects of hypoxia on the decision support medical monitoring system and related physiological variables and responses.
Reportable Outcomes

We will focus on new unreported/published data from the systemic epinephrine trials since the data from the hypoxia trials have been reported in the previous annual report. The rationale for this study was that combat injuries frequently evoke a sympathoexcitatory response and we wanted to mimic this response in the laboratory. As such, testing the decision support medical monitoring system and related physiological variables during these conditions was an important step for the continued development of the algorithm and understanding of hemodynamic decompensation during battlefield or trauma situations.

We have completed data analysis on 10 subjects. Data from these subjects show that during systemic epinephrine infusion total time to presyncope (figure 1) was similar to saline infusion (P>0.05). Examination of hemodynamic variables, showed mean blood pressure (figure 2) and total peripheral resistance (figure 6) were lower during LBNP with epinephrine vs. saline infusion (p<0.05). Heart rate (figure 3) and pulse pressure (figure 5) were higher during LBNP with epinephrine infusion vs. saline (p<0.05), while the decrease in stroke volume (figure 4) had a similar trajectory.
In addition, we assessed cerebral blood flow by insonating the middle cerebral artery and found no differences in middle cerebral artery velocity trajectories during LBNP with epinephrine infusion vs. saline infusion (figure 7).
Figure 7. Middle cerebral artery velocity trajectories during LBNP with epinephrine and saline infusion.
Conclusion
The last three years have been highly successful and we have completed key manuscripts related to the main aims of the funding cycle including publication of four papers in highly visible journals. Additional peer-reviewed manuscripts are in the process of being submitted on the influences of hypoxia and systemic epinephrine infusion on responses to simulated blood loss. These papers represent an important step forward in the understanding of hemodynamic decompensation during battlefield or trauma situations and the continued development of the Army’s compensatory reserve index algorithm.
References

Published and in preparation manuscripts:


• Johnson BD, van Helmond N, Holbein WW, Petersen-Jones H.G., Curry TB, Convertino VA, Joyner MJ. The role of the carotid bodies in mediating hemodynamic responses to lower body negative pressure during hypoxia, in preparation

Abstracts


Aarts HM, Petersen-Jones HG, Johnson BD, Curry TB, Joyner MJ. Cardiac baroreflex sensitivity during lower body negative pressure and acute hypoxia: fainters vs. nonfainters. Accepted for Experimental Biology 2017.
Participants and Other Organizations

The following individuals have worked on the project:

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Funding Support: N/A

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Funding Support: N/A

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Funding Support: N/A

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There were no other organizations involved as partners.
Appendices

Please see the attached original manuscripts of 4 published journal articles.
Noud van Helmond\textsuperscript{1}, Blair D. Johnson\textsuperscript{1,2}, Timothy B. Curry\textsuperscript{1}, Andrew P. Cap\textsuperscript{3}, Victor A. Convertino\textsuperscript{3}, & Michael J. Joyner\textsuperscript{1}

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Disclaimer: 
\textit{The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of the Army or the US Department of Defense.}

Conflicts of Interest and Source of Funding: No conflicts of interest. Support for this study was provided by U.S. Army MRMC Combat Casualty Care Research Program Grant W81XWH-11–1-0823 to MJJ, American Heart Association Midwest Affiliate Grant 13POST-14380027 to B.D.J., and by Dutch Heart Foundation E. Dekker Stipend 2012SB013 to N.V.H.

Running Head: WBC concentrations during LBNP and blood loss

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1. What is the central question of this study?

Is lower body negative pressure a useful surrogate to study white blood cell responses to hemorrhage in humans?

2. What is the main finding and its importance?

We found that lower body negative pressure appears to be a useful surrogate to study the early white blood cell mobilization response during blood loss.
ABSTRACT
Hypovolemia has been associated with an immune response that might be secondary to sympathoexcitation. We tested the hypothesis that simulated hypovolemia using lower body negative pressure (LBNP) and actual hypovolemia induced via experimental blood loss (BL) cause similar increases in white blood cell concentrations ([WBC]). We measured [WBC] and catecholamine concentrations in twelve men who performed a LBNP and BL protocol in a randomized order. We compared 45 mmHg of LBNP to 1000 mL of BL; therefore [WBC] and catecholamine concentrations were plotted against central venous pressure (CVP) to obtain stimulus-response relationships using the linear regression line slopes for both protocols. Mean regression line slopes were similar for total [WBC] (LBNP 183±46 vs. BL 155±109 mcL⁻¹ × mmHg⁻¹, p=0.15), neutrophils (LBNP 110±29 vs. BL 96±72 mcL⁻¹ × mmHg⁻¹, p=0.15) and lymphocytes (LBNP 65±21 vs. BL 59±38 mcL⁻¹ × mmHg⁻¹, p=0.90). Mean regression line slopes for adrenaline were similar (LBNP 15±5 vs. BL 16±4 pg × mL⁻¹ × mmHg⁻¹, p=0.84), and were steeper during LBNP for noradrenaline (LBNP 28±6 vs. BL 9±6 pg × mL⁻¹ × mmHg⁻¹, p=0.01). These data indicate that central hypovolemia elicits a relative leukocytosis with a predominantly neutrophil-based response. Additionally, our results indicate that LBNP models the stimulus-response relationship between CVP and [WBC] observed during BL.

Key words: Leukocytosis; Hemorrhage; Humans; Central Hypovolemia
INTRODUCTION

Hemorrhage is one of the leading causes of accidental death (Boulanger et al., 2007) and is the leading cause of preventable death on the battlefield (Eastridge et al., 2011; Eastridge et al., 2012). Trauma and hemorrhagic shock are associated with an acute increase in circulating leukocytes (Thommasen et al., 1986; Teggatz et al., 1987; Yanagawa et al., 2005), which might prevent infection and promote wound healing following tissue damage. Therefore, examining the leukocytosis response to blood loss (BL) is important to gain insight into mechanisms that may prevent or render hemorrhage victims prone to infection and impaired wound healing. Animal studies have evaluated the mechanisms of hemorrhagic leukocytosis (Musser, 1921; Gaylor et al., 1969); however, using invasive methods to experimentally induce BL and evaluate hemorrhage-induced leukocytosis is challenging to perform in humans.

Barcroft and colleagues were pioneers in studying the physiological responses to BL in human subjects. They sequestered blood in the legs using venous tourniquets placed around the thighs in combination with venesection. This technique allowed Barcroft and colleagues to induce fainting and quickly increase venous return by releasing the venous tourniquets to restore consciousness (Barcroft et al., 1944). Similar to this method, lower body negative pressure (LBNP) has emerged as a non-invasive surrogate to study many of the physiological responses to BL (Cooke et al., 2004; Hinojosa-Laborde et al., 2014; Johnson et al., 2014). LBNP sequesters circulating blood in the lower body thereby reducing central blood volume and mimicking hemodynamic and blood coagulation responses generated during BL (Cooke et al., 2004; Hinojosa-Laborde et al., 2014; Johnson et al., 2014; Rickards et al., 2015).

However, we are unaware of any data that supports the notion that LBNP influences circulating leukocytes to the same extent as BL. It has been suggested that catecholamines cause the leukocytosis associated with hypovolemic shock (Yanagawa et al., 2005). Since reductions in central blood volume by experimental BL or LBNP increase catecholamine concentrations to a similar extent (Cooke et al., 2004; Hinojosa-Laborde et al., 2014; Johnson et al., 2014), it seems reasonable to expect that LBNP
might elicit comparable changes in circulating leukocytes when the degree of central hypovolemia is similar between LBNP and BL.

To explore whether LBNP can be used as a model to study the leukocytosis associated with BL, we compared concentrations of circulating leukocytes during LBNP to those generated during BL in humans. We hypothesized that the stimulus-response relationships of central hypovolemia (i.e. central venous pressure) and catecholamine concentrations to circulating leukocytes during LBNP would be similar to those observed during BL for a given central hypovolemic stimulus.
MATERIALS AND METHODS

Ethical Approval

This study was approved by the Mayo Clinic Institutional Review Board and conformed to the standards set by the 2008 revision of the Declaration of Helsinki (Williams, 2008). Prior to participation, all subjects provided written informed consent after all procedures and study risks were fully explained.

Subjects

Twelve healthy men (age: 32 ± 6 years; height: 181.8 ± 6.8 cm; weight: 88.4 ± 8.8 kg; BMI: 26.7 ± 1.8 kg m⁻²) participated in this study. Subjects were non-smokers, and did not take any medications. All subjects reported to be free of cardiovascular, respiratory, neurologic, and metabolic disease. Following an overnight fast, subjects reported to the Clinical Research Trial Unit (CRTU) of the Mayo Clinic at 0700. Upon reporting to the CRTU, subjects consumed a snack (Cliff Bar; Shelton, CT, USA; 240 kcals) and drank 250 mL of water. Subjects were studied in the supine position in a temperature-controlled room (20-22°C).

Experimental Design

The study timeline is presented in Figure 1. The experimental design and selection of LBNP and BL protocols have been detailed in previously publications that focused on testing the hypotheses that hemodynamic (Johnson et al., 2014), coagulation (Helmond et al., 2015), and cerebral blood velocity (Rickards et al., 2015) responses would be similar between LBNP and BL. In the present paper, we uniquely test the hypothesis that the WBC response associated with hemorrhage will be similar during progressive reduction in central blood volume induced by LBNP.

Both protocols were performed on the same day and the order was randomized and counterbalanced. Briefly, subjects were studied supine and instrumented for blood removal (brachial vein catheter) and monitoring (brachial artery catheter and peripherally inserted central catheter (PICC)). After the first protocol, subjects rested quietly for 45-75 minutes in the supine position. The protocols were terminated
if mean arterial pressure fell by 30%, systolic blood pressure dropped below 80 mmHg, or the subject began to experience symptoms of pre-syncope or syncope. Arterial blood samples were collected at baseline and at the conclusion of each protocol. During the LBNP protocol, blood samples were collected shortly before suction was terminated. If a protocol was terminated early, blood samples were obtained immediately upon the decision to terminate the protocol.

**LBNP protocol**

Subjects laid in an LBNP chamber sealed at the iliac crest. The LBNP protocol was based on the first 3 stages of the protocol frequently used by the U.S. Army Institute of Surgical Research (Cooke *et al.*, 2004) (Figure 1). Following a 5-minute baseline period, the protocol commenced and consisted of 5-minute stages at 15, 30, and 45 mmHg of LBNP. Subjects were instructed not to move during the protocol.

**Blood Loss protocol**

Preservative/anticoagulant bags (63 mL anti-coagulant citrate phosphate dextrose solution) were positioned below the subject to enable blood transfer from the subject via gravity from a large bore intravenous catheter. Following a 5-minute baseline period, 3 aliquots of 333 mL of blood were removed. After each aliquot of blood was removed, the subject rested for a 5-minute period to emulate the timing of the LBNP protocol. The removed blood was kept in the study room (20-22°C) and was re-infused at a rate of 20 mL min⁻¹ into the antecubital vein following the BL protocol.

**Hemodynamic measurements**

Heart rate (HR) was measured from a 3-lead ECG (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). Blood pressure was measured beat-by-beat by arterial catheter. Central venous pressure (CVP) was measured using the PICC. The PICC was introduced through an antecubital vein and advanced to the level of the superior vena cava. Placement of the PICC was estimated using external measurement of the distance from the antecubital fossa to the manubrium and was verified by the identification of a typical CVP waveform. All lines were placed aseptically with local anesthesia. The arterial catheter and the
PICC were connected to pressure transducers (FloTrac, Edwards Lifesciences Corp., Irvine, CA, USA) placed at the mid-axillary line.

*Complete blood counts and Catecholamines*

Blood was collected from the brachial artery catheter for the measurement of complete blood cell and circulating catecholamine concentrations. The Department of Laboratory Medicine and Pathology and the Immunochemistry Core Laboratory of the CRTU of the Mayo Clinic Center for Clinical and Translational Science analyzed the blood samples for complete blood cell concentrations and circulating catecholamines, respectively. Blood samples collected in 3 mL EDTA tubes were analyzed for red blood cell concentrations [RBC], white blood cell concentrations [WBC] and WBC differential using an automated analyzer according to the RF/DC detection method (Sysmex XE-5000, Kobe, Japan). Plasma adrenaline and noradrenaline concentrations were determined from 4.5 mL of arterial blood using HPLC after prior alumina extraction (ESA Coulochem III, Dionex, Sunnyvale, CA, USA).

*Hemoconcentration measures*

Baseline total blood volume was estimated according to Retzlaff et al. (Retzlaff *et al.*, 1969) using the following equation:

\[
\text{Blood volume} = 31.9 \times \text{height (cm)} + 26.3 \times \text{weight (kg)} - 2402
\]

Estimated changes in blood volume and the estimated percentage change in plasma volume from pre to post LBNP and from pre to post BL were determined using the formula by Dill and Costill (Dill & Costill, 1974). Hemoglobin values were corrected for volume of blood withdrawn and baseline plasma percentage was defined as 1-hematocrit.

*Data and statistical analysis*
Hemodynamic data were collected and analyzed off-line using signal processing software (WinDaq, DATAQ Instruments, Akron, OH, USA). Hemodynamic data were analyzed and averaged over the last 2 minutes of baseline and final stages of LBNP and BL for statistical analysis. All hemodynamic signals were automatically peak-detected and manually verified. Stroke volume (SV) was determined using WinCPRS software (Absolute Aliens Oy, Turku, Finland) by selecting the area under the arterial blood pressure curve and calculated using Modelflow (Wesseling et al., 1993). Cardiac output was calculated as the product of heart rate and stroke volume. Protocol (LBNP/BL) × time (Baseline/Protocol termination) repeated measures ANOVA was used to determine if values obtained during the LBNP protocol were similar to values during the BL protocol. If a significant main or interaction effect was obtained, Tukey’s post hoc test was performed to determine where differences existed. If data were not normally distributed, the Wilcoxon Signed Rank test was used. As a post hoc test, we compared the relationship of white blood cell counts and catecholamines vs. hypovolemia during BL and LBNP to adjust for differences in hypovolemia. We performed this analysis by plotting white blood cell concentrations and catecholamine concentrations against CVP to obtain stimulus-response relationships using the linear regression line slopes as we (Johnson et al., 2014; Helmond et al., 2015) and others (Rea et al., 1991) have done. CVP decreases early and linearly throughout both LBNP and BL protocols (Gauer et al., 1956; Henry et al., 1956; Norsk et al., 1986; Hirsch et al., 1989; Rea et al., 1991; van Hoeyweghen et al., 2001; Hinojosa-Laborde et al., 2014; Johnson et al., 2014). To assess the relationship of the white blood cell concentrations vs. catecholamines during BL and LBNP, we performed a similar analysis by plotting the white blood cell concentrations that increased against catecholamine concentrations. Paired t-tests were used to determine if the slopes of these regression lines were different between protocols. Group data are presented as mean ± SE and P values are reported.
RESULTS

Of the twelve subjects, two did not complete both protocols (both subjects completed 667 mL of BL and 30 mmHg of LBNP); additionally, one subject did not complete the LBNP protocol (completed 30 mmHg of LBNP), and one subject did not complete the BL protocol (completed 333 mL of BL). These protocols were terminated early due to pre-syncope symptoms or syncope. Data obtained from the final completed stage were used for these subjects. The mean hemodynamic values obtained during both protocols are presented in Table 1 and are reported elsewhere (Johnson et al., 2014). Complete [WBC], catecholamine concentrations, and [RBC] at baseline and protocol termination are shown in Table 1. The mean [WBC] and catecholamine concentrations across the range of CVP during LBNP and BL are displayed in Figures 2 and 3. Regression line slopes produced from the stimulus-response relationships between the mean [WBC] and catecholamine concentrations are illustrated in Figure 4.

Effects of LBNP and BL on Hemodynamics

Table 1 shows that both LBNP and BL evoked pronounced hemodynamic changes from baseline to protocol termination. At baseline, CVP (p=0.024) was slightly lower during BL while SV (p=0.016), and CO (p=0.045) were slightly higher. Overall, 45 mmHg of LBNP caused greater changes in hemodynamic parameters than 1000 mL of BL. Specifically, at protocol termination, CVP (p<0.001), SV (p<0.001) and CO (p=0.002) were lower and HR was higher (p<0.001) during LBNP versus BL.

Effects of LBNP and BL on White Blood Cell Concentrations

Total [WBC] was increased at LBNP termination (p<0.001) and at BL termination (p=0.100) (Table 1). Total [WBC] was higher during LBNP versus BL at protocol termination (p=0.040). Neutrophil concentration was increased at LBNP termination (p=0.001) and at BL termination (p=0.080). Lymphocyte concentration was also increased at protocol termination in LBNP (p=0.005). Monocyte, eosinophil, and basophil concentrations remained relatively unchanged at both LBNP and BL.
termination. Importantly, the regression line slopes calculated from the relationship between the various [WBC] and CVP were not different between LBNP and BL.

**Effects of LBNP and BL on Catecholamine Concentrations**

Adrenaline (p<0.001 and p=0.002) and noradrenaline (p<0.001 and p=0.043) concentrations were both elevated at LBNP and BL protocol termination, respectively (Table 1). Noradrenaline concentrations were higher during LBNP versus BL at protocol termination (p=0.003). Regression line slopes produced from the stimulus-response relationship between adrenaline and CVP were not different during LBNP and BL. The noradrenaline response slopes were steeper during LBNP vs. BL (28±19 vs. 9±20 pg ×mL⁻¹ ×mmHg⁻¹, p=0.010) (Figure 3).

**Relationship of White Blood Cell Concentrations vs. Catecholamines**

Regression line slopes produced from the stimulus-response relationships between the total [WBC], neutrophils, lymphocytes and adrenaline were not different during LBNP and BL (Figure 4). Regression line slopes from the relationships between total [WBC], neutrophils, lymphocytes and noradrenaline were also not different during LBNP and BL.

**Effects of LBNP and BL on Hemoconcentration**

Several markers indicated that LBNP caused hemoconcentration, while BL induced hemodilution (Table 1). After LBNP, there were increases in hemoglobin (p=0.003) and hematocrit (p=0.001) and a decrease in estimated plasma volume (p=0.001) compared to baseline values. BL induced decreases in hemoglobin (p=0.006) and hematocrit (p=0.006) and an increase in estimated plasma volume (p=0.004) compared to baseline values. At protocol termination, hemoglobin (p=0.001) and hematocrit (p=0.001) were lower in BL versus LBNP and estimated plasma volume (p≤0.001) was greater in BL when compared to LBNP.
DISCUSSION

The results of this study indicate that BL and LBNP induce similar leukocyte response slopes across a wide range of CVP. A reduction in CVP resulting from central hypovolemia by LBNP induced a relative leukocytosis with a predominantly neutrophil-based response and a slight increase in lymphocytes. Additionally, neutrophil and lymphocyte concentrations were relatively unchanged during BL. This indicates that a greater hypovolemic stimulus, such as that which occurred during LBNP, is needed to increase neutrophil and lymphocyte numbers.

To our knowledge, this is the first experimental study reporting the early WBC mobilization in response to central hypovolemia induced by BL or LBNP. The increase in total leukocytes we found during LBNP is consistent with previous reports that found an increase in total leukocytes during a combination of LBNP and whole body heating (Meyer et al., 2013). The increase in neutrophils and lymphocytes we found during LBNP are consistent with the immune cell responses observed during hypovolemic shock in clinical settings (Thommasen et al., 1986; Teggatz et al., 1987; Yanagawa et al., 2005) and experimental hemorrhage in animals (Musser, 1921; Gaylor et al., 1969). The increase in circulating leukocytes is related to the sympathetic responses to trauma (Thommasen et al., 1986; Teggatz et al., 1987; Yanagawa et al., 2005) and may contribute to wound healing under these conditions (Benschop et al., 1996; Dhabhar et al., 2012). Our findings demonstrate that increases in WBC can be stimulated by a reduction in central blood volume without tissue injury. Previous investigations have described a pronounced leukocytosis with lymphocytosis and neutrophilia after subcutaneous injection of adrenaline in both animals (Frey, 1914) and humans (Loeper & Crouzon, 1904). Additionally, experimentally induced psychological stress (Dhabhar et al., 2012) and physical exercise (Pedersen & Hoffman-Goetz, 2000), both of which increase circulating catecholamines, lead to increases in lymphocyte and neutrophil concentrations. Therefore, it is probable that the elevated circulating catecholamines observed in both LBNP and BL protocols account for the rise in circulating neutrophils and lymphocytes.

The increase in circulating lymphocytes following an increase in adrenaline is mediated via activation of
β2-adrenoceptors, whereas α-adrenoceptor stimulation contributes to the increase in circulating neutrophils (Benschop et al., 1996; Sanders, 2006). We found significant increases in adrenaline after both LBNP and BL and the regression line slopes calculated from the relationship between the total [WBC], neutrophils, lymphocytes and adrenaline values were not different between protocols. Therefore, sympathoexcitation and the release of adrenaline likely contributed to the relative leukocytosis. Noradrenaline was also significantly elevated after both LBNP and BL, but noradrenaline has limited influence on leukocyte numbers (Benschop et al., 1996).

In addition to the greater hypovolemic stimulus that LBNP produced, we observed divergent effects on plasma volume during LBNP and BL. We found a decrease in plasma volume by ~4% during LBNP, and plasma volume increased by ~3% during BL. This is a direct result of how these protocols cause central hypovolemia. The suction applied during LBNP produces a pressure gradient that pulls fluid from the intravascular compartment to the extravascular space in the lower body resulting in hemoconcentration (Sander-Jensen et al., 1988; Ward et al., 2010; Cvirn et al., 2012). However, BL has the opposite effect. The reduction in circulating blood volume causes fluid to shift from the extravascular space to the intravascular space resulting in hemodilution (Riddez et al., 1998; Drobin & Hahn, 1999; Zaar et al., 2014). This divergent effect on plasma volume likely contributed to the greater increase in white blood cells during LBNP.

We observed a relative leukocytosis at the immediate termination of both LBNP and BL. The immune cell response to stress, exercise, and to adrenaline injection all show a biphasic pattern, with an initial lymphocytosis and a maximal response within 30 minutes of the stimulus. This increase is followed by a maximal rise in neutrophils that occurs between two and four hours following the stimulus (Samuels, 1951; Pedersen & Hoffman-Goetz, 2000; Dhabhar et al., 2012). Therefore, we might have observed a greater increase in neutrophil concentrations if we would have prolonged the hypovolemic exposures or postponed blood sample collection until 2-4 hours following each protocol. However, we did not find an order effect such that the observed responses were not dependent on whether LBNP or BL occurred
first. Therefore, a delay in blood sample collection would not have resulted in a marked increase in neutrophils following our protocols.

Methodological considerations

Several methodological considerations pertain and additional limitations have been considered elsewhere (Johnson et al., 2014; Helmond et al., 2015). First, we collected blood only at baseline and at the termination of each protocol. Collecting multiple samples throughout both protocols might have allowed us to identify whether a biphasic leukocyte response exists during central hypovolemia elicited by LBNP or BL. Second, the maximal hypovolemic stimulus during LBNP, as measured by the change in cardiovascular parameters likely was greater when compared to BL. Additionally, it is likely that the differing profiles of central blood volume reduction during BL (i.e., slower rate) vs. LBNP in the present study influenced the hemodynamic responses and leukocyte changes. Progressive LBNP was applied continuously without any break in the stimulus. In contrast, the BL protocol had intermittent reductions in central blood volume. The smaller changes in hemodynamic variables and leukocytes during the BL protocol might reflect the ability of compensatory responses to react to a slower rate of central blood volume reduction. Third, we have no direct recordings of sympathetic neural activity, which could have provided additional insight regarding interpretation of the magnitude of sympathetic nervous system activation during central hypovolemia. Fourth, catecholamine induced immune cell redistribution accompanies increases in immune function (Dhabhar et al., 2012). Therefore, it would have been informative if we had performed specific immune function tests in addition to determining cell concentrations.

CONCLUSIONS

Reductions in CVP elicit early relative leukocytosis with a predominantly neutrophil-based response. The stimulus-response slopes for leukocyte concentrations versus CVP were similar between the two protocols, which indicates that LBNP elicits a relative leukocytosis similar to BL within the range of central hypovolemia that we tested. Additionally, the increase in WBC during LBNP and/or BL can be
achieved in the absence of tissue injury. Therefore, LBNP appears to be a useful surrogate to study the early WBC mobilization response during BL.

**AUTHOR CONTRIBUTIONS**


**ACKNOWLEDGEMENTS**

We thank the subjects who participated in the study. We also thank Shelly K. Roberts, Sara C. Wolhart, Christopher P. Johnson, Margaret McGill-Zimny, and David Warren for their assistance.

**FUNDING**

Support for this study was provided by U.S. Army MRMC Combat Casualty Care Research Program Grant W81XWH-11–1-0823 to MJJ, American Heart Association Midwest Affiliate Grant 13POST-14380027 to B.D.J., and by Dutch Heart Foundation E. Dekker Stipend 2012SB013 to N.V.H.

**ABREVIATIONS**

LBNP: Lower body negative pressure; BL: blood loss; CVP: central venous pressure; PICC; peripherally inserted central catheter; WBC: white blood cell; RBC: red blood cell; HR: heart rate; MAP: mean arterial pressure; SV: stroke volume; CO: cardiac output; CRTU: Clinical Research Trial Unit; BMI: body mass index
Table 1. Effects of LBNP and BL on hemodynamic variables, white blood cell concentrations, catecholamine concentrations, red blood cell concentration and plasma volume.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
</tr>
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<tbody>
<tr>
<td><strong>Hemodynamic variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>LBNP 7.3 ± 2.2</td>
<td>-0.2 ± 2.0*</td>
</tr>
<tr>
<td></td>
<td>BL 6.1 ± 2.1</td>
<td>1.8 ± 2.7†</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>LBNP 60 ± 8.7</td>
<td>80 ± 17.5*</td>
</tr>
<tr>
<td></td>
<td>BL 60 ± 9.7</td>
<td>67 ± 9.1†</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>LBNP 93.5 ± 8.1</td>
<td>84.5 ± 10.1*</td>
</tr>
<tr>
<td></td>
<td>BL 91.8 ± 6.7</td>
<td>87.0 ± 9.5</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>LBNP 83.2 ± 9.4</td>
<td>54.1 ± 11.4*</td>
</tr>
<tr>
<td></td>
<td>BL 89.5 ± 9.4†</td>
<td>70.5 ± 9.4†</td>
</tr>
<tr>
<td>CO (L ×min⁻¹)</td>
<td>LBNP 5.0 ± 1.0</td>
<td>4.1 ± 0.3†</td>
</tr>
<tr>
<td></td>
<td>BL 5.3 ± 1.0†</td>
<td>4.7 ± 0.7†</td>
</tr>
<tr>
<td><strong>White blood cell concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total leukocytes (×10⁹ ×L⁻¹)</td>
<td>LBNP 5.6 ± 1.1</td>
<td>6.8 ± 1.8*</td>
</tr>
<tr>
<td></td>
<td>BL 5.2 ± 1.5</td>
<td>5.6 ± 2.1†</td>
</tr>
<tr>
<td>Neutrophils (×10⁹ ×L⁻¹)</td>
<td>LBNP 3.6 ± 1.2</td>
<td>4.3 ± 1.4*</td>
</tr>
<tr>
<td></td>
<td>BL 3.1 ± 1.4</td>
<td>3.4 ± 1.9</td>
</tr>
<tr>
<td>Lymphocytes (×10⁹ ×L⁻¹)</td>
<td>LBNP 1.4 ± 0.4</td>
<td>1.9 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td>BL 1.5 ± 0.4</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Monocytes (×10⁹ ×L⁻¹)</td>
<td>LBNP 0.41 ± 0.10</td>
<td>0.47 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>BL 0.44 ± 0.10</td>
<td>0.41 ± 0.07</td>
</tr>
<tr>
<td>Eosinophils (×10⁹ ×L⁻¹)</td>
<td>LBNP 0.13 ± 0.06</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>BL 0.15 ± 0.09</td>
<td>0.13 ± 0.08</td>
</tr>
<tr>
<td>Basophils (×10⁹ ×L⁻¹)</td>
<td>LBNP 0.02 ± 0.01</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>BL 0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>
Catecholamine concentrations

Noradrenaline (pg ×mL⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>LBNP</th>
<th>BL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>148 ± 70</td>
<td>354 ± 153*</td>
</tr>
</tbody>
</table>
| Adrenaline (pg ×mL⁻¹)
|       | 53 ± 26    | 144 ± 105**| 103 ± 61*|

Red blood cell concentration and plasma volume

Hemoglobin (g/dL)

<table>
<thead>
<tr>
<th></th>
<th>LBNP</th>
<th>BL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.2 ± 1.4</td>
<td>14.7 ± 1.2*</td>
</tr>
</tbody>
</table>
| Hematocrit (%)
|       | 14.3 ± 1.3 | 14.0 ± 1.3*†|
|       | 41 ± 2.8   | 42 ± 2.9*   |
| RBC (×10¹²×L⁻¹)
|       | 4.8 ± 0.4  | 5.0 ± 0.3*  |
|       | 4.8 ± 0.4  | 4.7 ± 0.4*†|
| Plasma volume (%)
|       | 59 ± 2.8   | 56 ± 3.3*   |
|       | 59 ± 2.9   | 61 ± 3.9*†  |

LBNP = lower body negative pressure; BL = blood loss; CVP = central venous pressure; HR = heart rate; MAP = mean arterial pressure; SV = stroke volume; CO = cardiac output.

Values are means ± standard deviation, n = 12.

* Different from Baseline (P < 0.05); † Different vs. LBNP
Figure 1. Timeline of the lower body negative pressure and blood loss protocols. The order of the protocols was randomized. When the lower body negative pressure protocol was performed first, 45 minutes of quiet rest was given between protocols to ensure hemodynamic variables returned to baseline. To allow for the reinfusion of removed blood, 75 minutes of quiet resting was given to allow for hemodynamic variables to return to baseline between protocols when blood loss occurred first. Blood was drawn at baseline and during the last stage of each protocol.

Figure 2. Mean ± SD white blood cell concentrations: (A) total white blood cell concentration, (B) neutrophil concentration, (C) lymphocyte concentration, (D) monocyte concentration, (E) eosinophil concentration, and (F) basophil concentration plotted against mean central venous pressure (CVP) ± SD at baseline and immediately after protocol termination during the LBNP and BL protocols. None of the response slopes were different between LBNP and BL protocols.

Figure 3. Mean ± SD (A) adrenaline and (B) noradrenaline concentrations plotted against mean CVP ± SD at baseline and immediately after protocol termination during the LBNP and BL protocols. Adrenaline response slopes were not different between LBNP and BL protocols and noradrenaline response slopes were steeper during LBNP (p=0.01).

Figure 4. Mean ± SD white blood cell concentrations plotted against mean ± SD catecholamine concentrations at baseline and immediately after protocol termination during the LBNP and BL protocols. (A) Total white blood cell concentration, (B) neutrophil concentration and (C) lymphocyte concentration plotted against adrenaline concentrations. (D) Total white blood cell concentration, (E) neutrophil concentration and (F) lymphocyte concentration plotted against noradrenaline concentrations. None of the response slopes were different between LBNP and BL protocols.
REFERENCES


Figure 2.
Figure 3.

A

Adrenaline (ng/mL)

CVP (mm Hg)

Mean LBNP slope: 14.7
Mean BL slope: 15.6

B

Noradrenaline (ng/mL)

CVP (mm Hg)

Mean LBNP slope: 27.8
Mean BL slope: 9.7
Figure 4.

A. Mean LBNP slope: 0.04
Mean BL slope: 0.03

B. Mean LBNP slope: 0.02
Mean BL slope: 0.03

C. Mean LBNP slope: 0.01
Mean BL slope: 0.01

D. Mean LBNP slope: 0.01
Mean BL slope: 0.02

E. Mean LBNP slope: <0.01
Mean BL slope: 0.02

F. Mean LBNP slope: <0.01
Mean BL slope: <0.01
Cerebral blood velocity regulation during progressive blood loss compared to lower body negative pressure in humans

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Running Title: Cerebral blood velocity and simulated hemorrhage

Manuscript word count: 4516
Abstract word count: 276
Number of figures: 5

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Abstract

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 3 levels of LBNP (5-min at -15, -30, -45 mmHg), and during 3 levels of blood loss (333, 667, 1000 ml). LBNP and blood loss conditions were randomized. Intra-arterial mean arterial pressure (MAP) was similar during LBNP compared with blood loss (p≥0.42). Central venous pressure (CVP; 2.8±0.7 vs. 4.0±0.8, 1.2±0.6 vs. 3.5±0.8, 0.2±0.9 vs. 2.1±0.9 mmHg for level 1, 2, and 3; p≤0.003) and stroke volume (71±4 vs. 80±3, 60±3 vs. 74±3, 51±2 vs. 68±4 ml for level 1, 2, and 3; p≤0.002) were lower during LBNP compared with blood loss. Despite differences in CVP, middle cerebral artery velocity (MCAv) and cerebrovascular conductance (CVC) were similar between LBNP and blood loss at each level (MCAv at level 3: 62±6 vs. 66±5 cm/s; p=0.37; CVC at level 3: 0.72±0.05 vs. 0.73±0.05 cm/s/mmHg; p=0.53). While the slope of the relationship between MAP and MCAv was slightly different between LBNP and blood loss (LBNP: 0.41 ±0.03 cm/s/mmHg vs. Blood Loss: 0.66 ± 0.04 cm/s/mmHg; 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 1000 ml follow a similar trajectory, and the relationship between arterial pressure and cerebral blood velocity are not altered from baseline under these conditions.

Key Words: simulated hemorrhage, cerebrovascular, hypovolemia
Introduction

Hemorrhage accounts for approximately one-third of all trauma related deaths (28), and 80% of potentially survivable battlefield injuries (15). As logistical and ethical constraints have often limited comprehensive assessment of the physiological responses to hemorrhage in humans, studies investigating the early detection and prevention of blood loss in humans have often used lower body negative pressure (LBNP) to simulate the hemodynamic effects of actual blood loss. LBNP elicits progressive reductions in central blood volume, reflected by decreases in central venous pressure (CVP), stroke volume (SV), and cardiac output (CO), eliciting baroreflex-mediated increases in heart rate (HR) and total vascular resistance, and the release of vasoactive and volume regulating hormones (11, 13, 18, 26, 35, 46, 47, 53). As reviewed by Cooke et al., in 2004, many of these hemodynamic adjustments associated with LBNP are similar to those induced by hemorrhage (13). While many studies have assessed the effects of blood loss on hemodynamic responses in humans, such as arterial pressure, HR, SV, sympathetic nerve activity and peripheral resistance (1, 2, 19, 39, 45, 50), few have investigated cerebral blood flow responses (7, 48). Inadequate cerebral blood flow and oxygenation is the final common pathway to loss of consciousness from blood loss, so represents an important area of investigation. Two studies (7, 48) have demonstrated progressive reductions in cerebral oxygenation assessed via near infrared spectroscopy (NIRS), following withdrawal of ≤500 ml of blood. No studies, to our knowledge, have investigated cerebral blood flow (or velocity) responses to hemorrhage of any magnitude in humans, or whether the effects of actual blood loss on cerebral blood flow regulation are analogous to simulated blood loss during LBNP.
A direct comparison of the physiological responses to LBNP and blood loss has been performed in a baboon model (21), and from these data, the estimated loss of blood in humans was calculated; approximately -70 mmHg LBNP equated to blood loss of 17.8 ml/kg, or ~0.25 ml/kg/mmHg LBNP. This study provided the basis for our work comparing simulated hemorrhage using LBNP with actual blood loss in adult men (25). While 1000 ml of blood loss elicited smaller reductions in CVPSV compared with -45 mmHg LBNP, between baseline and a loss of ~18% of total blood volume, the CVP, SV, HR, and mean arterial pressure (MAP) responses between LBNP and blood loss were linearly related (25). Importantly, this suggests that the hemodynamic responses to central hypovolemia associated with LBNP are similar to blood loss in adult men.

During progressive central hypovolemia using LBNP, middle cerebral artery velocity (MCAv) is initially maintained, followed by a progressive decrease until the onset of presyncope (3, 29, 41). As the inability to maintain adequate cerebral blood flow and oxygenation can determine tolerance to central hypovolemia (7, 29), the purpose of the present study was to compare the effects of actual graded blood loss to simulated hemorrhage using progressive LBNP on cerebral blood flow (velocity) regulation in humans.

Methods

Subjects. Nine healthy males were recruited for this study (age 31 ± 6 years; height 183 ± 7 cm; weight 89 ± 9 kg; body mass index 26.7 ± 1.8 kg/m²). These subjects were a sub-set of the 12 subjects reported in another publication focused on hemodynamic and hormonal responses...
this protocol (25). All subjects reported to be free of any cardiovascular, respiratory, neurologic, or metabolic disease. Subjects were non-obese (BMI < 30 kg/m$^2$), non-smokers, and were not taking any 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 Mayo Clinic at 0700 following an overnight fast. At this time, each subject consumed a small breakfast bar (Clif Bar; Shelton, CT, USA; 240 kcals) and drank 250 ml of water. Subjects were studied in the supine position in a temperature controlled room (20-22° C). 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 counter-balanced order. Figure 1 illustrates the study protocol. The goal of the experimental design was to elicit a wide range of CVP in both protocols. Based on approximations for comparing LBNP levels to blood loss (13), we chose the initial stages of the U.S. 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 (3 x 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 per the Hinojosa-Laborde et al., study in baboons where LBNP always followed blood loss (21). Either protocol was terminated early if: 1) 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 pre-syncope 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 3-lead electrocardiogram (ECG) was used to continuously record HR (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). Arterial oxygen saturation was monitored using a finger pulse oximeter and end-tidal CO$_2$ (ETCO$_2$) was collected from a nasal cannula (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). 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 ETCO$_2$ tracings were interfaced with a data acquisition system for offline analysis (WinDaq, DATAQ Instruments, Akron, OH, USA).

Cerebral blood velocity. Subjects were imaged using a 2-MHz Doppler probe (Transcranial Doppler (TCD), Neurovision System, Multigon, Yonkers, NY, USA) to estimate middle cerebral artery blood velocity (MCAv). The basal portion of the left MCA was insonated by placing the probe over the temporal bone just above the zygomatic arch in front of the ear. The Doppler signal was optimized by varying the sample volume depth in incremental steps and varying 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 Corp., Irvine, CA, USA) 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. Preservative/anticoagulant bags (63 mL anti-coagulant citrate phosphate dextrose solution) were placed below the level of the bed to allow blood to transfer 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. Following a 5 min baseline period, 3 aliquots of 333 ml of blood was removed as described. A 5 min measurement period separated 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 re-infused 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 3 stages of a commonly used 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 was collected at 500 Hz (WinDaq, DATAQ Instruments, Akron, OH, USA) and stored on a laboratory computer for off-line analysis with signal processing software (WinDaq, DATAQ Instruments, Akron, OH, USA; WinCPRS, Absolute Aliens, Turku, Finland). All variables of interest (HR, blood pressure, CVP, ETCO$_2$, 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, Absolute Aliens, Turku, Finland) based on the brachial arterial pressure
waveform (23). CO was derived using the calculated SV and HR obtained by ECG. A portion of this hemodynamic data is presented in a publication for N=12 (25), specifically the HR, MAP, SV, CO, and CVP responses. 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 interpolating linearly and resampling 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 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 versus LBNP as per Johnson et al. (25). Linear mixed effect model analysis with repeated measures was used to assess the relationship between mean MCAv versus MAP across LBNP and blood loss for all subjects; ETCO$_2$ was also included as a co-variate due to the independent effects of arterial CO$_2$ on mean MCAv and MAP. Condition × stage (2 × 4) repeated measures ANOVAs were used to determine if values obtained during the LBNP protocol were similar to the corresponding stages of the blood loss protocol. A one-way repeated measures ANOVA was used to compare the time of blood withdrawal across the 3 aliquots. If a significant main or 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 mean ± 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. The mean time for blood removal was 563 ± 49 sec for the first 333 ml, 489 ± 56 sec for the second 333 ml, and 467 ± 73 sec for the final 333 ml (P=0.195). Hematocrit increased with LBNP (baseline: 40.6 ± 0.9% vs.
termination: 41.9 ± 0.9; P=0.020) and decreased with hemorrhage (baseline: 40.8 ± 0.9% vs.
termination: 39.7 ± 0.9; 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 the 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 compared with 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 level 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 vs. 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 versus blood loss are presented in figure 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). ETCO_2 decreased at level 3 for the blood loss trial only, and respiration rate
decreased for the LBNP trial only.
Individual mean MCAv vs. MAP responses are presented in figure 3. There was inter-subject variability in these responses, and as a group, the slope of the line between MCAv and MAP was lower with LBNP compared with blood loss (LBNP: 0.41 ±0.03 cm/s/mmHg vs. blood loss: 0.66 ± 0.04 cm/s/mmHg; 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 cm/s/mmHg vs. 4.3 ± 2.4 cm/s/mmHg; P=0.23). LF and VLF power spectral density (PSD) for MAP and mean MCAv are shown in figure 4. There were no differences from baseline (P≥0.13) in PSD for MAP LF and VLF, or 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; figure 5). VLF coherence was consistently < 0.5 for both conditions across all levels, so phase and gain are not reported.

Discussion

This is the first study to systematically compare cerebral blood velocity responses between LBNP and actual hemorrhage in healthy human subjects. The key findings from this investigation are; 1) LBNP up to ~45 mmHg elicited greater reductions in central blood volume than hemorrhage up to ~1000 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 cerebrovascular conductance responses were similar between LBNP and blood loss conditions; and, 3) neither the LBNP nor
blood loss protocols induced changes in the relationship between MAP and mean MCAv as
determined by gain calculations in both the time domain and via transfer function analysis.

In 1940, Ebert and Stead reported the sequestration of approximately 15% of total
blood volume into the extremities (two legs and one arm) following rapid application of
tourniquets as a potential alternative to phlebotomy for the treatment of congestive heart
failure (16). 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 from human volunteers (i.e., one unit) suggested equivalency between one
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 of greater than one
unit (i.e., >500 ml) were performed in baboons (21) and in 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/mmHg LBNP, equating to blood loss of approximately 450,
1000, and 1600 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 two studies assessing cerebral oxygen saturation responses
(via NIRS) to blood loss protocols of ≤500 ml, Colier et al., (7) and Torella et al., (48) reported
increases in deoxy-hemoglobin concentration, and decreases in oxy-hemoglobin concentration and cerebral oxygen saturation. As NIRS measures a sample volume consisting of a mix of approximately 25% arterial and 75% venous blood (33, 38), decreases in oxy-hemoglobin and increases in deoxy-hemoglobin 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 ~1000 ml) in humans, and to compare these responses to LBNP. As reported for a larger group of subjects (N=12) (25), LBNP to -45 mmHg elicits greater reductions in central blood volume than 1000 ml of blood loss. As a consequence, mean MCAv was reduced by ~11% with LBNP compared with a decrease of just ~3% with blood loss, MAP decreased by ~8% (LBNP) and ~2% (blood loss), and the relationship between mean MCAv and MAP was lower for LBNP compared with blood loss (figure 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 figure 3, and the hemodynamic data presented by Johnson et al., (25), 1000 ml of blood loss implemented in the present protocol appears equivalent to LBNP of between -15 to -30 mmHg. This is in contrast to estimations using SV responses from baboons exposed to both LBNP and hemorrhage (0.25 ml/kg/mmHg, as described previously) (21), where -45 mmHg LBNP would be equivalent to 1000 ml of blood loss in the subjects used in the present investigation (i.e., body weight of approx. 90 kg). Prospective matching of both CVP responses and the time course of blood withdrawal and LBNP exposure between the two protocols, as per Hinojosa-Laborde et al., (21)
may address these differences in central hypovolemia observed in the current investigation, and allow for more accurate calculations of equivalency.

LF oscillatory power for both MAP and mean MCAv did not change from baseline under either LBNP or blood loss conditions. 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 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 only with LBNP and not blood loss as reported by Johnson et al., (25). The small subject number combined with high inter-subject variability in MAP LF responses under both protocols also contribute to this finding. As oscillations in arterial pressure are the primary driving factor for increased MCAv oscillations, it is not surprising that MCAv LF power did not change under either protocol.

Assessing the relationship between arterial pressure and cerebral blood velocity oscillations 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 (<0.5) across time and condition indicates an independence of flow from pressure within
332 this frequency range (54). While coherence between MAP and mean MCAv was consistently >
333 0.5 in the LF range, transfer function gain and phase did not change with either LBNP or blood
334 loss, and were not different between conditions. These findings are in contrast with a number
335 of studies that show either a reduction (41) or increase (55) in MAP-mean MCAv gain during
336 LBNP of similar magnitude. In particular, Zhang et al., (55) suggested that simultaneous
337 increases in the magnitude of oscillations in both arterial pressure and cerebral blood velocity
338 and the subsequent increase in MAP-mean MCAv gain, represented attenuated cerebral
339 autoregulation, that may, in turn, predispose individuals to presyncope. The stability of MAP-
340 mean MCAv gain and phase reported in the current investigation is most likely associated with
341 the stability of MAP and mean MCAv LF oscillations, and the high inter-subject variability
342 inherent in transfer function estimates of cerebral pressure-flow relationships, further
343 compounded by the small sample size utilized in this study. In the time domain, cerebral
344 autoregulation can also be assessed as the gain between changes in arterial pressure and
345 cerebral blood velocity (36, 40); in the present study this relationship was not altered under
346 either condition, and was not statistically distinguishable between conditions. Together, these
347 data suggest that cerebral pressure-flow relationships across multiple time scales (fast
348 component via transfer function analysis and slow component via time domain analysis) were
349 not affected by the magnitude of central hypovolemia induced by either LBNP or blood loss.
350 Other factors, including small reductions in arterial CO₂ and increased sympathetic drive may
351 also be contributing to the observed small decrease in MCAv with LBNP and blood loss.
352
353 **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, 1000 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 sub-maximal levels, differences in the time course of blood removal versus 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 the measurement of velocity is equivalent to flow as long as the caliber of the MCA does not change over the course of the intervention. While recent studies have indicated changes in MCA cross sectional area (CSA) with both increases (ETCO₂ ≥ 9 mmHg above baseline) and decreases in arterial CO₂ (ETCO₂ ≥ 13 mmHg below baseline) (14, 49), the magnitude of hypocapnia induced with both LBNP and blood loss in the current investigation (2-3 mmHg below baseline) was well below these levels. 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 render this limitation unlikely. Future assessment of cerebral blood flow of the extracranial feeding arteries (e.g., internal carotid artery, vertebral artery) (22, 37, 43, 52), and/or use of transcranial color-coded Doppler (TCCD) ultrasound (34, 51) during this type of investigation would allow for direct assessment of cerebral blood flow without relying on the assumption of constant arterial caliber. 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 compared with 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 about tolerance to central hypovolemia (24, 30, 32, 41). Inclusion of 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 1000 ml follow a similar trajectory, and the relationship between arterial pressure and cerebral blood velocity are not altered under these conditions. Careful matching of both the magnitude of central hypovolemia (e.g., via CVP) and 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.

Acknowledgements
The authors would like to thank Shelly Roberts, Sarah Wolhart, Timothy Curry, John Eisenach,
Christopher Johnson, Pamela Engrav, Branton Walker, Jennifer Taylor, and Luke Matzek for
their continued assistance throughout the project, and Yu Chieh Tzeng, Ph.D. for his valuable
advice on the linear mixed effect models analysis. The views, opinions, and/or findings
contained in this article are those of the authors and should not be construed as an official
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Sources of Funding
Funding provided by U.S. Army MRMC Combat Casualty Care Research Program Grants #
W81XWH-11-1-0823 (MJJ) and # W81XWH-11-2-0137 (CAR), NIH AG038067 (JNB), American
Heart Association Midwest Affiliate Grants 13POST-14380027 (BDJ) and 14PRE-18040000 (REH).

"This publication was made possible by CTSA Grant Number UL1 TR000135 from the National
Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of
Health (NIH), and from the US Army. Its contents are solely the responsibility of the authors and
do not necessarily represent the official view of NIH, the Department of the Army, or the
Department of Defense."
Conflict of Interest

None.
Figure Legends

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

Figure 2. Linear regression for amalgamated values for central venous pressure (CVP, Panel A), and stroke volume (SV, Panel B) responses between lower body negative pressure (LBNP) and blood loss conditions.

Figure 3. Individual plots of mean arterial pressure (MAP) versus mean middle cerebral artery velocity (MCAv) for all 9 subjects for LBNP (blue circles) and blood loss (red circles). Group responses are presented in the lower right panel (N=9).

Figure 4. Low frequency (LF) and very low frequency (VLF) power spectral density for mean arterial pressure (MAP; Panels A and C), mean middle cerebral artery velocity (MCAv; Panels B and D) during lower body negative pressure (LBNP) and blood loss. Data are mean ± SE.

Figure 5. Low frequency (LF) coherence, phase and gain between mean arterial pressure (MAP) and mean middle cerebral artery velocity (MCAv) during lower body negative pressure (LBNP) and blood loss. Data are mean ± SE.
### 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><strong>MAP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td></td>
<td>-15</td>
<td>-30</td>
<td>-45</td>
</tr>
<tr>
<td>Blood Loss</td>
<td></td>
<td>-333</td>
<td>-667</td>
<td>-1000</td>
</tr>
<tr>
<td><strong>CVP, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</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><strong>SV, mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</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><strong>HR, beats/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></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><strong>CO, L/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</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><strong>Mean MCAv, cm/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</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><strong>CVC, cm/s/mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</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><strong>ETCO₂, mmHg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</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><strong>Respiration rate, n</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>15 ± 1</td>
<td>13 ± 1†</td>
<td>13 ± 1†</td>
<td>14 ± 1†</td>
</tr>
<tr>
<td>Blood Loss</td>
<td>13 ± 1*</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 1</td>
</tr>
</tbody>
</table>

Mean ± SEM. Data are calculated from the final 3-min of each level. 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 carbon dioxide. *p<0.05 vs. LBNP at the same level; †p<0.05 vs. baseline of the same protocol.


FIGURE 1

LBNP Protocol

Baseline 5 min 5 min 5 min 45 min-75 min rest

Level (mmHg)

-15 -30 -45

Blood Loss Protocol

Baseline 5 min 5 min 5 min 5 min

Level (mL)

-333 -667 -1000
FIGURE 2

A

Blood Loss CVP (mmHg)

LBNP CVP (mmHg)

\[ y = 0.56x + 2.36 \]
\[ R^2 = 0.96 \]

B

Blood Loss SV (ml)

LBNP SV (ml)

\[ y = 0.55x + 40.96 \]
\[ R^2 = 0.99 \]
#1
\[ y = 1.4211x - 51.424 \quad R^2 = 0.6601 \]
\[ y = -2.0387x + 234.89 \quad R^2 = 0.0526 \]

#2
\[ y = 0.3888x + 36.424 \quad R^2 = 0.4035 \]
\[ y = 0.3889x + 33.975 \quad R^2 = 0.0014 \]

#3
\[ y = 0.6926x + 22.221 \quad R^2 = 0.9963 \]
\[ y = 0.8029x - 1.3634 \quad R^2 = 0.97 \]

#4
\[ y = 0.9434x - 34.74 \quad R^2 = 0.7908 \]
\[ y = 1.9297x - 127.22 \quad R^2 = 0.8428 \]

#5
\[ y = 1.6605x - 67.089 \quad R^2 = 0.9813 \]
\[ y = 2.9268x - 187.63 \quad R^2 = 0.5591 \]

#6
\[ y = 0.6418x + 11.903 \quad R^2 = 0.9613 \]
\[ y = 0.886x - 15.07 \quad R^2 = 0.9702 \]

#7
\[ y = 0.8452x + 0.316 \quad R^2 = 0.6518 \]
\[ y = 1.9765x - 129.27 \quad R^2 = 0.8839 \]

#8
\[ y = 0.6807x + 9.6175 \quad R^2 = 0.9065 \]
\[ y = -0.1954x + 96.76 \quad R^2 = 0.3644 \]

#9
\[ y = 0.1922x + 27.561 \quad R^2 = 0.0569 \]
\[ y = 1.3683x - 66.241 \quad R^2 = 0.9286 \]
FIGURE 4

A

B

C

D

MAP LF (mmHg²)

MCAv LF (cm/s²)

MAP VLF (mmHg²)

MCAv VLF (cm/s²)

Level

Level

Level

Level

Baseline 1 2 3

Baseline 1 2 3

Baseline 1 2 3

Baseline 1 2 3

LBNP  Blood Loss

Baseline 1 2 3

Baseline 1 2 3

Baseline 1 2 3

Baseline 1 2 3
FIGURE 5

A  
[Bar graph showing LF coherence with LBNP and Blood Loss levels.]

B  
[Bar graph showing LF Phase (degrees) with LBNP and Blood Loss levels.]

C  
[Bar graph showing LF Gain (cm/s/mmHg) with LBNP and Blood Loss levels.]
Coagulation Changes during Lower Body Negative Pressure and Blood Loss in Humans

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ABSTRACT

We tested the hypothesis that markers of coagulation activation are greater during lower body negative pressure (LBNP) than those obtained during blood loss (BL). We assessed coagulation using both standard clinical tests and thrombelastography in 12 men who performed a LBNP and BL protocol in a randomized order. LBNP consisted of 5-minute stages at 0, -15, -30, and -45 mmHg of suction. BL included 5 minutes at baseline and following three stages of 333 mL of blood removal (up to 1000 mL total). Arterial blood draws were performed at baseline and after the last stage of each protocol. We found that LBNP to -45mmHg is a greater central hypovolemic stimulus vs. BL, therefore the coagulation markers were plotted against central venous pressure (CVP) to obtain stimulus-response relationships using the linear regression line slopes for both protocols. Paired t-tests were used to determine if the slopes of these regression lines fell on similar trajectories for each protocol. Mean regression line slopes for coagulation markers vs. CVP fell on similar trajectories during both protocols, except for TEG α° angle (-0.42 ± 0.96 during LBNP vs. -2.41 ± 1.13 °/mmHg during BL, p<0.05). During both LBNP and BL coagulation was accelerated as evidenced by shortened R-times (LBNP 9.9 ± 2.4 to 6.2 ± 1.1 min, BL 8.7 ± 1.3 to 6.4 ± 0.4 min, both p<0.05). Our results indicate that LBNP models the general changes in coagulation markers observed during BL.

Key words: Blood Coagulation, Hemorrhage, Lower Body Negative Pressure, Blood Coagulation Tests, Humans, Central Hypovolemia
NEW AND NOTEWORTHY

Our study provides noteworthy data that directly compares blood coagulation activation induced by lower body negative pressure to those observed during blood loss in conscious humans.
INTRODUCTION

Hemorrhage is one of the leading causes of accidental death (1) and is the leading cause of death on the battlefield (8, 9). Activation of the coagulation system is vital following a hemorrhagic injury to reduce the risk of exsanguination. Consequently, studying the activation of the coagulation system during blood loss (BL) is of upmost importance so new therapies and treatment algorithms, such as fluid resuscitation, can be developed. However, using invasive methods to experimentally induce BL is challenging to perform in humans.

Lower body negative pressure (LBNP) is a technique that is used as a non-invasive surrogate to study many of the physiological responses to BL (4, 15, 18). LBNP sequesters circulating blood in the lower body thereby reducing central blood volume and mimicking hemodynamic responses generated during BL (4, 15, 18). However, it is unclear if markers of coagulation system activation respond similarly during these protocols. Reductions in central blood volume by LBNP (38) or orthostatic stress (10, 21, 36) activate the coagulation cascade, therefore it is likely that central hypovolemia during BL elicits comparable changes in coagulation when the degree of central hypovolemia is similar between LBNP and BL.

In spite of the similarities between the hemodynamic responses to LBNP and BL, these protocols cause central hypovolemia in fundamentally different ways that might cause differential coagulation responses. The suction applied during LBNP produces a pressure gradient that pulls fluid from the intravascular compartment to the extravascular space in the lower body resulting in hemoconcentration (5, 29, 34). Plasma protein concentration and blood viscosity both increase, which creates a procoagulant milieu due to increased interactions between coagulation factors and cellular contributors to coagulation (12, 17, 21). However, BL
has the opposite effect. The reduction in circulating blood volume causes fluid to shift from the extravascular space to the intravascular space resulting in hemodilution (7, 27, 39) and a lower blood viscosity (3). The divergent hematocrit and viscosity responses to LBNP and BL may differentially influence coagulation responses during these two protocols, despite similar hemodynamic responses.

To explore whether LBNP can be used as a model for BL in studies of coagulation activation during BL, we compared markers of coagulation activation during LBNP to those generated during BL in humans. We hypothesized that the stimulus-response relationships of central hypovolemia to coagulation responses during LBNP would be greater than those observed during BL for a given central hypovolemic stimulus due to the increases in blood viscosity and hemoconcentration during LBNP.
METHODS

Subjects

Twelve healthy men (age: 32 ± 2 years; height: 181.8 ± 2.0 cm; weight: 88.4 ± 2.5 kg; BMI: 26.7 ± 0.5 kg/m²) participated in this study, which was approved by the Institutional Review Board. Prior to participation, all subjects provided written informed consent after all procedures and study risks were fully explained. Subjects were non-obese (BMI < 30), non-smokers, did not take any medications and all subjects reported to be free of cardiovascular, respiratory, neurologic, and metabolic disease. Following an overnight fast, subjects reported to the Clinical Research Trial Unit (CRTU) of Mayo Clinic at 07:00. Upon reporting to the CRTU, subjects consumed a small breakfast bar (Cliff Bar; Shelton, CT, USA; 240 kcals) and drank 250 mL of water. Subjects were studied in the supine position in a temperature-controlled room (20-22°C).

Experimental Design

The study timeline is presented in Figure 1. The experimental design and selection of LBNP and BL protocols have been detailed previously and the comprehensive hemodynamic and circulating catecholamine responses to these protocols have been reported (18, 26). Briefly, the objective of this analysis was to determine if changes in coagulation markers, obtained from our previous investigations (18, 26), were similar across a broad range of CVP elicited by LBNP and BL. Both protocols were performed on the same day and the order was randomized. Subjects were supine for 60-90 minutes prior to initiating the first protocol (≥ 30 minutes following invasive instrumentation). After the first protocol, subjects rested quietly for 45-75 minutes in the supine position. A longer duration was needed after the BL protocol to allow for blood re-
infusion. Arterial blood samples were collected at baseline and at the conclusion of each protocol. During the LBNP protocol, blood samples were collected shortly before suction was terminated. The protocols were terminated if mean arterial pressure fell by 30%, systolic blood pressure dropped below 80 mmHg, or the subject began to experience symptoms of pre-syncope or syncope.

**LBNP protocol**

Subjects laid in an LBNP chamber sealed at the iliac crest. The LBNP protocol was based on the first 3 stages of the protocol frequently used by the U.S. Army Institute of Surgical Research (4) (Figure 1). Following a 5-minute baseline period, the protocol commenced and consisted of 5-minute stages at 15, 30, and 45 mmHg of LBNP. Subjects were instructed not to move throughout the protocol.

**Blood Loss protocol**

A 14-gauge catheter was inserted into an antecubital vein for blood removal during the BL protocol. Preservative/anticoagulant bags (63 mL anti-coagulant citrate phosphate dextrose solution) were positioned below the subject to facilitate blood transfer from the subject to the blood collection bags via gravity. Following a 5-minute baseline period, 3 aliquots of 333 mL of blood were removed. A 5-minute period separated each aliquot to emulate the LBNP stages. In two subjects, a blood pressure cuff was inflated around the upper arm to 40 mmHg to enhance the rate of blood removal and this cuff pressure was released prior to all measurements. As blood was collected, it was weighed to determine the volume of blood removed by multiplying the weight of the blood by 1.06 mL/g. The removed blood was kept in the study room (20-22°C) and was re-infused at a rate of 20 mL/min into the antecubital vein following the BL protocol.
**Hemodynamic measurements**

Heart rate (HR) was measured from a 3-lead ECG (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). Arterial blood pressure was measured beat-by-beat by a brachial artery catheter. Central venous pressure (CVP) was measured using a peripherally inserted central catheter (PICC). All lines were placed aseptically with local anesthesia by anesthesiologists. The PICC was introduced through an antecubital vein and advanced to the level of the superior vena cava. Placement of the PICC was estimated using external measurement of the distance from the antecubital fossa to the manubrium and was verified by the identification of a typical CVP waveform. The arterial catheter and the PICC were connected to pressure transducers (FloTrac, Edwards Lifesciences Corp., Irvine, CA, USA) placed at the mid-axillary line). Intra-arterial pressures were consistent with Riva-Rocci blood pressures.

**Hemoconcentration Measures**

Blood samples were analyzed by the Immunochemistry Core Laboratory of the CRTU of the Mayo Clinic Center for Clinical and Translational Science. Blood samples collected in 3 mL EDTA tubes were analyzed for hemoglobin (Hb), hematocrit (Hct), red blood cell count (RBC) and platelet count. Total blood volume at baseline ($BV_0$) was estimated according to Retzlaff et al. (25) using the following equation:

$$BV_0 = 31.9 \times \text{height (cm)} + 26.3 \times \text{weight (kg)} - 2402$$

Changes in blood volume and the estimated percentage change in plasma volume from pre to post LBNP and from pre to post BL ($\%dPV$) were determined using the formula by Dill
and Costill(6). Changes in hemoglobin were corrected for the amount of blood withdrawn and baseline plasma percentage was defined as 1-Hct.

Hemostatic Activity of Arterial Blood

Prothrombin Time (PT) and Activated Partial Thrombin Time (APTT). Arterial blood was drawn into 3 mL sodium citrate tubes. Samples were centrifuged for 10 minutes at 3000 x g. Platelet-poor plasma was aliquoted into tubes and stored in a freezer at -80°C until assayed. Assays were performed using a coagulation analyzer (STA-R Evolution, France) and Prothrombin time (PT) and activated partial thrombin time (APTT) were determined by standard coagulometric methods using standard reagents (PT = HemosIL RecombiPlasTin 2G; APTT = HemosIL SynthASil, Instrumentation Laboratory, Bedford, MA, USA).

Whole Blood Thromboelastography (TEG). TEG was performed on 1.5 mL of citrated whole arterial blood using a TEG 5000 device (Haemonetics Corp., Braintree, MA, USA) within four minutes of blood sampling. Samples were activated with kaolin and the analyzer produced a graphical representation of clot formation, strength, and breakdown. We recorded the following values: R, the period of time from initiation of the test to initial fibrin formation; K, time of beginning of clot formation until the amplitude of the thromboelastogram reaches 20 mm; α angle, the angle between the line in the middle of the TEG tracing and the line tangential to the developing ‘body’ of the TEG tracing which is reflective of the rate of fibrin polymerization; maximum amplitude (MA), expressing the maximum strength in millimeters of the final clot;
and lysis 30 (LY30) and lysis 60 (LY60) which reflect fibrinolysis and are expressed as the percent decrease in amplitude at 30 and 60 minutes, respectively, after MA.

**Catecholamines**

Plasma epinephrine and norepinephrine concentrations were determined from 4.5 mL of arterial blood using HPLC after prior alumina extraction (ESA Coulochem III, Dionex, Sunnyvale, CA, USA).

**Data and statistical analysis**

Data were collected and analyzed off-line using signal processing software (WinDaq, DATAZ Instruments, Akron, OH, USA). Hemodynamic data were analyzed and averaged over the last 2 minutes of baseline and final stages of LBNP and BL for statistical analysis. All hemodynamic signals were automatically peak-detected and manually checked. Stroke volume (SV) was determined using WinCPRS software (Absolute Aliens, Oy, Finland) by selecting the area under the arterial blood pressure curve and calculated using Modelflow (35), which simulates flow using a three-element Windkessel model. Cardiac output was calculated as the product of heart rate and stroke volume. Protocol (LBNP/BL) × time (Baseline/Protocol termination) repeated measures ANOVA was used to determine if values obtained during the LBNP protocol were similar to values during the BL protocol. If a significant main or interaction effect was obtained, Tukey’s post hoc test was performed to determine where differences existed. If data were not normally distributed the Wilcoxon Signed Rank test was used. As a post hoc test, we compared the relationship between coagulation markers and hypovolemia during BL and LBNP to adjust for differences in hypovolemia. We performed this analysis by plotting the
coagulation markers against CVP to obtain stimulus-response relationships using the linear regression line slopes as we (18) and others (24) have done previously. Previous experimental investigations have found that CVP decreases early and linearly during both LBNP and BL protocols (11, 14-16, 18, 22, 24, 31). Paired t-tests were used to determine if the slopes of these regression lines fell on similar trajectories between the two protocols. Group data are presented as mean ± SE. P values are reported.
RESULTS

Of the 12 subjects, 2 subjects did not complete both protocols (both subjects completed 667 mL of BL and 30 mmHg of LBNP); additionally, one subject did not complete the LBNP protocol (completed 30 mmHg of LBNP), and one subject did not complete the BL protocol (completed 333 mL of BL). These protocols were terminated early due to pre-syncope symptoms or syncope. Data obtained from the final completed stage were used for these subjects. The mean time for 1000 mL of blood removal was 1402 ± 157 seconds (~ 43 mL/min). The mean hemodynamic values obtained during both protocols are presented in Table 1 and are reported elsewhere\(^1\). The mean TEG coagulation values across the range of CVP during LBNP and BL are displayed in Figure 2. Changes in complete blood counts are shown in Table 2. The mean standard coagulation tests and the TEG lysis values at baseline and protocol termination are displayed in Tables 3 and 4. The mean catecholamine concentrations are presented in Table 5.

Effects of LBNP and BL on Hemodynamics

Table 1 shows that both LBNP and BL evoked pronounced hemodynamic changes from baseline to protocol termination. At baseline, CVP (LBNP 7.3 ± 0.6 BL 6.1 ± 0.6 mmHg, p = 0.024) was slightly lower during BL while SV (LBNP 83.2 ± 2.7 BL 89.5 ± 2.7 mL, p = 0.016), and CO (LBNP 5.0 ± 0.3 BL 5.3 ± 0.3 L/min, p = 0.045) were slightly higher. At protocol termination, CVP (LBNP -0.2 ± 0.6 BL 1.8 ± 0.8 mmHg, p ≤ 0.001), SV (LBNP 54.1 ± 3.3 BL 70.5 ± 2.7 mL, p ≤ 0.001) and CO (LBNP 4.1 ± 0.1 BL 4.7 ± 0.2 L/min, p = 0.002) were lower during LBNP, and HR was higher (LBNP 80 ± 5.1 BL 67 ± 2.6 bpm, p ≤ 0.001) versus BL. Overall, 45 mmHg of LBNP caused greater changes in hemodynamic parameters than 1000 mL of BL.
Effects of LBNP and BL on Hemoconcentration

As we expected, several markers indicated that LBNP caused hemoconcentration, while BL induced hemodilution (Table 2). After LBNP there was an increase in hemoglobin (14.2 ± 0.4 to 14.7 ± 0.4 g/dL, p = 0.003) and hematocrit (41 ± 0.8 to 42 ± 0.8 %, p = 0.001) and a decrease in estimated plasma volume (59 ± 0.8 to 56 ± 0.9 %, p ≤ 0.001) compared to baseline values. BL induced a decrease in hemoglobin (14.3 ± 0.4 to 14.0 ± 0.4 g/dL, p = 0.006) and hematocrit (41 ± 0.8 to 40 ± 0.9 %, p = 0.006) and an increase in estimated plasma volume (59 ± 0.9 to 61 ± 1.1 %, p = 0.004) compared to baseline values. At protocol termination, hemoglobin (p ≤ 0.001) and hematocrit (p ≤ 0.001) were lower in BL versus LBNP and estimated plasma volume (p ≤ 0.001) was greater in BL when compared to LBNP.

Effects of LBNP and BL on Standard Laboratory Coagulation Tests

Mean PT (12.2 ± 0.2 to 12.0 ± 0.1 s, Wilcoxon signed rank post hoc test p = 0.026) and APTT (32.2 ± 0.7 to 31.0 ± 0.8 s, Wilcoxon signed ranked post hoc test p = 0.047) were quicker after LBNP vs. baseline (Table 3).

Effects of LBNP and BL on TEG values

At protocol termination, R times were quicker versus baseline for both LBNP and BL protocols (LBNP 9.9 ± 2.4 to 6.2 ± 1.1 BL 8.7 ± 1.3 to 6.4 ± 0.4 min, Wilcoxon signed rank post hoc test p = 0.037 and p = 0.039, Figure 2) and these relative changes were not different from each other. Regression line slopes produced from the relationship between TEG measures and CVP fell on similar trajectories during LBNP and BL, except for the slope of α angle vs. CVP (-0.42 ± 0.96 during LBNP vs. -2.41 ± 1.13 °/mmHg during BL, p = 0.046).
Effects of LBNP and BL on Catecholamine Levels

Epinephrine (LBNP 53 ± 7 to 144 ± 30 BL 49 ± 7 to 103 ± 19 pg/mL, p ≤ 0.001 and p = 0.002) and norepinephrine (LBNP 148 ± 20 to 354 ± 44BL 155 ± 22 to 211 ± 29 pg/mL, p ≤ 0.001 and p = 0.043) concentrations were both elevated at protocol termination in both LBNP and BL protocols (Table 5). Norepinephrine levels were higher during LBNP versus BL at protocol termination (p = 0.003).
The general results of this study indicate that BL and LBNP induce similar coagulation response trajectories across a wide range of CVP. Only the slope of TEG α angle was statistically different between protocols.

Central hypovolemia, induced by either BL or LBNP, alters blood coagulation status, which is evidenced by a reduction in R-time obtained from TEG. The greater degree of hypovolemia induced by LBNP in this study also demonstrated coagulation activation by reducing PT and APTT. This is in line with the reported activation of coagulation during LBNP (38) and other orthostatic challenges (10, 21, 36). Because direct vascular damage due to arterial and venous catheter placement was likely minimal in our study, it is probable that other factors contributed to the coagulation response. The increase in circulating catecholamines in both LBNP and BL protocols may have contributed to the hypercoagulable milieu. Intravenous administration of epinephrine has been shown to accelerate blood coagulation (2, 33). Additionally, hemostatically active von Willebrand factor, clotting factor VIII, and tissue-type plasminogen activator are released from endothelial cells (32) or the spleen (19) into the circulation via stimulation of endothelial β2-adrenoreceptors (32). This mechanism of epinephrine-induced release of coagulation factors has also been implicated during other orthostatic challenges (10, 36). Splenic release of platelets has also been found following adrenergic stimulation (19). Therefore, it is likely that sympathoexcitation and release of epinephrine during BL and LBNP contribute to the coagulation response. We found significant increases in epinephrine after both LBNP and BL, suggesting that this hormone plays an important role in activating the coagulation system.
We observed a very small decrease in PT and APTT times during LBNP. Other investigators have also found a decrease in PT time during orthostatic challenges (21). Our observation is likely due in part to a reduction in plasma volume by ~4% during LBNP. However, plasma volume increased by ~3% during BL. This might explain the small increase in PT and almost no change in APTT from baseline to protocol termination during BL (Table 3).

Because of the divergent effects of LBNP and BL on plasma volume, it appears as though plasma markers of coagulation might not be appropriate to assess coagulation during LBNP and experimental BL.

Data obtained from TEG analysis of whole blood might be a better method to assess changes in coagulation than plasma markers due to the changes in plasma volume during LBNP and BL that we observed. TEG analysis has also been shown to be a better indicator of hemostasis than PT (20, 23). Recently, Zaar et al. demonstrated a reduction in time to fibrin formation after LBNP to presyncope demonstrated by shortened R-time (37). However, PT and APTT were unaffected. TEG R-times were shortened during both LBNP and BL protocols in our study. As little as ~300 mL of blood loss during surgery (30) and 480 mL of blood removal (28) have both been shown to reduce R-time and increase α angle, or the rate of clot formation. In another study by Zaar and colleagues (39), a graded reduction in R-time from 450 mL to 900 mL of blood removal as well as an increase in α angle was observed, but only following 900 mL of blood loss. However, we did not observe a large increase in the α angle following 1000 mL of BL or following LBNP. This discrepancy might have occurred due to differences in the rate of blood removal (~43 mL/min in our study vs. ~30 mL/min). Additionally, we removed blood into 3 separate 333 mL aliquots whereas Zaar et al. (39) used two 450 mL aliquots to protocol completion when compared to our protocol. Although α angle was not statistically
distinguishable from baseline to protocol termination in both LBNP and BL protocols, the stimulus-response trajectory of CVP-α angle was steeper during BL when compared to LBNP. This discrepancy is primarily due to the differences in CVP achieved at the end of each protocol, as α angle was not different between protocols (Figure 2). Contrary to a recent study that found increased LY 60 (37) after LBNP, we did not find any differences in TEG measured fibronolysis (Table 4). This may have been the result of a large interindividual variability in TEG lysis values.

The more robust change in whole blood TEG-R time after both LBNP and BL compared to the very subtle change in platelet-poor plasma based assays PT and APTT after LBNP indicates that platelets contribute significantly to coagulation acceleration during central hypovolemia. Consistent with this idea, platelet activation, demonstrated by increased exposure of active glycoprotein 2b/3a, has been shown after LBNP (37). We observed an increase in platelet count after both LBNP and BL. This increase occurred despite hemodilution during BL, which suggests that platelets were released from the spleen.

Methodological considerations

Several methodological considerations pertain to our study. First, we collected blood only at baseline and at the termination of each protocol. Collecting multiple samples throughout both protocols would have allowed us to identify if a graded hypercoagulable state exists throughout a range of central hypovolemia within each subject (30, 39). Second, we did not match CVP between protocols. The goal of our study was to determine if changes in coagulation markers were similar across a broad range of central hypovolemia elicited by LBNP and BL. However, LBNP caused a greater reduction in central blood volume indicated by lower CVP, stroke
volume, and cardiac output values as well as higher heart rate and norepinephrine values when compared to BL. If we had matched CVP between the two protocols, we might have been able to provide additional information about how comparable the coagulation responses are throughout LBNP and BL. Third, we have no direct recordings of sympathetic nerve activity; this would have provided additional information regarding the contribution of the sympathetic nervous system in the activation of blood coagulation during central hypovolemia. Fourth, the protocol times were not matched. The time between the first and second blood draw was 20 minutes during the LBNP protocol and approximately 45 minutes during the BL protocol. This could introduce a difficulty in interpreting the results if there were a time effect on coagulation in the subjects due to prolonged rest in a supine position. However when we compared the baseline TEG R values of the first protocol that subjects underwent versus the baseline values of the second protocol, the R times were statistically indistinguishable (paired t-test $p = 0.219$), suggesting that supine position did not contribute significantly to observed changes in coagulation status. Fourth, subjects were randomized to LBNP and BL and underwent both protocols on the same day. Our assumption was that baseline cardiovascular and coagulation variables would not be different, regardless of protocol randomization order. We tested our assumption and performed paired t-tests on LBNP and BL Baseline hemodynamic and coagulation variables. We found that subjects who performed LBNP first had slightly lower CVP (~1.5 mmHg) and slightly higher SV (~10 mL) at baseline BL ($p = 0.025$ and $p = 0.032$ respectively). Perhaps this had a lasting effect on the greater increase in catecholamines during LBNP on cardiac contractility. This small order effect might explain the slight differences in these hemodynamic parameters we found between baselines. Finally, the method of Dill and Costill (6) was used for determinations of relative plasma volume changes. This requires that the
distribution of red cells throughout the vascular bed is similar between LBNP and BL (13). However, the distribution of red blood cells throughout the vasculature might have been different between protocols leading to underestimation of changes in plasma volume.

CONCLUSIONS

Our results indicate that 45 mmHg of LBNP elicited slightly greater increases in plasma measures of coagulation (PT and APTT) than 1000 mL of BL. When coagulation activation was measured in whole blood by TEG, we saw a robust change in R-time during both protocols. This indicates that cellular contributions to the coagulation response during central hypovolemia are important. The stimulus-response trajectories for most markers of coagulation versus CVP were similar between the two protocols, which indicates that acceleration of the coagulation system is comparable between LBNP and BL within the range of central hypovolemia that we tested. Therefore, LBNP appears to be a useful surrogate to study the coagulation system during BL.

ACKNOWLEDGEMENTS

We thank the subjects who participated in this study. We also thank Christopher P. Johnson, Margaret McGill-Zimny, Shelly K. Roberts, David Warren, and Sarah C. Wolhart for their assistance.

GRANTS

Support for this study was provided by U.S. Army MRMC Combat Casualty Care Research Program Grant W81XWH-11–1-0823, American Heart Association Midwest Affiliate Grant 13POST-14380027 to B.D.J., and by Dutch Heart Foundation E. Dekker Stipend 2012SB013 to N.V.H.
Table 1. Changes in hemodynamic variables with LBNP and BL.

<table>
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<tr>
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<th>Baseline</th>
<th>Termination</th>
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<tr>
<td>CVP (mmHg)</td>
<td></td>
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<tr>
<td>LBNP</td>
<td>7.3 ± 0.6</td>
<td>-0.2 ± 0.6*</td>
</tr>
<tr>
<td>BL</td>
<td>6.1 ± 0.6†</td>
<td>1.8 ± 0.8*†</td>
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<tr>
<td>HR (bpm)</td>
<td></td>
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<tr>
<td>LBNP</td>
<td>60 ± 2.5</td>
<td>80 ± 5.1*</td>
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<tr>
<td>BL</td>
<td>60 ± 2.8</td>
<td>67 ± 2.6*†</td>
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<tr>
<td>MAP (mmHg)</td>
<td></td>
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<tr>
<td>LBNP</td>
<td>93.5 ± 2.3</td>
<td>84.5 ± 4.7*</td>
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<tr>
<td>BL</td>
<td>91.8 ± 1.9</td>
<td>87.0 ± 2.7</td>
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<tr>
<td>SV (mL)</td>
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<tr>
<td>LBNP</td>
<td>83.2 ± 2.7</td>
<td>54.1 ± 3.3*</td>
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<tr>
<td>BL</td>
<td>89.5 ± 2.7†</td>
<td>70.5 ± 2.7†</td>
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<tr>
<td>CO (L/min)</td>
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<tr>
<td>LBNP</td>
<td>5.0 ± 0.3</td>
<td>4.1 ± 0.1*</td>
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<tr>
<td>BL</td>
<td>5.3 ± 0.3†</td>
<td>4.7 ± 0.2*†</td>
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LBNP = lower body negative pressure; BL = blood loss.
Values are means ± standard error, n = 12.
*Different from Baseline (P < 0.05); †Different vs. LBNP
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<th>Baseline</th>
<th>Termination</th>
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<tr>
<td><strong>Hgb (g/dL)</strong></td>
<td></td>
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<tr>
<td>LBNP</td>
<td>14.2 ± 0.4</td>
<td>14.7 ± 0.4*</td>
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<tr>
<td>BL</td>
<td>14.3 ± 0.4</td>
<td>14.0 ± 0.4*†</td>
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<tr>
<td><strong>Hct (%)</strong></td>
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<tr>
<td>LBNP</td>
<td>41 ± 0.8</td>
<td>42 ± 0.8*</td>
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<tr>
<td>BL</td>
<td>41 ± 0.8</td>
<td>40 ± 0.9*†</td>
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<tr>
<td>*<em>RBC (<em>10^12/L)</em></em></td>
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<tr>
<td>LBNP</td>
<td>4.8 ± 0.1</td>
<td>5.0 ± 0.1*</td>
</tr>
<tr>
<td>BL</td>
<td>4.8 ± 0.1</td>
<td>4.7 ± 0.1*†</td>
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<tr>
<td><strong>Plasma volume (%)</strong></td>
<td></td>
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<tr>
<td>LBNP</td>
<td>59 ± 0.8</td>
<td>56 ± 0.9*</td>
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<tr>
<td>BL</td>
<td>59 ± 0.9</td>
<td>61 ± 1.1*†</td>
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<tr>
<td>*<em>Platelet count (<em>10^9/L)</em></em></td>
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<tr>
<td>LBNP</td>
<td>194 ± 7</td>
<td>212 ± 10*</td>
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<tr>
<td>BL</td>
<td>186 ± 9</td>
<td>200 ± 11*</td>
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LBNP = lower body negative pressure; BL = blood loss.
Values are means ± standard error, n = 12.
*Different from Baseline (P < 0.05); †Different from lower body negative pressure (P < 0.05).
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<thead>
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<tr>
<td>PT (s)</td>
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<tr>
<td>LBNP</td>
<td>12.2 ± 0.2</td>
<td>12.0 ± 0.1*</td>
</tr>
<tr>
<td>BL</td>
<td>12.1 ± 0.2</td>
<td>12.2 ± 0.3</td>
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<tr>
<td>APTT (s)</td>
<td></td>
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<tr>
<td>LBNP</td>
<td>32.2 ± 0.7</td>
<td>31.0 ± 0.8*</td>
</tr>
<tr>
<td>BL</td>
<td>32.6 ± 0.9</td>
<td>32.4 ± 0.9</td>
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LBNP = lower body negative pressure; BL = blood loss. Values are means ± standard error, n = 12. *Different from Baseline (P < 0.05)
Table 4. Effects of LBNP and BL on clot lysis measures.

<table>
<thead>
<tr>
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<th>Baseline</th>
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<tr>
<td>LY30 (%)</td>
<td></td>
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</tr>
<tr>
<td>LBNP</td>
<td>1.6 ± 0.4</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td>BL</td>
<td>2.3 ± 1.1</td>
<td>3.1 ± 1.6</td>
</tr>
<tr>
<td>LY60 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>5.6 ± 1.1</td>
<td>7.5 ± 2.5</td>
</tr>
<tr>
<td>BL</td>
<td>6.1 ± 1.9</td>
<td>7.4 ± 2.5</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure; BL = blood loss. Values are means ± standard error, n = 12.
Table 5. Effects of LBNP and BL on catecholamine levels.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>148 ± 20</td>
<td>354 ± 44*</td>
</tr>
<tr>
<td>BL</td>
<td>155 ± 22</td>
<td>211 ± 29*†</td>
</tr>
<tr>
<td>Epinephrine (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>53 ± 7</td>
<td>144 ± 30*</td>
</tr>
<tr>
<td>BL</td>
<td>49 ± 7</td>
<td>103 ± 19*</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure; BL = blood loss. Values are means ± standard error, n = 12.
*Different from Baseline (P < 0.05); †Different from lower body negative pressure (P < 0.05).
Figure 1. Timeline of the lower body negative pressure and blood loss protocols. The order of the protocols was randomized. When the lower body negative pressure protocol was performed first, 45 minutes of quiet rest was given between protocols to ensure hemodynamic variables returned to baseline. To allow for the reinfusion of removed blood, 75 minutes of quiet resting was given to allow for hemodynamic variables to return to baseline between protocols when blood loss occurred first. Blood was drawn at baseline and during the last stage of each protocol.

Figure 2. Mean ± SEM TEG values (A) R, (B) K, (C) alpha angle, and (D) MA plotted against mean CVP ± SEM at baseline and immediately after protocol termination during the LBNP and BL protocols. All response trajectories were similar between LBNP and BL protocols with the exception of alpha angle, which was steeper during BL versus LBNP.

*Different versus BL; p = 0.046.
REFERENCES


Randomized first protocol
45-75 minutes rest
in between protocols

LBNP (mmHg)

BL (total in mL)
Figure 2.
Reductions in Central Venous Pressure by Lower Body Negative Pressure or Blood Loss Elicit Similar Hemodynamic Responses

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Running Head: Hemodynamic responses during LBNP and blood loss

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ABSTRACT
The purpose of this study was to compare hemodynamic and blood analyte responses to reduced central venous pressure (CVP) and pulse pressure (PP) elicited during graded LBNP to those observed during graded BL in conscious humans. We hypothesized that the stimulus-response relationships of CVP and PP to hemodynamic responses during LBNP would mimic those observed during BL. We assessed CVP, PP, heart rate, mean arterial pressure (MAP), and other hemodynamic markers in 12 men during LBNP and BL. Blood samples were obtained for analysis of catecholamines, hematocrit, hemoglobin, arginine vasopressin, and blood gases. LBNP consisted of 5-minute stages at 0, 15, 30, and 45 mmHg of suction. BL consisted of 5 minutes at baseline and following three stages of 333 mL of hemorrhage (1000 mL total). Individual $r^2$ values and linear regression slopes were calculated to determine if the stimulus (CVP and PP) - hemodynamic response trajectories were similar between protocols. The CVP-MAP trajectory was the only CVP-response slope that was statistically different during LBNP when compared to BL (0.93 ± 0.27 vs. 0.13 ± 0.26; $P = 0.037$). The PP-heart rate trajectory was the only PP-response slope that was statistically different during LBNP when compared to BL (-1.85 ± 0.45 vs. -0.46 ± 0.27; $P = 0.024$). Norepinephrine, hematocrit, and hemoglobin were all lower at termination in the BL protocol when compared to LBNP ($P < 0.05$). Consistent with our hypothesis, LBNP mimics the hemodynamic stimulus-response trajectories observed during BL across a significant range of CVP in humans.

Key Words: hemorrhage, central hypovolemia, heart rate, blood pressure, stroke volume
INTRODUCTION

Hemorrhage is one of the main causes of death associated with civilian trauma (16, 33, 35) and it is the leading cause of potentially survivable death on the battlefield (3, 14). Therefore, identifying physiological changes in response to blood loss (BL) is important because it can promote timely assessment of patient status and appropriate triage. Clinical studies of BL are difficult due to the heterogeneity of patients, injuries, volume of blood lost, and resuscitation efforts. Standardized laboratory studies where graded hypovolemia is induced via BL or dehydration provide a standardized way of measuring the effects of hypovolemia; however the removal of an adequate volume of blood to mimic clinically relevant hemorrhage in conscious humans in a laboratory is invasive and may not be practical. Therefore, lower body negative pressure (LBNP) is frequently used to simulate BL in conscious humans. The application of LBNP results in a central volume shift to the lower body which creates central hemodynamic conditions that are thought to mimic those obtained during actual BL (12). Recent evidence indicates that LBNP is a valid surrogate to simulate hemodynamic responses to BL in anesthetized baboons (23). Data obtained from human experiments also suggest that LBNP creates a hemodynamic environment that is similar to BL (20, 30, 36). In this context, it has been proposed, based on a review of LBNP and BL studies, that LBNP creates similar compensatory and hemodynamic responses as BL (12). However, a direct comparison of physiological responses during LBNP and BL have only been conducted in two previous studies, both of which involved only mildly reduced blood volume of 450 ml (20, 30). Notably, these studies did not compare hemodynamic responses throughout progressive reductions in circulating blood volume to responses obtained during graded LBNP.

Reductions in central blood volume not only cause changes in hemodynamics but blood analyte responses as well. Central hypovolemia generated by LBNP or BL is a strong activator
of the sympathetic nervous system and increases circulating catecholamines (11, 13, 15, 24, 28). Additionally, arterial and atrial mechanical stretch receptors sense the decrease in blood pressure during acute reductions in central blood volume and initiate the release of volume-regulating hormones, such as arginine vasopressin (1, 2, 11, 18, 24, 37). Therefore, it is plausible that blood analyte responses to LBNP are comparable to the blood analyte responses observed during BL. However, similar to the lack of a comparison of hemodynamic adjustments between LBNP and BL, a direct comparison of blood analyte responses to LBNP and BL has not been fully elucidated.

Despite the idea that LBNP mimics BL, a direct comparison of multiple hemodynamic and blood analyte responses to reductions in central venous pressure (CVP) and pulse pressure obtained by graded LBNP and graded BL has not been performed in conscious humans. The purpose of this study was to compare hemodynamic responses elicited during a bout of graded LBNP to those observed during graded BL in conscious humans. We hypothesized that hemodynamic responses to graded LBNP (0, 15, 30, & 45 mmHg of LBNP) would mimic hemodynamic responses observed during graded BL (0, 333, 667, 1000 ml of BL) across a wide range of CVP and pulse pressure, and these responses would be strongly correlated between the two protocols. Additionally, we hypothesized that the blood analyte responses to LBNP and BL would be similar.
METHODS

Subjects. Twelve healthy men (age: 32 ± 2 years; height: 181.8 ± 2.0 cm; weight: 88.4 ± 2.5 kg; BMI: 26.7 ± 0.5 kg/m²) were recruited to participate in this study. All subjects reported to be free of any cardiovascular, respiratory, neurologic, or metabolic disease. Subjects were required to be non-obese (BMI < 30), non-smokers, and not taking any medications. Subjects reported to the Clinical Research Unit at Mayo Clinic at 0700 following an overnight fast. At this time, subjects consumed a small breakfast bar (Cliff Bar; Shelton, CT, USA; 240 kcals) and drank 250 ml of water. Subjects were studied in the supine position in a temperature controlled room (20-22° C). Prior to the study day, all subjects provided written informed consent after all procedures and risks of the study were fully explained and the study was approved by the Institutional Review Board. 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 BL protocol.

Experimental Design. Subjects underwent a LBNP and a BL protocol on the same day in a randomized order. The goal of the experimental design was to create a wide range of CVP in both protocols. Based on recommendations for equating LBNP levels to BL (12), we selected the initial stages of the U.S. Army Institute for Surgical Research LBNP protocol and stepwise reductions in blood volume that would closely mirror CVP at each LBNP stage and allow hemodynamic conditions to stabilize. The order of the protocols was randomized; therefore we were unable to match CVP values between LBNP and BL due to subject safety. Prior to the first perturbation, subjects were supine for 60-90 minutes (at least 30 minutes following invasive instrumentation). Subjects rested quietly in the supine position for 45-75 minutes following the first protocol. A longer duration of rest was required after the BL protocol to allow for the re-
infusion of blood prior to the LBNP protocol. Arterial blood samples were obtained at baseline and at the termination of each protocol to measure circulating catecholamines, hematocrit, hemoglobin, blood gases, bicarbonate, and circulating arginine vasopressin. The protocols were terminated early if 1) mean arterial pressure fell by 30%, 2) systolic blood pressure dropped below 80 mmHg, or 3) the subject began to experience symptoms of pre-syncope or syncope. Figure 1 illustrates the study timeline.

Measurements and Procedures.

Heart rate and arterial oxygen saturation. A 3-lead EKG was used to continuously record heart rate and arterial oxygen saturation was obtained using a finger pulse oximeter (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). The EKG and pulse oximeter tracings were interfaced with a personal computer for continuous measurements.

Central venous pressure. A 16-gauge peripherally inserted central catheter was introduced into an antecubital vein under local anesthesia (2% lidocaine) using aseptic techniques and advanced until an appropriate CVP waveform was obtained. This catheter was connected to a high-resolution transducer (FloTrac, Edwards Lifesciences Corp., Irvine, CA, USA) positioned at heart level and interfaced with a personal computer for continuous measurement of central venous pressure.

Blood removal. A second 14-gauge catheter was placed in an antecubital vein to facilitate blood removal for the BL protocol. The catheter was placed under local anesthesia (2% lidocaine) using aseptic techniques. Preservative/anticoagulant bags (63 ml anti-coagulant citrate phosphate dextrose solution) were placed below the subject to allow blood to transfer 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 and this
cuff pressure was released during all measurements. As blood was being collected, it was weighed to determine the volume of blood removed by multiplying the weight of the blood by 1.06 ml/g. The removed blood was kept in the study room (20-22°C) and the temperature of the blood was allowed fluctuate. At the end of the BL protocol, blood was re-infused at a rate of 20 ml/min into the antecubital vein.

**Intra-arterial blood pressure, stroke volume, and cardiac output.** A 20-gauge, 5 cm catheter was placed into a brachial artery under local anesthesia (2% lidocaine) using aseptic techniques and ultrasound guidance. The catheter was attached to a high-resolution transducer (FloTrac, Edwards Lifesciences Corp., Irvine, CA, USA) positioned, at heart level and interfaced with a personal computer to obtain continuous beat by beat arterial pressure waveforms. Pulse pressure was calculated as the difference between systolic and diastolic blood pressure. Model flow analysis software (WinCPRS, Absolute Aliens Oy, Turku, Finland) was used to calculate beat by beat stroke volume and cardiac output (38). Total peripheral resistance was calculated as mean arterial pressure divided by cardiac output.

**Blood sampling and oxygen delivery.** Arterial blood samples were collected at baseline and at the termination of each protocol for the measurement of the partial pressure of oxygen and carbon dioxide, pH, bicarbonate, hematocrit, hemoglobin, catecholamines, and arginine vasopressin. Blood samples were analyzed by the Immunochemistry Core Laboratory of the Clinical Research Unit of the Mayo Clinic CTSA. Oxygen delivery was calculated as: \[1.39 \times \text{hemoglobin concentration} \times \text{arterial oxygen saturation} + (0.003 \times \text{partial pressure of oxygen})\] \times \text{cardiac output}.

**LBNP protocol.** Subjects laid in an airtight LBNP chamber sealed at the iliac crest. The LBNP protocol was based on the first 3 stages of the protocol commonly used by the U.S. Army
Institute for Surgical Research (5, 7, 9, 22, 31, 32). The protocol consisted of a 5 minute baseline period followed by 5 minutes at 15, 30, and 45 mmHg of suction. Subjects were not allowed to cross their legs and were instructed to refrain from contracting any muscles in the lower body throughout the protocol.

**BL protocol.** Following a 5 minute baseline period, 3 aliquots of 333 ml of blood were removed via gravity from an antecubital vein. A 5 minute period separated each aliquot to emulate the three stages of LBNP. The removed blood was stored in standard preservative/anticoagulant bags and was periodically agitated to prevent clotting. Subjects were instructed not to cross their legs or contract any muscles in their lower body throughout the protocol.

**Data and Statistical Analysis.**

Data were collected and variables were analyzed off-line using signal processing software (WinDaq, DATAZ Instruments, Akron, OH, USA). Data were analyzed and averaged over the last 2 minutes of each stage for statistical analysis. To explore the relationship between BL and LBNP, individual subject $r^2$ values and linear regression line slopes were calculated for each variable for both protocols. Paired t-tests were used to determine if the $r^2$ values and slopes of the regression lines of the hemodynamic variables fell on similar trajectories throughout a range of CVP and pulse pressure during each protocol. If data were not normally distributed, the Wilcoxon Signed Rank test was used. The amalgamated $r^2$ value and linear regression line slopes were also calculated using linear regression analysis using group mean values obtained at each stage versus the group mean CVP and pulse pressure obtained during each stage of both protocols. Protocol × stage (2 × 4) repeated measures ANOVA was used to determine if values obtained during the LBNP protocol were similar to the corresponding stages
of the BL protocol. If a significant main or interaction effect was obtained, Tukey’s post hoc analysis was performed to determine where differences existed. Group data are presented as mean ± SE. The alpha level was set at 0.05.
RESULTS

Of the 12 subjects who volunteered to participate in this study, 2 subjects did not complete both protocols (both subjects completed 667 ml of BL and 30 mmHg of LBNP); additionally, one subject did not complete the LBNP protocol (completed 30 mmHg of LBNP), and one subject did not complete the BL protocol (completed 333 ml of BL). These protocols were terminated early due to pre-syncope symptoms or syncope. Data obtained from subjects who did not complete 667 ml of BL or 30 mmHg of LBNP were excluded from regression analyses. A sample size of 8 subjects (age: 32 ± 3 years; height: 185.3 ± 1.8 cm; weight: 91.3 ± 3.4 kg; BMI: 26.6 ± 0.8 kg/m$^2$) was used for ANOVA analyses due to the missing data points. The mean time for blood removal was 483 ± 163 seconds (~41 ml/minute) for the three aliquots. The first aliquot took 538 ± 134 seconds (~37 ml/minute), the second aliquot took 468 ± 160 seconds (~43 ml/minute), and the final aliquot took 436 ± 194 seconds (~46 ml/minute). The time to fill each aliquot was not statistically distinguishable ($P = 0.068$). The correlation of the amalgamated hemodynamic values obtained during BL and LBNP are presented in Figure 2. Tables 1 and 2 display the mean and range of individual $r^2$ values of the hemodynamic variables versus CVP and pulse pressure, respectively. The mean and range of individual regression line slope values of hemodynamic variables versus CVP and pulse pressure are presented in Tables 1 and 2 as well. The mean and individual hemodynamic values generated at each stage across the range of CVP and pulse pressure during LBNP and BL are displayed in Figures 3 and 4, respectively. The mean hemodynamic values obtained at each stage during both protocols are presented in Table 3. The mean and range of individual regression line slope values achieved by plotting blood analyte responses against CVP and pulse pressure are displayed in Table 4. The mean blood analyte data obtained at baseline and at protocol termination are presented in Table
5. The mean and individual catecholamine values generated at baseline and at protocol termination plotted against CVP and pulse pressure are displayed in Figure 5.

   *Central venous pressure.* There was a strong correlation for the amalgamated CVP values between BL and LBNP \( (r^2 = 0.99) \) (Figure 2A).

   *Heart rate.* There was a strong correlation for the amalgamated heart rate values between BL and LBNP \( (r^2 = 0.97) \) (Figure 2B). Individual \( r^2 \) values \( (P = 0.371) \) and regression line slopes \( (P = 0.158) \) generated from the relationships between heart rate and CVP from each protocol were statistically similar between BL and LBNP (Table 1). Individual \( r^2 \) values \( (P = 0.010) \) and regression line slopes \( (P = 0.024) \) produced from the relationships between heart rate and pulse pressure from both protocols were statistically greater in LBNP when compared to BL (Table 2).

   *Mean arterial pressure.* There was a good correlation for the amalgamated MAP values between BL and LBNP \( (r^2 = 0.74) \) (Figure 2C). Individual \( r^2 \) values \( (P = 0.007) \) and regression line slopes \( (P = 0.037) \) produced from the relationships between MAP and CVP from each protocol were statistically greater in LBNP when compared to BL (Table 1). Individual \( r^2 \) values \( (P = 0.902) \) and regression line slopes \( (P = 0.567) \) produced from the relationships between MAP and pulse pressure from each protocol were not statistically similar between BL and LBNP (Table 2).

   *Pulse Pressure.* There was a strong correlation for the amalgamated PP values between BL and LBNP \( (r^2 = 0.99) \) (Figure 2D). Individual \( r^2 \) values \( (P = 0.113) \) and regression line slopes \( (P = 0.105) \) calculated from the relationships between PP and CVP from each protocol were statistically similar between BL and LBNP (Table 1).

   *Arterial oxygen saturation, blood gases, hematocrit, and hemoglobin.* Individual \( r^2 \)
values (P = 0.733) and regression line slopes (P = 0.999) calculated from the relationships between arterial oxygen saturation and CVP from the LBNP and BL protocols were not distinguishable (Table 1). Individual $r^2$ values (P = 0.311) and regression line slopes (P = 0.102) generated from the relationship between arterial oxygen saturation and pulse pressure from each protocol were not statistically distinguishable between BL and LBNP (Table 2). The arterial partial pressure of oxygen, partial pressure of carbon dioxide, and pH responses were similar between BL and LBNP at baseline and protocol termination (Table 5). The regression line slopes for the arterial partial pressure of oxygen, partial pressure of carbon dioxide, and pH were not different between BL and LBNP when the responses were plotted against CVP or pulse pressure (P > 0.05). The regression line slopes of hematocrit plotted against CVP and pulse pressure were different between BL and LBNP (P = 0.002 and P < 0.001, respectively) (Table 4). The regression line slopes of hemoglobin plotted against CVP and pulse pressure were also different between protocols (P = 0.001 and P = 0.027, respectively) (Table 4).

**Stroke volume.** There was a strong correlation for the amalgamated stroke volume values between BL and LBNP ($r^2 = 0.98$) (Figure 2E). Individual $r^2$ values (P = 0.232) and regression line slopes (P = 0.636) produced from the relationships between stroke volume and CVP from each protocol were statistically similar between BL and LBNP (Table 1). Individual $r^2$ values (P = 0.978) and regression line slopes (P = 0.922) obtained from the relationships between stroke volume and pulse pressure from each protocol were statistically similar between BL and LBNP (Table 2).

**Cardiac output.** There was a good correlation for the amalgamated cardiac output values between BL and LBNP ($r^2 = 0.80$). Individual $r^2$ values (P = 0.433) and regression line slopes (P = 0.642) generated from the relationships between cardiac output and CVP from each protocol...
were statistically similar between BL and LBNP (Table 1). Individual $r^2$ values ($P = 0.945$) and regression line slopes ($P = 0.121$) produced from the relationships between cardiac output and pulse pressure from each protocol were statistically similar between BL and LBNP (Table 2).

**Oxygen delivery.** The regression line slope generated from the relationship between oxygen delivery and CVP during LBNP was not statistically different from the slope obtained during BL ($P = 0.164$) (Table 4). The regression line slope produced from the relationship between oxygen delivery and pulse pressure was also statistically indistinguishable between LBNP and BL ($P = 0.064$) (Table 4).

**Total peripheral resistance.** There was a modest correlation for the amalgamated total peripheral resistance values between BL and LBNP ($r^2 = 0.53$) (Figure 2F). Individual $r^2$ values ($P = 0.907$) and regression line slopes ($P = 0.124$) produced from the relationships between total peripheral resistance and CVP from each protocol were statistically similar between BL and LBNP (Table 1). Individual $r^2$ values ($P = 0.364$) and regression line slopes ($P = 0.849$) generated from the relationships between stroke volume and pulse pressure from each protocol were statistically similar between BL and LBNP (Table 2).

**Catecholamines.** The regression line slope generated from the relationship between norepinephrine and CVP during LBNP was steeper than the slope obtained during BL ($P = 0.011$) (Table 4). The regression line slopes produced from the relationships between norepinephrine and pulse pressure from each of the protocols were not statistically distinguishable ($P = 0.129$) (Table 4). The regression line slope produced from the relationship between epinephrine and CVP ($P = 0.816$) and between epinephrine and pulse pressure ($P = 0.470$) were not different between protocols.

**Arginine vasopressin.** The regression line slopes obtained from plotting arginine
vasopressin against CVP were not statistically distinguishable between BL and LBNP (P = 0.152) (Table 4). Additionally, the regression line slopes generated between arginine vasopressin and pulse pressure were not different between protocols (P = 0.936) (Table 4).
DISCUSSION

The overarching results of this investigation indicate that LBNP elicits similar hemodynamic stimulus-response relationships as BL throughout the ranges of CVP and pulse pressure that were attained. That is, with the exception of heart rate and MAP, the relationship between indices of central blood volume (i.e. CVP and pulse pressure) and hemodynamic responses produced by stepwise decreases in circulating blood volume were mimicked by progressive reductions in LBNP. This is demonstrated by the similar response trajectories across the wide range of CVP and pulse pressures that were achieved for multiple hemodynamic variables between the two protocols. Therefore, our results provide the first direct comparison of data from human subjects who have undergone more than 450 ml of BL and LBNP. Furthermore, these data support our hypothesis that LBNP models multiple hemodynamic responses induced by hemorrhage.

Heart rate during the LBNP and BL protocols followed similar trajectories throughout the range of CVP. Rea et al. (30) found that heart rate increased 3 bpm following only 450 ml of BL (reduced CVP by ~2.4 mmHg), whereas heart rate remained unchanged during 15 mmHg of LBNP (reduced CVP by ~3.8 mmHg). These small changes in heart rate following a modest volume of BL suggest that heart rate might respond differently to BL when compared to LBNP. As a clinical perspective, an elevation in heart rate caused by hemorrhage is a tool used to assess patient status in trauma situations. However, the results presented here reinforce the idea that tachycardic heart rates may not necessarily reflect the severity of BL or predict impending hemodynamic collapse (8, 34). Removing up to 17% of total blood volume and reducing CVP to as low as -2 mmHg, did not elicit a heart rate over 100 bpm in any subject during the BL protocol. Furthermore, only one subject achieved a heart rate above 100 bpm during the last two
stages of the LBNP protocol. In this context, an increase in total peripheral resistance appears to be a main contributor to the defense of MAP during central hypovolemia. Our data support this idea as we observed an increase in total peripheral resistance while MAP remained unchanged during the first two stages of the LBNP and BL protocols. Additionally, it has been shown that muscle sympathetic nerve activity increases while heart rate and MAP remain stable during low levels of LBNP (30).

Although MAP correlated well between LBNP and BL, the response trajectories across a wide range of CVP differed statistically between the protocols. MAP was statistically unaltered throughout the early stages of both BL and LBNP and was only lower than baseline during the final stage of both protocols. This observation is consistent with previous reports which indicate that MAP is well-defended in spite of progressive reductions in central blood volume (6, 10). The well-defended MAP during the BL protocol also highlights the concept that using MAP to monitor patients during hemorrhage may not provide accurate information regarding hemodynamic stability (10). In this context, of the three subjects who did not complete the entire BL protocol due to pre-syncope symptoms or syncope did not exhibit unusually low MAP. The final MAP prior to protocol termination in these subjects was 75 mmHg following 333 ml of BL, and 72 and 85 mmHg following 667 ml of BL. Furthermore, MAP was either unchanged or slightly increased following 1000 ml of BL in 5 subjects.

Importantly, stroke volume was well correlated between LBNP and BL and the stroke volume response trajectories across the range of CVP were remarkably similar between the LBNP and BL protocol. Reductions in stroke volume are an early indicator that central blood volume has decreased and stroke volume declines during progressive reductions in LBNP (4, 5, 7, 20, 22, 29, 31, 32). However, previous studies have not compared stroke volume during
graded LBNP to graded BL in humans. In baboons, decreases in stroke volume were nearly identical during LBNP and BL across an extensive range of CVP (23). Our results in humans reinforce the baboon data indicating that stroke volume during graded LBNP accurately models the changes in stroke volume obtained during graded BL. Additionally, aside from CVP, stroke volume was the first hemodynamic variable measured that was statistically different from baseline following 667 ml of BL. In this context, stroke volume also had the greatest decrease from baseline to protocol termination in the subjects who were unable to complete LBNP and BL protocols. In these non-finishers, stroke volume fell by 10-36% before LBNP protocol termination and stroke volume decreased by 16-25% prior to the cessation of the BL protocol. Therefore, these data support the idea that monitoring stroke volume during BL provides an accurate reflection of decreases in blood volume (26) and tracking stroke volume might provide caregivers vital hemodynamic information that could be used to prevent cardiovascular collapse.

We found statistically different hematocrit and hemoglobin responses to the LBNP and BL protocols. These findings are similar to those observed in anesthetized baboons (23). The reduction in hematocrit and hemoglobin during BL represents a shift of fluid from the extravascular to the intravascular space to counteract the reduction in circulating blood volume (1, 25, 27). Whereas the increase in hematocrit and hemoglobin during LBNP is likely due to a plasma volume shift from the intravascular to extravascular space in the lower body as a result of the large pressure gradient which occurs during LBNP. The differences in hemoglobin, hematocrit, and cardiac output between the protocols generated a lower calculated oxygen delivery during LBNP when compared to BL. However, it is doubtful that the disparities in oxygen delivery caused significant physiological changes in tissue oxygenation during LBNP and BL. This is reinforced by our observation that blood pH and the partial pressure of carbon
dioxide were unaffected in both protocols.

The circulating catecholamine responses in our LBNP and BL protocols differ from those obtained in baboons (23). We did not observe a statistically significant increase in circulating norepinephrine during the BL protocol but we did observe an increase during LBNP. The mean norepinephrine response in the subjects who completed the BL protocol (65% increase) was nearly 100% lower than the mean values obtained during the LBNP protocol (162% increase). Interestingly, the three subjects that did not complete the BL protocol had abnormally low norepinephrine responses (change from baseline values were -47%, -15%, and 7%). Regardless of the statistically similar epinephrine responses, it is plausible that our BL protocol did not activate the sympathetic nervous system to the same extent as the LBNP protocol. This finding is consistent with our observation that total peripheral resistance was consistently lower during BL when compared to the LBNP protocol.

Despite lower CVP and pulse pressure during the last LBNP stage when compared to the final BL stage, we observed statistically similar arginine vasopressin responses to both protocols. The increase in arginine vasopressin in both protocols is not surprising (1, 2, 11, 18, 24, 37). However, when baboons underwent LBNP and BL protocols and CVP and pulse pressure were matched between protocols, the arginine vasopressin response was lower during LBNP when compared to BL (23). It was speculated that arginine vasopressin may be differentially released between the protocols possibly due to a decrease in oxygen delivery during BL as a result of decreases in hematocrit, hemoglobin, and central venous oxygen saturation (23). Our data contrast this idea as the arginine vasopressin response was statistically similar between LBNP and BL even though oxygen delivery was greater in BL when compared to LBNP. Previous reports have suggested that arginine vasopressin is associated with pre-syncope symptoms (2, 11,
Therefore, we examined the six individual protocols (3 LBNP and 3 BL) that were not completed and found that five of the six arginine vasopressin responses were considerably large. The mean increase of arginine vasopressin in these individual protocols was nearly 5 times the mean value obtained from the subjects that completed the protocols. In this context, arginine vasopressin might be an additional marker that can be used in conjunction with the monitoring of other hemodynamic variables, like stroke volume, that would give caregivers insight on patient stability during hemorrhagic trauma.

Limitations

Several limitations pertain to our study. First, we removed blood volume using three equal aliquots that were not based on a percentage of total blood volume. LBNP protocols are also not based on body size and it is difficult to measure the volume of blood that shifts from the thorax to the capacitance vessels in the legs during LBNP and this volume likely varies from person to person. In this context, LBNP might substantially impede the mobilization of sequestered blood in the leg capacitance vessels to the central circulation via changes in intrathoracic pressure during breathing when compared with BL. That is, the respiratory pump might lose its effectiveness in aiding venous return for a given reduction in central blood volume during LBNP; whereas changes in intrathoracic pressures during BL are not competing against lower body suction for blood volume. This effect might contribute to the divergent stimulus-response trajectories between LBNP and BL in some hemodynamic variables. The physiological consequence of this potential sequestration effect during LBNP needs to be compared with absolute reductions in blood volume during BL to fully elucidate the potential impact it has on the respiratory pump. Second, we did not take all subjects in both protocols to tolerance due to subject safety. Therefore, it remains unclear if the response trajectories remain similar between
LBNP and BL at lower levels of central hypovolemia. Third, we were unable to match the rate of negative pressure change during the LBNP protocol to the rate of blood removal during BL because we randomized the order of the protocols. This temporal difference between protocols may have differentially influenced hemodynamic adjustments to changes in central blood volume during the BL protocol when compared to the LBNP protocol. However, we allowed three minutes after progressing to the next LBNP stage and following the removal of each aliquot of blood to reach a stable hemodynamic state prior to data analysis. Fourth, we only collected blood at baseline and at the termination of each protocol. Therefore, we cannot discern if the blood analyte responses are linear throughout the CVP and pulse pressures obtained during each protocol. Fifth, we did not test women. Due to a lower total blood volume in women, removing 1000 ml of blood represents a greater percentage of total blood volume and thus increases the risk of cardiovascular collapse. Interestingly however; we may have observed differential responses between men and women because women typically have a lower orthostatic stress tolerance than men (17) and hemodynamic responses to orthostatic stress are different between sexes (19). Furthermore, young women appear to regulate blood pressure differently than young men (21), which may influence hemodynamic responses to central hypovolemia.
CONCLUSIONS

We observed striking similarities between LBNP and BL in the stimulus-response relationships of central venous pressure and pulse pressure to hemodynamic responses. As such, LBNP mimics the trajectories of the hemodynamic responses observed during BL across a significant range of central venous pressure and pulse pressure in humans. Therefore, our data support the hypothesis that LBNP adequately reflects the hemodynamic responses observed during BL.

ACKNOWLEDGMENTS

We would like to thank the subjects for their participation in this study. We also thank Margaret McGill-Zimny and David Warren for their assistance on the blood loss protocol. This study was funded by U.S. Army MRMC Combat Casualty Care Research Program Grant # W81XWH-11-1-0823. BDJ was supported by American Heart Association Midwest Affiliate 13POST14380027. NVH was supported by Dutch Heart Foundation E. Dekker stipend 2012SB013.

DISCLOSURES

The authors have no conflicts of interest to report.
REFERENCES


Table 1. Mean and range of individual $r^2$ values and the mean and range of individual regression line slope values of hemodynamic variables versus central venous pressure.

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r^2$</th>
<th>$r^2$ range</th>
<th>Slope</th>
<th>Slope range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.67</td>
<td>0.14 - 0.95</td>
<td>-2.42</td>
<td>-4.58 - -0.46</td>
</tr>
<tr>
<td>BL</td>
<td>0.54</td>
<td>0.02 - 0.98</td>
<td>-1.53</td>
<td>-5.29 - 0.12</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.68</td>
<td>0.23 - 0.99</td>
<td>0.93</td>
<td>0.08 - 3.48</td>
</tr>
<tr>
<td>BL</td>
<td>0.35†</td>
<td>&lt;0.01 - 0.66</td>
<td>0.13†</td>
<td>-1.30 - 2.12</td>
</tr>
<tr>
<td>Pulse Pressure</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.72</td>
<td>0.01 - 0.97</td>
<td>1.81</td>
<td>0.03 - 4.37</td>
</tr>
<tr>
<td>BL</td>
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<td>&lt;0.01 - 0.92</td>
<td>1.23</td>
<td>0.09 - 3.63</td>
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<tr>
<td>$S_aO_2$</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.52</td>
<td>0.03 - 0.95</td>
<td>-0.42</td>
<td>-0.28 - 0.23</td>
</tr>
<tr>
<td>BL</td>
<td>0.46</td>
<td>0.01 - 0.98</td>
<td>-0.04</td>
<td>-0.68 - 0.32</td>
</tr>
<tr>
<td>Stroke Volume</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.85</td>
<td>0.44 - 0.99</td>
<td>3.69</td>
<td>1.86 - 5.26</td>
</tr>
<tr>
<td>BL</td>
<td>0.73</td>
<td>&lt;0.01 - 0.93</td>
<td>3.59</td>
<td>0.25 - 5.74</td>
</tr>
<tr>
<td>Cardiac Output</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.68</td>
<td>0.18 - 0.99</td>
<td>0.10</td>
<td>-0.02 - 0.24</td>
</tr>
<tr>
<td>BL</td>
<td>0.61</td>
<td>&lt;0.01 - 0.98</td>
<td>0.10</td>
<td>-0.13 - 0.31</td>
</tr>
<tr>
<td>TPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.51</td>
<td>0.24 - 0.99</td>
<td>-0.26</td>
<td>-0.63 - 0.17</td>
</tr>
<tr>
<td>BL</td>
<td>0.53</td>
<td>0.02 - 0.99</td>
<td>0.07</td>
<td>0.75 - 0.99</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure; BL = blood loss; MAP = mean arterial pressure; $S_aO_2$ = arterial oxygen saturation; TPR = total peripheral resistance.
†Different from lower body negative pressure (P < 0.05).
Table 2. Mean and range of individual $r^2$ values and the mean and range of individual regression line slope values of hemodynamic variables versus pulse pressure.

<table>
<thead>
<tr>
<th>Variable</th>
<th>LBNP</th>
<th>BL</th>
<th>Slope</th>
<th>Slope range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.86</td>
<td>0.57 - 0.98</td>
<td>-1.85</td>
<td>-6.12 - 0.38</td>
</tr>
<tr>
<td>BL</td>
<td>0.57†</td>
<td>0.03 - 0.99</td>
<td>-0.46†</td>
<td>-2.19 - 0.76</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.70</td>
<td>0.04 - 1.00</td>
<td>0.43</td>
<td>-0.29 - 1.95</td>
</tr>
<tr>
<td>BL</td>
<td>0.43</td>
<td>&lt;0.01 - 0.66</td>
<td>0.67</td>
<td>-0.20 - 1.52</td>
</tr>
<tr>
<td>$S_aO_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.59</td>
<td>&lt;0.01 - 0.99</td>
<td>0.08</td>
<td>-0.14 - 1.05</td>
</tr>
<tr>
<td>BL</td>
<td>0.42</td>
<td>&lt;0.01 - 0.99</td>
<td>0.01</td>
<td>-0.40 - 0.31</td>
</tr>
<tr>
<td>Stroke Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.86</td>
<td>0.49 - 0.99</td>
<td>2.76</td>
<td>1.16 - 8.94</td>
</tr>
<tr>
<td>BL</td>
<td>0.86</td>
<td>0.29 - 1.00</td>
<td>2.17</td>
<td>1.02 - 4.74</td>
</tr>
<tr>
<td>Cardiac Output</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.69</td>
<td>&lt;0.01 - 0.96</td>
<td>0.06</td>
<td>&lt;0.01 - 0.11</td>
</tr>
<tr>
<td>BL</td>
<td>0.66</td>
<td>0.03 - 0.99</td>
<td>0.09</td>
<td>&lt;0.01 - 0.22</td>
</tr>
<tr>
<td>TPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>0.52</td>
<td>&lt;0.01 - 0.96</td>
<td>-0.19</td>
<td>-0.68 - 0.04</td>
</tr>
<tr>
<td>BL</td>
<td>0.64</td>
<td>&lt;0.01 - 0.99</td>
<td>-0.18</td>
<td>-0.47 - 0.12</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure; BL = blood loss; MAP = mean arterial pressure; $S_aO_2$ = arterial oxygen saturation; TPR = total peripheral resistance.

†Different from lower body negative pressure (P < 0.05).
Table 3. Hemodynamic responses during each stage of lower body negative pressure and blood loss.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>7.1 ± 0.9</td>
<td>2.6 ± 0.9</td>
<td>0.8 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-0.6 ± 0.8&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL</td>
<td>5.9 ± 0.8</td>
<td>3.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.2 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>62 ± 2.8</td>
<td>64 ± 3.8</td>
<td>71 ± 5.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>83 ± 6.3&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL</td>
<td>59 ± 3.4</td>
<td>60 ± 3.0</td>
<td>63 ± 2.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>67 ± 3.5&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>95 ± 3.2</td>
<td>93.0 ± 3.3</td>
<td>92.3 ± 3.3</td>
<td>87.3 ± 4.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL</td>
<td>91.4 ± 2.7</td>
<td>91.0 ± 2.4</td>
<td>90.3 ± 3.0</td>
<td>89.2 ± 2.7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>66.2 ± 4.3</td>
<td>62.2 ± 4.3</td>
<td>57.4 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.7 ± 4.9&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL</td>
<td>63.8 ± 3.9</td>
<td>61.9 ± 3.4</td>
<td>58.6 ± 4.1</td>
<td>55.7 ± 4.1&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>S&lt;sub&gt;a&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>96.5 ± 0.7</td>
<td>97.0 ± 0.5</td>
<td>96.9 ± 0.4</td>
<td>96.9 ± 0.3</td>
</tr>
<tr>
<td>BL</td>
<td>97.5 ± 0.3</td>
<td>97.5 ± 0.3</td>
<td>97.4 ± 0.3</td>
<td>98.1 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SV (ml/beat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>82.8 ± 3.1</td>
<td>72.8 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.9 ± 3.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>50.8 ± 3.7&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>88.8 ± 3.1</td>
<td>83.8 ± 2.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>77.6 ± 3.4&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>70.6 ± 4.0&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>5.2 ± 0.4</td>
<td>4.6 ± 0.2</td>
<td>4.5 ± 0.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.1 ± 0.2&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.3 ± 0.4</td>
<td>5.1 ± 0.3</td>
<td>4.8 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.7 ± 0.2&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>TPR (mmHg/l/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>18.9 ± 0.9</td>
<td>20.4 ± 0.8</td>
<td>21.0 ± 1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.6 ± 1.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>BL&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.9 ± 1.2</td>
<td>18.2 ± 1.0</td>
<td>18.9 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.3 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure: Stage 1 = 15 mmHg, Stage 2 = 30 mmHg, Stage 3 = 45 mmHg.
BL = blood loss: Stage 1 = 333 ml, Stage 2 = 667 ml, Stage 3 = 1000 ml.
CVP = central venous pressure; MAP = mean arterial pressure; PP = pulse pressure; S<sub>a</sub>O<sub>2</sub> = arterial oxygen saturation; SV = stroke volume; CO = cardiac output; TPR = total peripheral resistance.
Values are means ± standard error, n = 8.
<sup>a</sup>Different from Baseline (P < 0.05).
<sup>b</sup>Different from Stage 1 (P < 0.05).
<sup>c</sup>Different from Stage 2 (P < 0.05).
<sup>d</sup>Different from lower body negative pressure (P < 0.05).
<sup>e</sup>Stage main effect, different from Baseline (P < 0.05).
<sup>f</sup>Stage main effect, different from Stage 1 (P = 0.025).
Table 4. Mean and range of individual regression line slope values of blood analyte and oxygen delivery responses versus central venous pressure and pulse pressure.

<table>
<thead>
<tr>
<th></th>
<th>Central Venous Pressure</th>
<th>Pulse Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Slope range</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>-27.8</td>
<td>-80.7 - 0.6</td>
</tr>
<tr>
<td>BL</td>
<td>-8.7†</td>
<td>-32.7 - 39.5</td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>-13.5</td>
<td>-48.3 - 0.0</td>
</tr>
<tr>
<td>BL</td>
<td>-14.5</td>
<td>-36.3 - 1.2</td>
</tr>
<tr>
<td>Hematocrit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>-0.22</td>
<td>-0.58 - 0.07</td>
</tr>
<tr>
<td>BL</td>
<td>0.24†</td>
<td>-0.33 - 1.18</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>-0.07</td>
<td>-0.18 - 0.05</td>
</tr>
<tr>
<td>BL</td>
<td>0.08†</td>
<td>-0.17 - 0.30</td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasopressin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>-2.1</td>
<td>-7.8 - 0.1</td>
</tr>
<tr>
<td>BL</td>
<td>-4.5</td>
<td>-20.8 - 0.6</td>
</tr>
<tr>
<td>Oxygen Delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>17.3</td>
<td>-10.6 - 74.2</td>
</tr>
<tr>
<td>BL</td>
<td>38.5</td>
<td>-25.8 - 137.3</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure; BL = blood loss.
†Different from lower body negative pressure (P < 0.05).
Table 5. Blood analyte and oxygen delivery responses at baseline and the termination of lower body negative pressure and blood loss protocols.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Norepinephrine (pg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>148 ± 20</td>
<td>354 ± 44^</td>
</tr>
<tr>
<td>BL</td>
<td>155 ± 22</td>
<td>211 ± 29†</td>
</tr>
<tr>
<td><strong>Epinephrine (pg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>53 ± 7</td>
<td>144 ± 30*</td>
</tr>
<tr>
<td>BL</td>
<td>49 ± 7</td>
<td>103 ± 19*</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>40.8 ± 0.8</td>
<td>42.4 ± 0.8^</td>
</tr>
<tr>
<td>BL</td>
<td>41.1 ± 0.8</td>
<td>40.3 ± 0.9^†</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dl)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>14.2 ± 0.4</td>
<td>14.7 ± 0.4^</td>
</tr>
<tr>
<td>BL</td>
<td>14.3 ± 0.4</td>
<td>14.0 ± 0.4^†</td>
</tr>
<tr>
<td><strong>Oxygen (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>99.5 ± 2.9</td>
<td>97.8 ± 3.1</td>
</tr>
<tr>
<td>BL</td>
<td>105.1 ± 3.5</td>
<td>103.8 ± 2.7</td>
</tr>
<tr>
<td><strong>Carbon Dioxide (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>42.1 ± 0.9</td>
<td>41.7 ± 1.0</td>
</tr>
<tr>
<td>BL</td>
<td>41.5 ± 0.6</td>
<td>41.0 ± 0.7</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>7.42 ± 0.01</td>
<td>7.41 ± 0.01</td>
</tr>
<tr>
<td>BL</td>
<td>7.42 ± 0.01</td>
<td>7.42 ± 0.01</td>
</tr>
<tr>
<td><strong>Bicarbonate (mmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>26.2 ± 0.2</td>
<td>25.8 ± 0.4*</td>
</tr>
<tr>
<td>BL</td>
<td>26.2 ± 0.3</td>
<td>25.8 ± 0.3*</td>
</tr>
<tr>
<td><strong>Arginine Vasopressin (pg/ml)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>2.8 ± 0.7</td>
<td>19.1 ± 6.2*</td>
</tr>
<tr>
<td>BL</td>
<td>3.4 ± 0.7</td>
<td>13.5 ± 4.0*</td>
</tr>
<tr>
<td><strong>Oxygen Delivery (ml/minute)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>948 ± 57</td>
<td>809 ± 32*</td>
</tr>
<tr>
<td>BL†</td>
<td>1036 ± 59</td>
<td>926 ± 37*</td>
</tr>
</tbody>
</table>

LBNP = lower body negative pressure; BL = blood loss.
Values are means ± standard error, n = 12.
*Stage main effect, different from Baseline (P < 0.05).
^Different from Baseline (P < 0.05).
†Different from lower body negative pressure (P < 0.05).
**Figure 1.** Timeline of the lower body negative pressure and blood loss protocols. The order of the protocols was randomized. When the lower body negative pressure protocol was performed first, 45 minutes of quiet rest was given between protocols to ensure hemodynamic variables returned to baseline. To allow for the reinfusion of removed blood, 75 minutes of quiet resting was given to allow for hemodynamic variables to return to baseline between protocols when blood loss occurred first. Arterial blood was drawn at baseline and during the last stage of each protocol for blood gases, pH, bicarbonate, catecholamines, hematocrit, hemoglobin, and arginine vasopressin.

**Figure 2.** Correlation of the amalgamated hemodynamic values obtained during lower body negative pressure versus blood loss. BL = blood loss; LBNP = lower body negative pressure.

**Figure 3.** The mean and individual hemodynamic values obtained at each stage across the range of central venous pressures during the lower body negative pressure and blood loss protocols.

**Figure 4.** The mean and individual hemodynamic values obtained at each stage across the range of pulse pressures during the lower body negative pressure and blood loss protocols.

**Figure 5.** The mean and individual catecholamine values obtained at baseline and protocol termination across the range of central venous pressures and pulse pressures during the lower body negative pressure and blood loss protocols.
Figure 1.
Figure 3.
Figure 4.
Figure 5.