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: October 2015

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	15 Sep 2014 - 14 Sep 2015

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-2-0038

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A high fraction of both battlefield and civilian trauma deaths are caused by hemorrhage and subsequent cardiovascular collapse. It is estimated that □35% 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

16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	74	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

	Page
Introduction……..	**4**
Keywords ……..	**5**
Body…….	**5**
Key Research Accomplishments……	**4**
Reportable Outcomes……	**8**
Conclusion……	**8**
References……	**9**
Participants……	**10**
Appendices……	**11**

Introduction

This report details results from the last year 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 year we have published two 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.

Richards CA, Johnson BD, Harvey RE, Convertino VA, Joyner MJ, Barnes JN. Cerebral blood velocity regulation during progressive blood loss compared with lower body negative pressure in humans. J Appl Physiol 2015 Sep 15; 9119(6):677-85.

Van Helmond N, Johnson BD, Curry TB, Cap AP, Convertino VA, Joyner MJ. Coagulation Changes during Lower Body Negative Pressure and Blood Loss I Humans. Am J Physiol Heart Circ. Physiol. 2015 Sep 14 [Epub ahead of print]

These papers were published in the Journal of Applied Physiology and the American Journal of Physiology-Heart and Circulatory Physiology and are included as an additional attachment to this report. They are additional data explorations from our study:
“Reductions in central venous pressure by lower body negative pressure or blood loss elicit similar hemodynamic responses.” Johnson et al, J Appl Physiol 2014 PMID 24876357.

Keywords

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

Body, Key research accomplishments & reportable outcomes

The abstract from these papers summarizes key accomplishments and outcomes.

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 (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 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.

Next Steps

Additional data analysis and manuscript preparation/submission is in process related to the blood reinfusion, heart rate variability, cardiac baroreflex sensitivity, from our first study. Our second study is on the effects of hypoxia on responses to simulated blood loss is underway with anticipated data collection completed by late 2015. We will also be starting a third protocol in early 2016 evaluating the effects of systemic epinephrine on the decision support medical monitoring system and related physiological variables and responses. The rationale for this study is that combat injuries frequently evoke a sympathoexcitatory response and we want to mimic this response in the laboratory. As such, testing this monitoring technology during these conditions is an important step for the continued development of the algorithm. We are also under active discussion with our Army colleagues about extension of the algorithm to the operating rooms and ICUs and are considering additional validation studies with radial artery catheters.

Reportable Outcomes

We will focus on new unreported/published data from the hypoxia trials since the relevant cerebral blood flow and coagulation data have been published. We have completed 8 subjects so far and the remaining 2 subjects are scheduled for the first half of October. Data from the 8 completed subjects show that under hypoxic conditions, total time to presyncope (figure 1) was significantly ($p<0.05$) shorter than during normoxia.

Figure 1: Time to syncope during normoxia and hypoxia

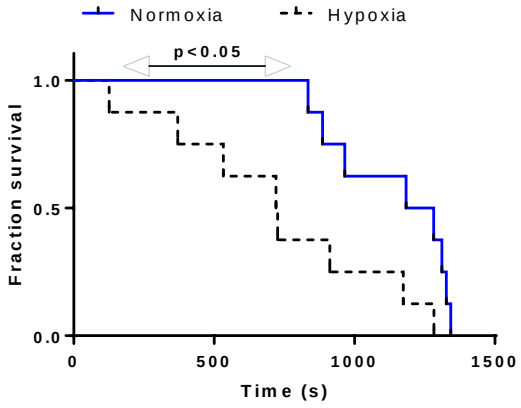


Figure 2: MAP response to LBNP during normoxia and hypoxia

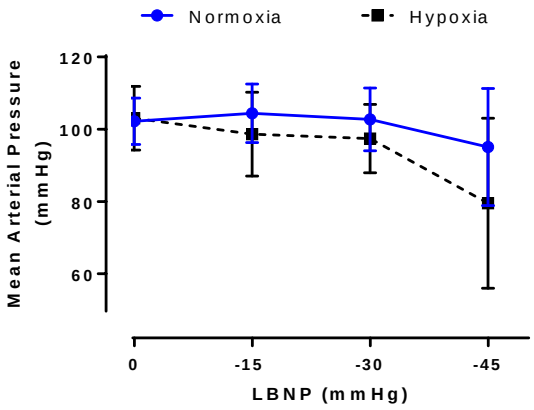


Figure 3: Change in CVP in response to LBNP during normoxia and hypoxia

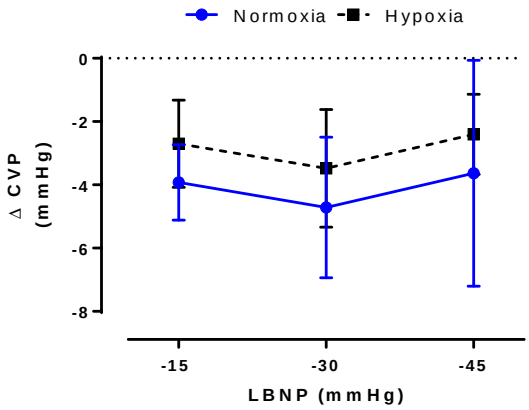
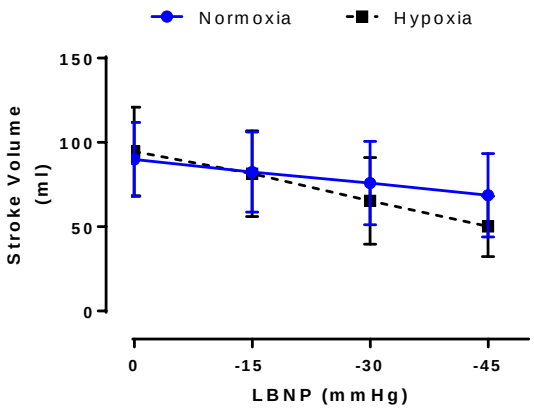


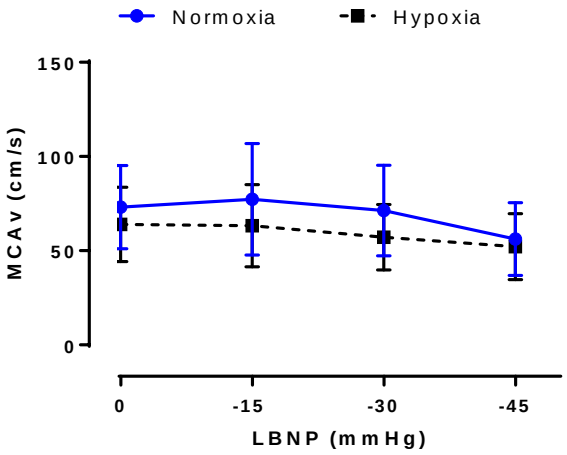
Figure 4: SV response to LBNP during normoxia and hypoxia



Further examination of hemodynamic variables, showed mean arterial pressure (MAP; figure 2) central venous pressure (CVP; figure 3), stroke volume (SV; figure 4) and middle cerebral artery velocity (MCAv; figure 5) had similar trajectories during LBNP under normoxic and hypoxic conditions.

Similarly, all other hemodynamic variables that were recorded followed a similar trajectory with no clear difference between normoxia and hypoxia.

Figure 5: MCAV response to LBNP during normoxia and hypoxia



Conclusion

The last year has been highly successful and we have completed key manuscripts related the main aims of the initial funding cycle including publication of two key papers in highly visible journals. We are continuing our secondary data analysis of this initial study and finishing the hypoxia studies and planning a third study to mimic the sympathoexcitation associated with combat a condition that might affect the performance of the monitoring device and CRI algorithm. Additional peer-reviewed manuscripts are in the process of being generated and additional follow-up studies are in the process of being conducted. Importantly these include ongoing discussion with our Army colleagues about next steps and expansion of our evaluation of CRI to operating room and ICU environments.

References

Published and in prep manuscripts

- Johnson BD, van Helmond N, Curry TB, van Buskirk CM, Convertino VA, & Joyner MJ. Reductions in Central Venous Pressure by Lower Body Negative Pressure or Blood Loss Elicit

Similar Hemodynamic Responses. *Journal of Applied Physiology*, 117(2): 131-141, 2014. doi:10.1152/japplphysiol.00070.2014.

- Rickards CA, Johnson BD, Harvey RE, Convertino VA, Joyner MJ, & Barnes JN. Cerebral Blood Flow Regulation during Progressive Blood Loss Compared to Lower Body Negative Pressure in Humans. *Journal of Applied Physiology*, 119(6): 677-685, 2015. **doi:**10.1152/japplphysiol.00127.2015
- van Helmond N, Johnson BD, Curry TB, Convertino VA, & Joyner MJ. Coagulation Changes during Lower Body Negative Pressure and Blood Loss in Humans. *American Journal of Physiology-Heart and Circulatory Physiology*, in press, doi:10.1152/ajpheart.00435.2015 .
- Johnson BD, van Helmond N, Johnson MC, Curry TB, van Buskirk CM, Convertino VA, & Joyner MJ. Hemodynamic changes during whole blood reinfusion following blood loss, in prep.
- Johnson BD, van Helmond N, Effertz CM, Johnson MC, Curry TB, van Buskirk CM, Convertino VA, & Joyner MJ. Heart rate variability and cardiac baroreflex sensitivity during lower body negative pressure and blood loss, in prep.

Abstracts

- Johnson BD, van Helmond N, Curry TB, Convertino VA, & Joyner MJ. Hemodynamic responses during lower body negative pressure and hemorrhage in humans. *FASEB J* 27:1206.3, 2013.
- van Helmond N, Johnson BD, Curry TB, Convertino VA, & Joyner MJ. The association between pulse pressure and stroke volume during lower body negative pressure and graded hemorrhage. *FASEB J* 27:1206.4, 2013.
- Johnson BD, van Helmond N, Curry TB, Convertino VA, & Joyner MJ. Relationship of Central Venous Pressure to Hemodynamic Responses during Lower Body Negative Pressure and Hemorrhage. *Medicine and Science in Sports & Exercise* 45 (5S), S39, 2013.

- van Helmond N, Johnson BD, Effertz CM, Curry TB, & Joyner MJ. Heart rate variability during graded hemorrhage in humans. *Clinical Autonomic Research* 23: 284, 2013.
- Effertz CM, Johnson BD, van Helmond N, Convertino VA, Joyner MJ, & Curry TB. Cardiac baroreflex sensitivity during lower body negative pressure and graded hemorrhage in humans. *Clinical Autonomic Research* 23: 282, 2013.
- Johnson BD, van Helmond N, Curry TB, Convertino VA, & Joyner MJ. Catecholamine responses to central hypovolemia induced by lower body negative pressure and blood loss in humans. *FASEB J*, 28:707.2, 2014.
- Effertz CM, Johnson BD, Convertino VA, Joyner MJ, & Curry TB. Changes to erythrocyte markers during lower body negative pressure and graded blood loss in humans. *FASEB J*, 28:707.3, 2014.
- Barnes JN, Johnson BD, Convertino VA, Joyner MJ, & Rickards CA. Cerebral blood flow regulation during blood loss compared to lower body negative pressure in humans. *FASEB J*, 28:1068.9, 2014.
- Johnson BD, van Helmond N, Curry TB, Convertino VA, & Joyner MJ. The effects of blood re-infusion on hemodynamics following blood removal. *FASEB J*, 29:823.4, 2015.
- van Helmond N, Johnson BD, Curry TB, Cap AP, Convertino VA, & Joyner MJ. Blood coagulation during lower body negative pressure vs. blood loss in humans. *FASEB J*, 29:823.3, 2015.
- van Helmond N, Curry TB, Cap AP, Convertino VA, Joyner MJ, & Johnson BD. Is the immune response to lower body negative pressure similar to blood loss? in prep for Experimental Biology

Participants and Other Collaborating Organizations

The following individuals have worked on the project: Drs. Michael Joyner (PI), Timothy Curry (CO-I),Walter Holbein (Post-Doc), Blair Johnson (Post-Doc), Chris Johnson (Research Tech), Andrew

Johnson (Research Tech) Maja Johnson (Research Tech), Katherine Malterer (Research Tech), Mike Mozer (Research Tech), Shelly Roberts (Head Nurse), Sarah Wolhart (Nurse), and Nancy Meyer (Scheduler). In the summer of 2015 Mike Mozer and Maja Johnson left Mayo to go to medical school. As a result, the following techs starting working on the project: Lauren Newhouse and Humphrey Petersen-Jones. In addition, in July 2015 the lab hired a new nurse named Jasmin Mccabe who has been working on this project.

There were no other organizations involved as partners.

Appendices

Please see the attached original copy of two journal articles.

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 ($p \geq 0.42$). 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; $p \leq 0.003$) 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; $p \leq 0.002$) 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 ($P \geq 0.10$). 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/m^2). These subjects were a sub-set of the 12 subjects reported in another publication focused on hemodynamic and hormonal responses to

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 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

123 below 80 mmHg; or 3) the subject began to experience symptoms of pre-syncope or syncope.
124 Hematocrit was measured from arterial blood samples collected during the baseline period and
125 at the termination of each experimental protocol.

126

127 *Measurements and Procedures.*

128 *Hemodynamic Monitoring*. Subjects were positioned in the supine posture on an
129 adjustable bed. A 3-lead electrocardiogram (ECG) was used to continuously record HR
130 (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). Arterial oxygen saturation was monitored
131 using a finger pulse oximeter and end-tidal CO_2 ($ETCO_2$) was collected from a nasal cannula
132 (Cardiocap/5, Datex-Ohmeda, Louisville, CO, USA). A 20-gauge, 5 cm catheter was placed into
133 the brachial artery under local anesthesia (2% lidocaine) using aseptic techniques and
134 ultrasound guidance. The catheter was attached to a high-resolution transducer positioned at
135 heart level to obtain continuous brachial arterial pressure waveforms. Continuous
136 hemodynamic, oxygen saturation, and $ETCO_2$ tracings were interfaced with a data acquisition
137 system for offline analysis (WinDaq, DATAQ Instruments, Akron, OH, USA).

138 *Cerebral blood velocity.* Subjects were imaged using a 2-MHz Doppler probe
139 (Transcranial Doppler (TCD), Neurovision System, Multigon, Yonkers, NY, USA) to estimate
140 middle cerebral artery blood velocity (MCAv). The basal portion of the left MCA was insonated
141 by placing the probe over the temporal bone just above the zygomatic arch in front of the ear.
142 The Doppler signal was optimized by varying the sample volume depth in incremental steps and
143 varying the angle of insonation to obtain the best-quality signal. Once the optimal signal was

144 determined, the probe was secured with a headband device to maintain a constant angle
145 throughout the protocol.

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

153 *Blood removal*. A 14-gauge catheter was placed in an antecubital vein to facilitate blood
154 removal for the blood loss protocol. The catheter was placed under local anesthesia (2%
155 lidocaine) using aseptic techniques. Preservative/anticoagulant bags (63 mL anti-coagulant
156 citrate phosphate dextrose solution) were placed below the level of the bed to allow blood to
157 transfer from the subject to the blood collection bags via gravity. In two subjects, a blood
158 pressure cuff was inflated around the upper arm to 40 mmHg to enhance the rate of blood
159 removal; this cuff pressure was released during all subsequent hemodynamic measurements.
160 As blood was being collected, it was weighed to determine the volume of blood removed by
161 multiplying the weight of the blood by a factor of 1.06 ml/g. The removed blood was kept in
162 the study room (20-22°C), the temperature of the blood was allowed to fluctuate, and the
163 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.

227 termination: 41.9 ± 0.9; P=0.020) and decreased with hemorrhage (baseline: 40.8 ± 0.9% vs.
228 termination: 39.7 ± 0.9; P=0.001). Hemodynamic responses are shown in Table 1. MAP
229 decreased between baseline and level 3 only during the LBNP trial (P=0.001). There were no
230 differences in MAP between the LBNP and blood loss trials at any level (P≥0.42). At each level,
231 CVP decreased below baseline in both LBNP and blood loss protocols, but values were
232 consistently higher during blood loss compared with LBNP (P≤0.003). During the LBNP trial, SV
233 and CO were lower than baseline at every level, but for the blood loss trial SV was reduced
234 during level 2 and 3 only and CO did not decrease below baseline values. Consistent with the
235 CVP responses, SV and CO were higher during the blood loss vs. LBNP trial at each level of the
236 protocol, except baseline. HR was higher than baseline for levels 2 and 3 of LBNP and during
237 level 3 of blood loss; in response to the greater reduction in central blood volume, HR was
238 higher during levels 2 and 3 of the LBNP trial compared with the blood loss trial. The CVP and
239 SV responses during LBNP versus blood loss are presented in figure 2; both amalgamated r^2
240 values were ≥0.80, but the slopes were <0.6, reflecting the differences in central blood volume
241 reduction between conditions.

242 Cerebral blood velocity and CVC responses to LBNP and blood loss are shown in table 1.
243 Mean MCAv decreased by 11±3% and 3±4% for the LBNP and blood loss protocols (P=0.44), but
244 was statistically distinguishable from baseline at the final level of the LBNP protocol only
245 (P=0.002). CVC did not change, and responses were similar between LBNP and blood loss trials
246 (P≥0.47). $ETCO_2$ decreased at level 3 for the blood loss trial only, and respiration rate
247 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)

311 may address these differences in central hypovolemia observed in the current investigation,
312 and allow for more accurate calculations of equivalency.

313 LF oscillatory power for both MAP and mean MCAv did not change from baseline under
314 either LBNP or blood loss conditions. The stability of MAP LF was unexpected based on
315 previously observed increases in MAP LF with LBNP of similar magnitude and duration (4, 5, 41,
316 55). LF oscillations in arterial pressure are primarily modulated by the baroreflex, evidenced by
317 a strong association with LF power in muscle sympathetic nerve activity (MSNA), which in turn,
318 is related to higher absolute MSNA (12, 27). As such, baroreflex-mediated sympathoexcitation
319 with LBNP-induced hypotension increases MSNA, and LF power in both MSNA and arterial
320 pressure (12). The very mild reductions in MAP (-8 and -2 mmHg) by the final level of LBNP and
321 blood loss in the current study may not have been sufficient to elicit increases in MSNA, hence
322 there was no increase in MSNA LF or, subsequently, MAP LF. This speculation is supported, in
323 part, by an increase in circulating norepinephrine only with LBNP and not blood loss as reported
324 by Johnson et al., (25). The small subject number combined with high inter-subject variability in
325 MAP LF responses under both protocols also contribute to this finding. As oscillations in arterial
326 pressure are the primary driving factor for increased MCAv oscillations, it is not surprising that
327 MCAv LF power did not change under either protocol.

328 Assessing the relationship between arterial pressure and cerebral blood velocity
329 oscillations via transfer function analysis in the VLF and LF ranges has been interpreted as an
330 index of cerebral autoregulation (54). The low coherence between MAP and mean MCAv in the
331 VLF (<0.5) across time and condition indicates an independence of flow from pressure within

this frequency range (54). While coherence between MAP and mean MCAv was consistently > 0.5 in the LF range, transfer function gain and phase did not change with either LBNP or blood loss, and were not different between conditions. These findings are in contrast with a number of studies that show either a reduction (41) or increase (55) in MAP-mean MCAv gain during LBNP of similar magnitude. In particular, Zhang et al., (55) suggested that simultaneous increases in the magnitude of oscillations in both arterial pressure and cerebral blood velocity and the subsequent increase in MAP-mean MCAv gain, represented attenuated cerebral autoregulation, that may, in turn, predispose individuals to presyncope. The stability of MAP-mean MCAv gain and phase reported in the current investigation is most likely associated with the stability of MAP and mean MCAv LF oscillations, and the high inter-subject variability inherent in transfer function estimates of cerebral pressure-flow relationships, further compounded by the small sample size utilized in this study. In the time domain, cerebral autoregulation can also be assessed as the gain between changes in arterial pressure and cerebral blood velocity (36, 40); in the present study this relationship was not altered under either condition, and was not statistically distinguishable between conditions. Together, these data suggest that cerebral pressure-flow relationships across multiple time scales (fast component via transfer function analysis and slow component via time domain analysis) were not affected by the magnitude of central hypovolemia induced by either LBNP or blood loss. Other factors, including small reductions in arterial CO_2 and increased sympathetic drive may also be contributing to the observed small decrease in MCAv with LBNP and blood loss.

Methodological Considerations

Many of the key methodological considerations associated with the design of this study have been addressed by Johnson et al., (25) including removal of absolute blood volumes (i.e., 333, 666, 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 ($ETCO_2$ ≥ 9 mmHg above baseline) and decreases in arterial CO_2 ($ETCO_2$ ≥ 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 Department of the Army position, or decision, unless so designated by other official documentation. Approved for public release; distribution unlimited.

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.

Tables

Table 1 Physiological Responses to LBNP and Blood Loss

Hypovolemic stress	**Baseline**	**Level 1**	**Level 2**	**Level 3**
LBNP (mmHg)	**--**	***-15***	***-30***	***-45***
Blood Loss (mL)	**--**	***-333***	***-667***	***-1000***
MAP, mmHg				
• LBNP	94 ± 3	91 ± 3	87 ± 5	86 ± 4†
• Blood Loss	93 ± 3	92 ± 2	90 ± 3	91 ± 3
CVP, mmHg				
• LBNP	7.4 ± 0.9	2.8 ± 0.7†	1.2 ± 0.6†	0.2 ± 0.9†
• Blood Loss	6.5 ± 0.8	4.0 ± 0.8*†	3.5 ± 0.8*†	2.1 ± 0.9*†
SV, mL				
• LBNP	81 ± 4	71 ± 4†	60 ± 3†	51 ± 2†
• Blood Loss	85 ± 5	80 ± 3*	74 ± 3*†	68 ± 4*†
HR, beats/min				
• LBNP	57 ± 3	60 ± 2	67 ± 3†	76 ± 4†
• Blood Loss	57 ± 3	58 ± 2	61 ± 2*	65 ± 3*†
CO, L/min				
• LBNP	4.6 ± 0.3	4.2 ± 0.2†	3.9 ± 0.2†	3.8 ± 0.2†
• Blood Loss	4.8 ± 0.3	4.7 ± 0.3*	4.5 ± 0.2*	4.4 ± 0.3*
Mean MCAv, cm/s				
• LBNP	70.0 ± 4.2	69.3 ± 4.3	65.2 ± 4.3	61.5 ± 5.8†
• Blood Loss	69.5 ± 5.1	69.6 ± 5.3	67.7 ± 5.0	66.5 ± 5.2
CVC, cm/s/mmHg				
• LBNP	0.75 ± 0.04	0.77 ± 0.05	0.76 ± 0.05	0.72 ± 0.05
• Blood Loss	0.75 ± 0.04	0.75 ± 0.05	0.75 ± 0.04	0.73 ± 0.05
$ETCO_2$, mmHg				
• LBNP	40 ± 2	40 ± 2	39 ± 2	38 ± 3
• Blood Loss	41 ± 2	40 ± 2	39 ± 2	38 ± 3†
Respiration rate, n				
• LBNP	15 ± 1	13 ± 1†	13 ± 1†	14 ± 1†
• Blood Loss	13 ± 1*	13 ± 1	13 ± 1	12 ±1

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; $ETCO_2$=end-tidal carbon dioxide. *p<0.05 vs. LBNP at the same level; †p<0.05 vs. baseline of the same protocol.

1. **Barcroft H, Edholm OG, McMichael J, and Sharpey-Schafer EP**. Posthaemorrhagic fainting study by cardiac output and forearm flow. *Lancet* i: 489-491, 1944.
2. **Bassin R, Vladeck BC, Kark AE, and Shoemaker WC**. Rapid and slow hemorrhage in man. I. Sequential hemodynamic responses. *Ann Surg* 173: 325-330, 1971. 1397391.
3. **Bondar RL, Kassam MS, Stein F, Dunphy PT, Fortney S, and Riedesel ML**. Simultaneous cerebrovascular and cardiovascular responses during presyncope. *Stroke* 26: 1794-1800, 1995.
4. **Brown CM, Dutsch M, Hecht MJ, Neundorfer B, and Hilz MJ**. Assessment of cerebrovascular and cardiovascular responses to lower body negative pressure as a test of cerebral autoregulation. *J Neurol Sci* 208: 71-78, 2003.
5. **Brown CM, Dutsch M, Ohring S, Neundorfer B, and Hilz MJ**. Cerebral autoregulation is compromised during simulated fluctuations in gravitational stress. *Eur J Appl Physiol* 91: 279-286, 2004.
6. **Brown E, Goei JS, Greenfield AD, and Plassaras GC**. Circulatory responses to simulated gravitational shifts of blood in man induced by exposure of the body below the iliac crests to sub-atmospheric pressure. *J Physiol* 183: 607-627, 1966. PMC1357510.
7. **Colier WN, Binkhorst RA, Hopman MT, and Oeseburg B**. Cerebral and circulatory haemodynamics before vasovagal syncope induced by orthostatic stress. *Clin Physiol* 17: 83-94, 1997.
8. **Convertino VA, Grudic G, Mulligan J, and Moulton S**. Estimation of individual-specific progression to impending cardiovascular instability using arterial waveforms. *Journal of applied physiology (Bethesda, Md : 1985)* 115: 1196-1202, 2013.
9. **Convertino VA, Rickards CA, Lurie KG, and Ryan KL**. Hyperventilation in response to progressive reduction in central blood volume to near syncope. *Aviat Space Environ Med* 80: 1012-1017, 2009.
10. **Convertino VA, Ryan KL, Rickards CA, Cooke WH, Metzger A, Holcomb JB, Adams BD, and Lurie KG**. Inspiratory resistance maintains arterial pressure during central hypovolemia: implications for treatment of patients with severe hemorrhage. *Crit Care Med* 35: 1145-1152, 2007.
11. **Convertino VA, and Sather TM**. Vasoactive neuroendocrine responses associated with tolerance to lower body negative pressure in humans. *Clin Physiol* 20: 177-184, 2000.
12. **Cooke WH, Rickards CA, Ryan KL, Kuusela TA, and Convertino VA**. Muscle sympathetic nerve activity during intense lower body negative pressure to presyncope in humans. *J Physiol (Lond)* 587: 4987-4999, 2009. PMC2770161.
13. **Cooke WH, Ryan KL, and Convertino VA**. Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *J Appl Physiol* 96: 1249-1261, 2004.
14. **Coverdale NS, Gati JS, Opalevych O, Perrotta A, and Shoemaker JK**. Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *J Appl Physiol* 117: 1090-1096, 2014. PMID: 25012027.
15. **Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, Mallett O, Zubko T, Oetjen-Gerdes L, Rasmussen TE, Butler FK, Kotwal RS, Holcomb JB, Wade C, Champion H, Lawnick M, Moores L, and Blackbourne LH**. Death on the battlefield (2001-2011): Implications for the future of combat casualty care. *J Trauma Acute Care Surg* 73: S431-437, 2012.
16. **Ebert RV, and Stead EA**. The effect of the application of tourniquets on the hemodynamics of the circulation. *J Clin Invest* 19: 561-567, 1940. PMC434991.
17. **Giller CA**. The frequency-dependent behaviours of cerebral autoregulation. *Neurosurgery* 27: 362-368, 1990.
18. **Greenleaf JE, Petersen TW, Gabrielsen A, Pump B, Bie P, Christensen NJ, Warberg J, Videbaek R, Simonson SR, and Norsk P**. Low LBNP tolerance in men is associated with attenuated activation of the renin-angiotensin system. *Am J Physiol* 279: R822-829, 2000.
19. **Hanson JM, Van Hoeyweghen R, Kirkman E, Thomas A, and Horan MA**. Use of stroke distance in the early detection of simulated blood loss. *J Trauma* 44: 128-134, 1998.

20. **Hinojosa-Laborde C, Rickards CA, Ryan KL, and Convertino VA**. Heart Rate Variability during Simulated Hemorrhage with Lower Body Negative Pressure in High and Low Tolerant Subjects. *Front Physiol* 2: 85, 2011. 3221414.
21. **Hinojosa-Laborde C, Shade RE, Muniz GW, Bauer C, Goei KA, Pidcoke HF, Chung KK, Cap AP, and Convertino VA**. Validation of lower body negative pressure as an experimental model of hemorrhage. *J Appl Physiol* 116: 406-415, 2014. 4073981.
22. **Huang SY, Moore LG, McCullough RE, McCullough RG, Micco AJ, Fulco C, Cymerman A, Manco-Johnson M, Weil JV, and Reeves JT**. Internal carotid and vertebral arterial flow velocity in men at high altitude. *J Appl Physiol* 63: 395-400, 1987.
23. **Jellema WT, Imholz BPM, Van Goudoever J, Wesseling KH, and Van Lieshout JJ**. Finger arterial versus intrabrachial pressure and continuous cardiac output during head-up tilt testing in healthy subjects. *Clin Sci* 91: 193-200, 1996.
24. **Jeong SM, Shibata S, Levine BD, and Zhang R**. Exercise plus volume loading prevents orthostatic intolerance but not reduction in cerebral blood flow velocity after bed rest. *Am J Physiol Heart Circ Physiol* 302: H489-497, 2012.
25. **Johnson BD, van Helmond N, Curry TB, van Buskirk CM, Convertino VA, and Joyner MJ**. Reductions in central venous pressure by lower body negative pressure or blood loss elicit similar hemodynamic responses. *J Appl Physiol* 117: 131-141, 2014.
26. **Johnson JM, Rowell LB, Niederberger M, and Eisman MM**. Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures. *Circ Res* 34: 515-524, 1974.
27. **Julien C**. The enigma of Mayer waves: Facts and models. *Cardiovasc Res* 70: 12-21, 2006.
28. **Kauvar DS, Lefering R, and Wade CE**. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma* 60: S3-11, 2006.
29. **Levine BD, Giller CA, Lane LD, Buckey JC, and Blomqvist CG**. Cerebral versus systemic hemodynamics during graded orthostatic stress in humans. *Circulation* 90: 298-306, 1994.
30. **Lewis NC, Bain AR, MacLeod DB, Wildfong KW, Smith KJ, Willie CK, Sanders ML, Numan T, Morrison SA, Foster GE, Stewart JM, and Ainslie PN**. Impact of hypocapnia and cerebral perfusion on orthostatic tolerance. *J Physiol* 592: 5203-5219, 2014.
31. **Lewis NC, Smith KJ, Bain AR, Wildfong KW, Numan T, and Ainslie PN**. Impact of transient hypotension on regional cerebral blood flow in humans. *Clin Sci (Lond)* 129: 169-178, 2015.
32. **Lucas RA, Pearson J, Schlader ZJ, and Crandall CG**. Hypercapnia-induced increases in cerebral blood flow do not improve lower body negative pressure tolerance during hyperthermia. *Am J Physiol Regul Integr Comp Physiol* 305: R604-609, 2013. PMC3763041.
33. **Madsen PL, and Secher NH**. Near-infrared oximetry of the brain. *Prog Neurobiol* 58: 541-560, 1999.
34. **Martin PJ, Evans DH, and Naylor AR**. Measurement of blood flow velocity in the basal cerebral circulation: advantages of transcranial color-coded sonography over conventional transcranial Doppler. *J Clin Ultrasound* 23: 21-26, 1995.
35. **Murray RH, Thompson LJ, Bowers JA, and Albright CD**. Hemodynamic effects of graded hypovolemia and vasodepressor syncope induced by lower body negative pressure. *Am Heart J* 76: 799-811, 1968.
36. **Novak V, Novak P, Spies JM, and Low PA**. Autoregulation of cerebral blood flow in orthostatic hypotension. *Stroke* 29: 104-111, 1998.
37. **Ogoh S, Sato K, Nakahara H, Okazaki K, Subudhi AW, and Miyamoto T**. Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. *Exp Physiol* 98: 692-698, 2013. PMID: 23143991.
38. **Pollard V, Prough DS, DeMelo AE, Deyo DJ, Uchida T, and Stoddart HF**. Validation in volunteers of a near-infrared spectroscope for monitoring brain oxygenation in vivo. *Anesth Analg* 82: 269-277, 1996.

39. **Rea RF, Hamdan M, Clary MP, Randels MJ, Dayton PJ, and Strauss RG**. Comparison of muscle sympathetic responses to hemorrhage and lower body negative pressure in humans. *J Appl Physiol* 70: 1401-1405, 1991.
40. **Rickards CA, Cohen KD, Bergeron LL, Burton BL, Khatri PJ, Lee CT, Ryan KL, Cooke WH, Doerr DF, and Convertino VA**. Cerebral blood flow response and its association with symptoms during orthostatic hypotension. *Aviat Space Environ Med* 78: 653-658, 2007.
41. **Rickards CA, Ryan KL, Cooke WH, and Convertino VA**. Tolerance to central hypovolemia: the influence of oscillations in arterial pressure and cerebral blood velocity. *J Appl Physiol* 111: 1048-1058, 2011. PMID: 21799129.
42. **Ryan KL, Cooke WH, Rickards CA, Lurie KG, and Convertino VA**. Breathing through an inspiratory threshold device improves stroke volume during central hypovolemia in humans. *J Appl Physiol* 104: 1402-1409, 2008.
43. **Sato K, Fisher JP, Seifert T, Overgaard M, Secher NH, and Ogoh S**. Blood flow in internal carotid and vertebral arteries during orthostatic stress. *Exp Physiol* 97: 1272-1280, 2012.
44. **Serrador JM, Picot PA, Rutt BK, Shoemaker JK, and Bondar RL**. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31: 1672-1678, 2000.
45. **Shenkin HA, Cheney RH, Govons SR, Hardy JD, and Fletcher AG**. On the diagnosis of hemorrhage in man - a study of volunteers bled large amounts. *Am J Med Sci* 208: 421-436, 1944.
46. **Stevens PM, and Lamb LE**. Effects of lower body negative pressure on the cardiovascular system. *Am J Cardiol* 16: 506-515, 1965.
47. **Summers RL, Ward KR, Witten T, Convertino VA, Ryan KL, Coleman TG, and Hester RL**. Validation of a computational platform for the analysis of the physiologic mechanisms of a human experimental model of hemorrhage. *Resuscitation* 80: 1405-1410, 2009. PMC3042239.
48. **Torella F, Cowley RD, Thorniley MS, and McCollum CN**. Regional tissue oxygenation during hemorrhage: can near infrared spectroscopy be used to monitor blood loss? *Shock* 18: 440-444, 2002.
49. **Verbree J, Bronzwaer AS, Ghariq E, Versluis MJ, Daemen MJ, van Buchem MA, Dahan A, Van Lieshout JJ, and van Osch MJ**. Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra high-field MRI. *J Appl Physiol* 117: 1084-1089, 2014. PMID: 25190741.
50. **Wallace JP, and Sharpey-Schafer EP**. Blood changes following controlled hemorrhage in man. *Lancet* 2: 393-395, 1941.
51. **Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK, and Ainslie PN**. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods* 196: 221-237, 2011. PMID: 21276818.
52. **Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA, and Ainslie PN**. Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol* 590: 3261-3275, 2012. PMC3459041.
53. **Wolthuis RA, Bergman SA, and Nicogossian AE**. Physiological effects of locally applied reduced pressure in man. *Physiol Rev* 54: 566-595, 1974.
54. **Zhang R, Zuckerman JH, Giller CA, and Levine BD**. Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol* 274: H233-241, 1998.
55. **Zhang R, Zuckerman JH, and Levine BD**. Deterioration of cerebral autoregulation during orthostatic stress: insights from the frequency domain. *J Appl Physiol* 85: 1113-1122, 1998.

FIGURE 1

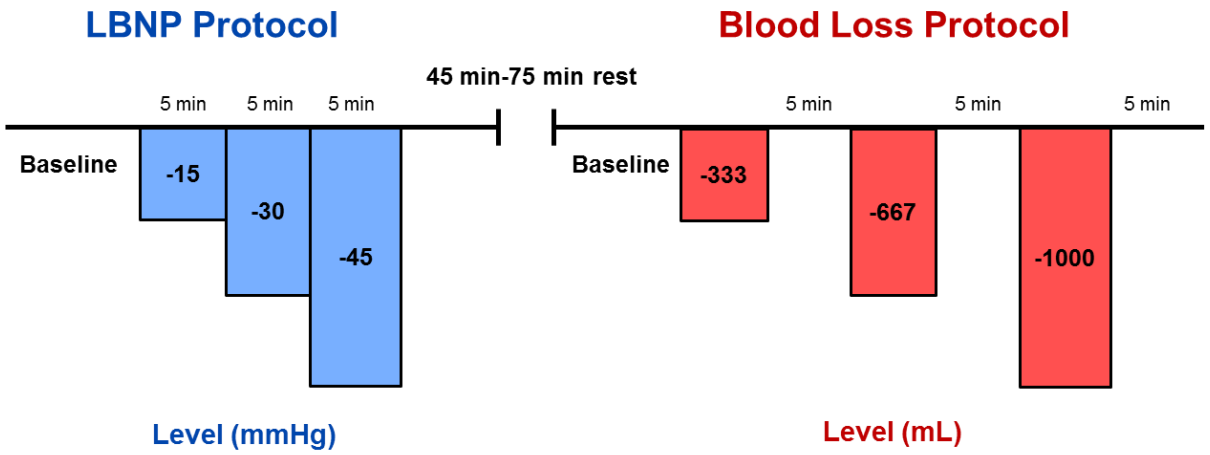
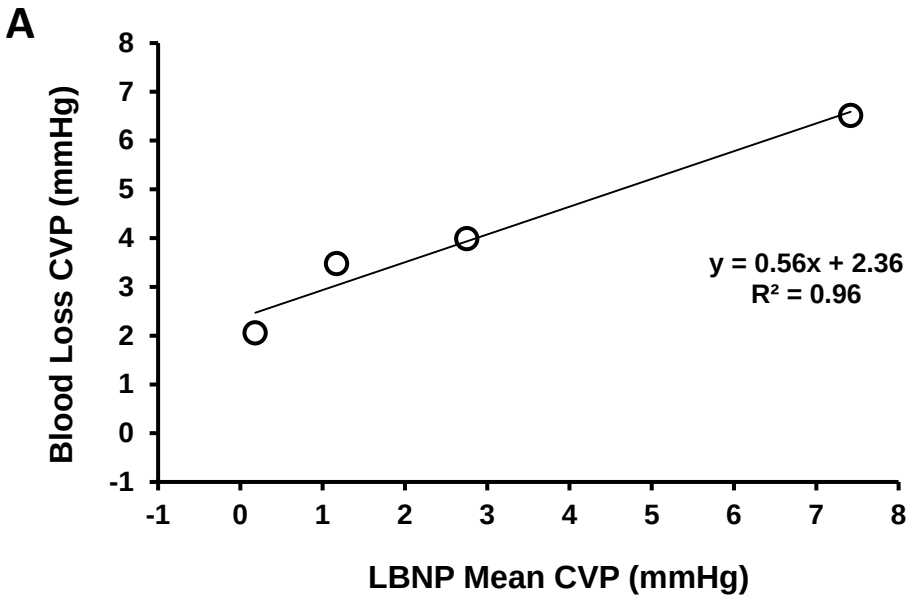
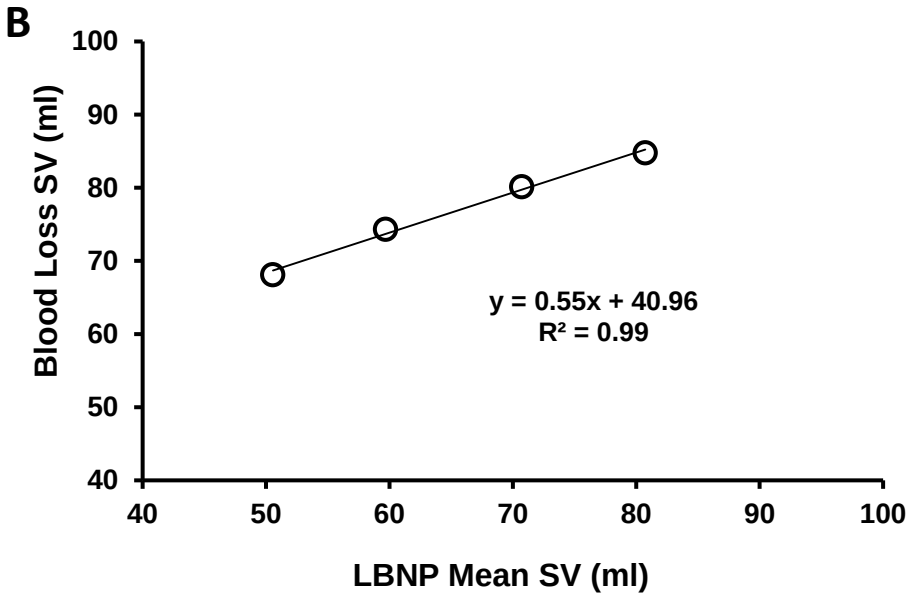
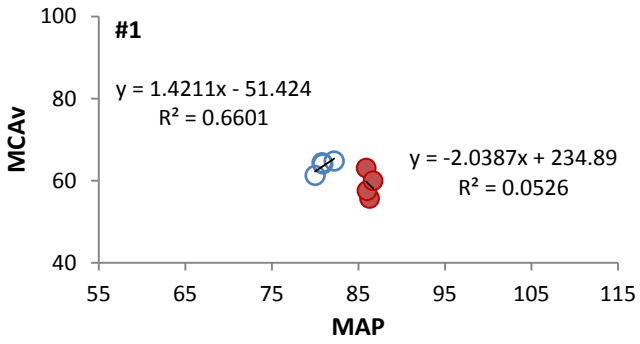
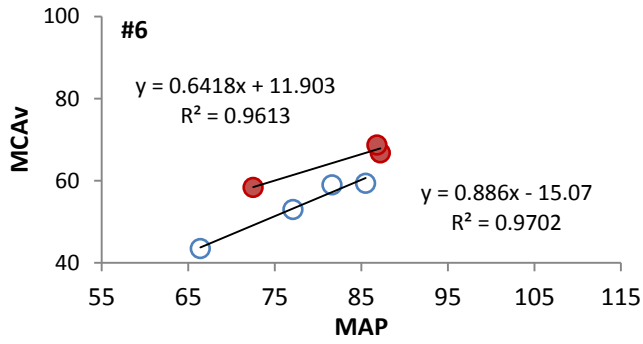

LBNP Protocol
Blood Loss Protocol
45 min-75 min rest
5 min
5 min
5 min
5 min
5 min
5 min
Baseline
Baseline
-15
-30
-45
-333
-667
-1000
Level (mmHg)
Level (mL)

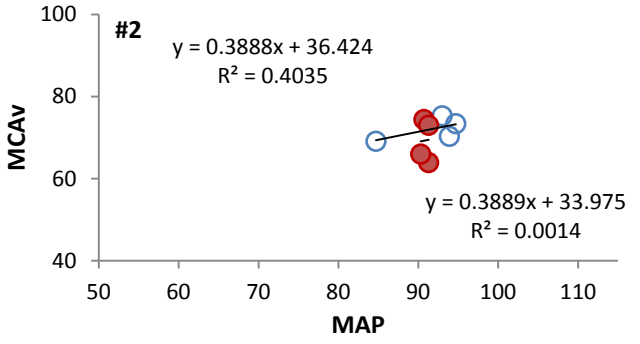
FIGURE 2

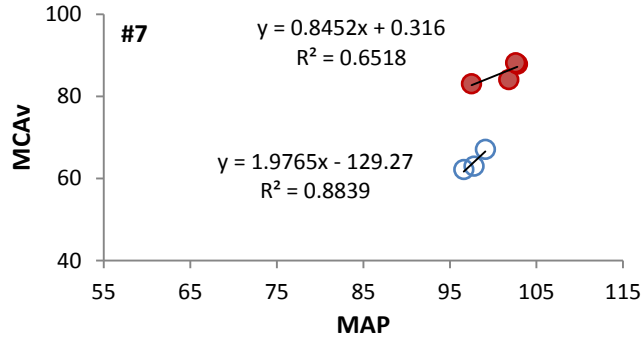

A
Blood Loss CVP (mmHg)
8
7
6
5
4
3
2
1
0
-1
-1
0
1
2
3
4
5
6
7
8
y = 0.56x + 2.36
R² = 0.96
LBNP Mean CVP (mmHg)

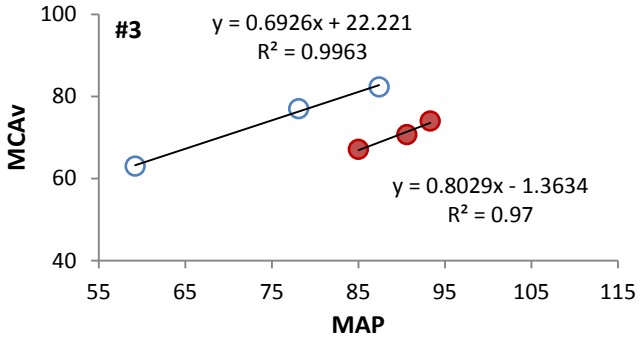

B
Blood Loss SV (ml)
100
90
80
70
60
50
40
40
50
60
70
80
90
100
y = 0.55x + 40.96
R² = 0.99
LBNP Mean SV (ml)

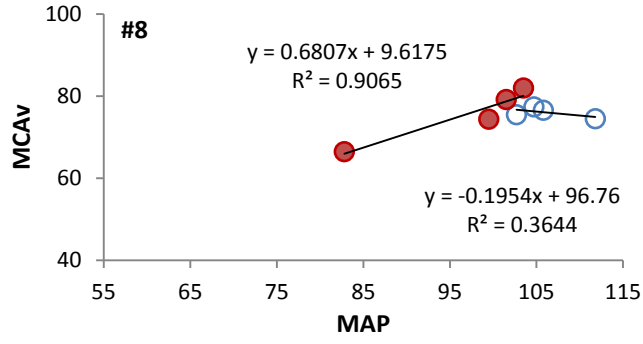

#1
y = 1.4211x - 51.424
R² = 0.6601
y = -2.0387x + 234.89
R² = 0.0526
MCAv
MAP

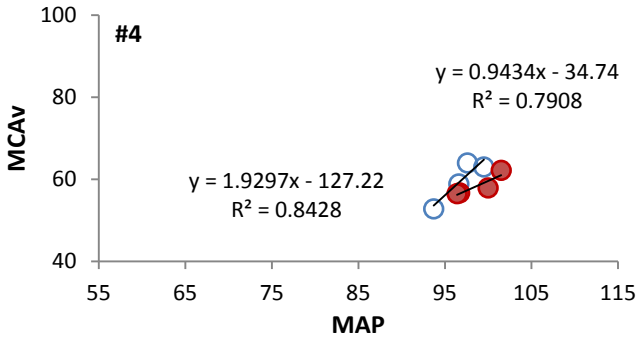

#6
y = 0.6418x + 11.903
R² = 0.9613
y = 0.886x - 15.07
R² = 0.9702
MCAv
MAP

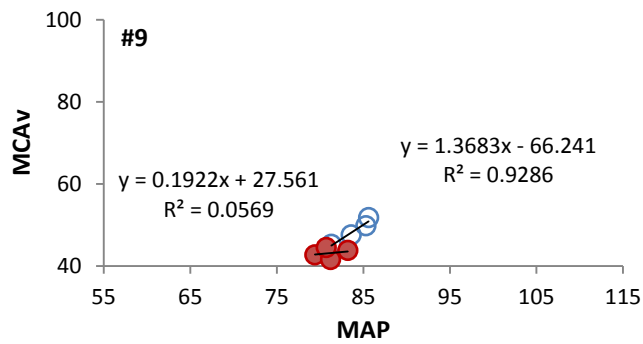

#2
y = 0.3888x + 36.424
R² = 0.4035
y = 0.3889x + 33.975
R² = 0.0014
MCAv
MAP

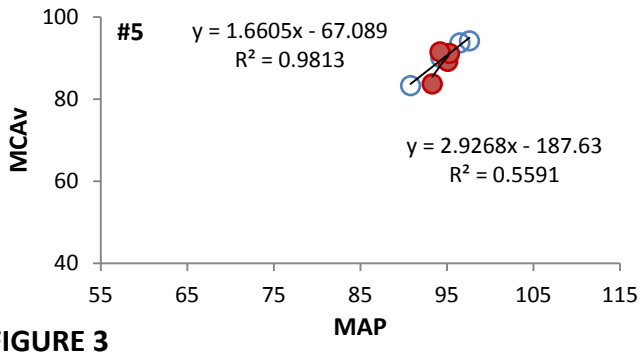

#7
y = 0.8452x + 0.316
R² = 0.6518
y = 1.9765x - 129.27
R² = 0.8839
MCAv
MAP


#3
y = 0.6926x + 22.221
R² = 0.9963
y = 0.8029x - 1.3634
R² = 0.97
MCAv
MAP


#8
y = 0.6807x + 9.6175
R² = 0.9065
y = -0.1954x + 96.76
R² = 0.3644
MCAv
MAP


#4
y = 0.9434x - 34.74
R² = 0.7908
y = 1.9297x - 127.22
R² = 0.8428
MCAv
MAP


#9
y = 1.3683x - 66.241
R² = 0.9286
y = 0.1922x + 27.561
R² = 0.0569
MCAv
MAP


#5
y = 1.6605x - 67.089
R² = 0.9813
y = 2.9268x - 187.63
R² = 0.5591
MCAv
MAP

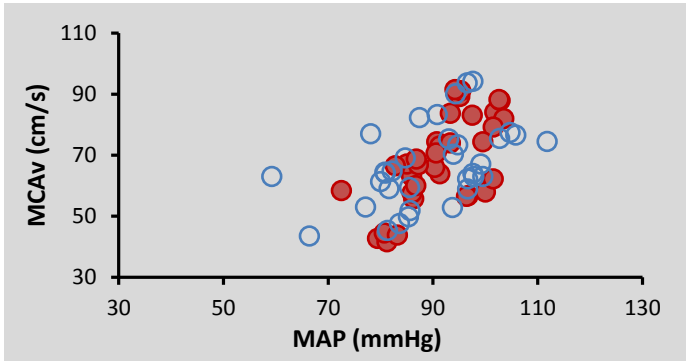

MCAv (cm/s)
MAP (mmHg)

FIGURE 3

FIGURE 4

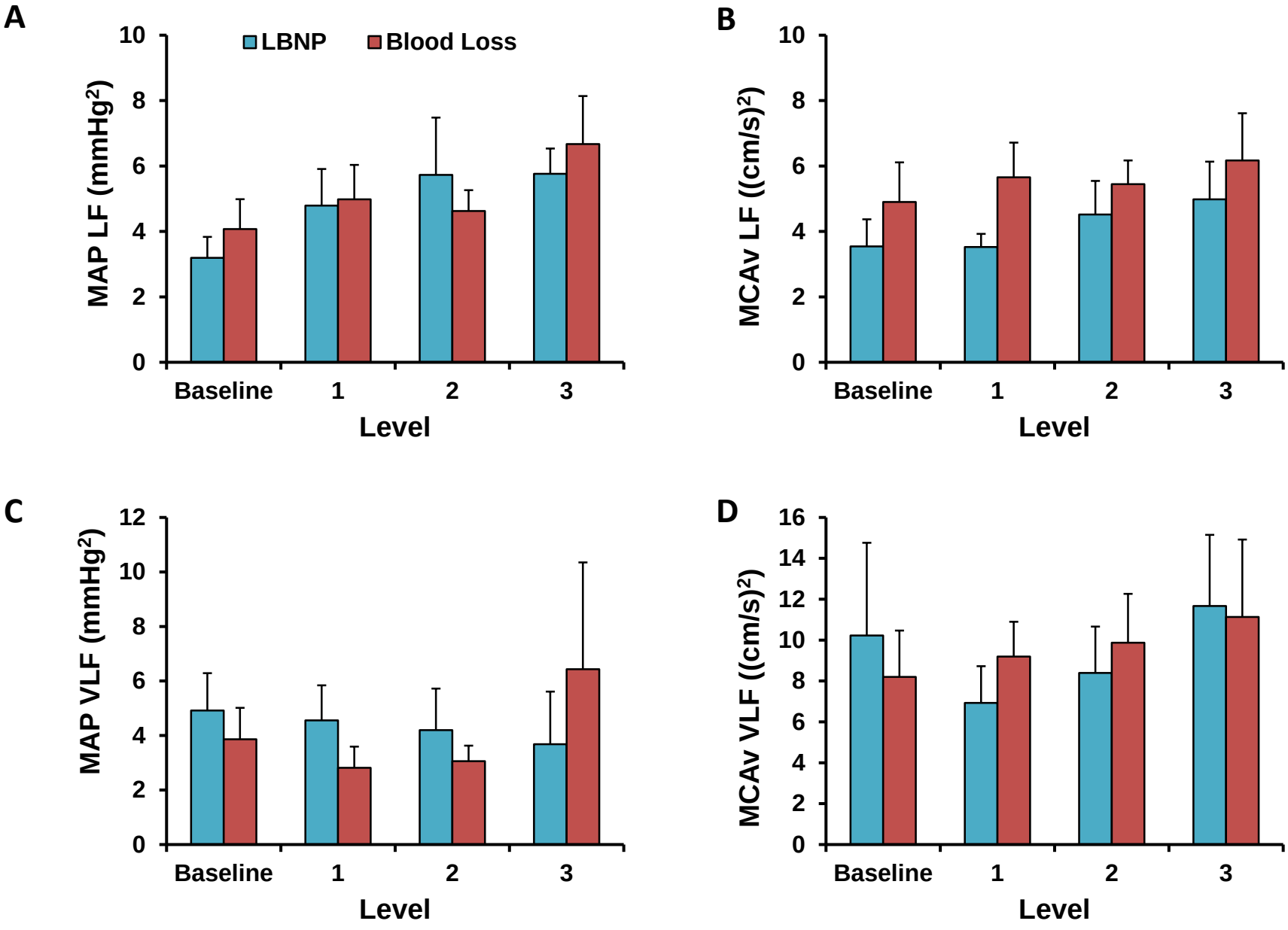
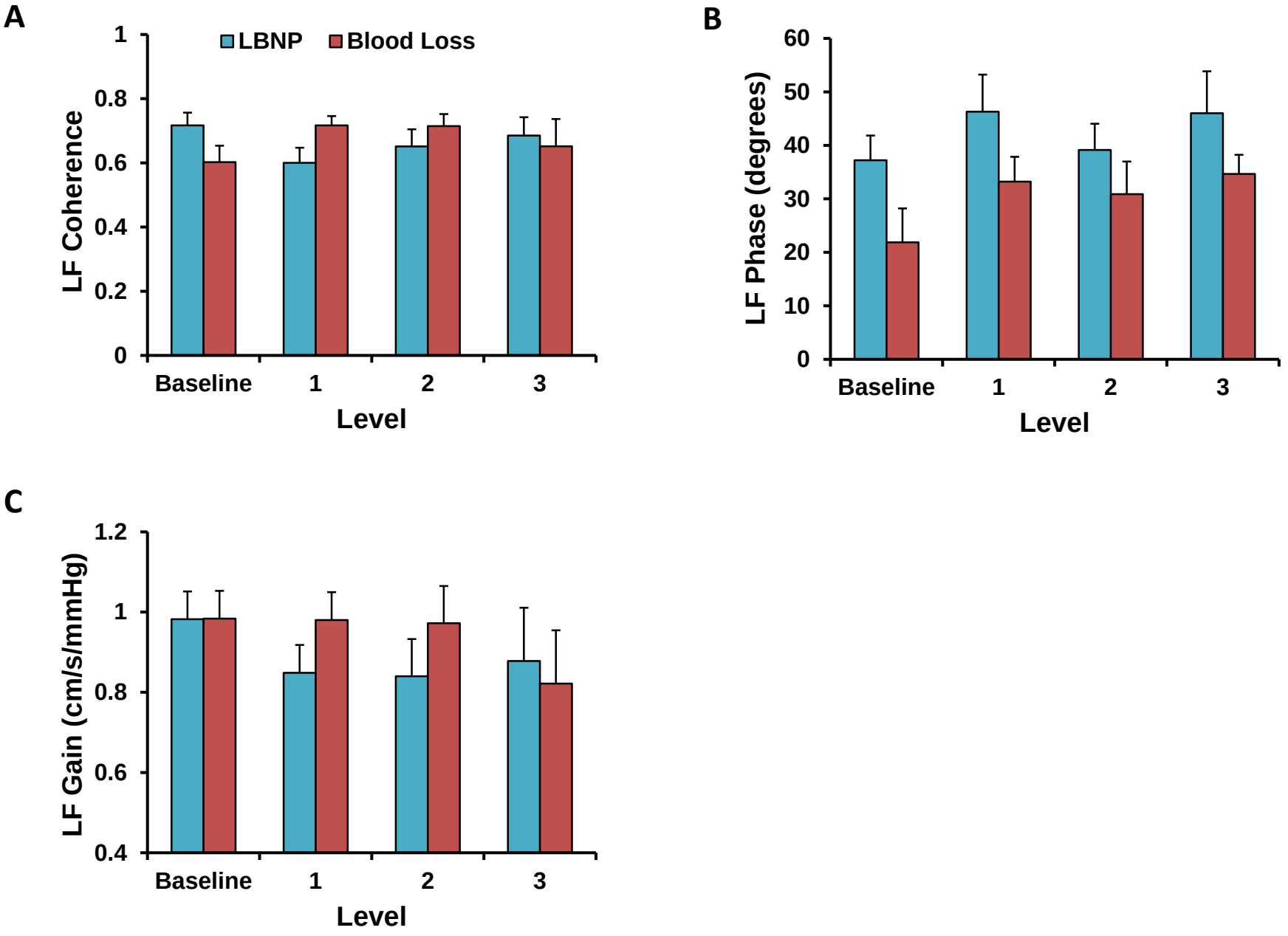

A
LBNP
Blood Loss
MAP LF (mmHg²)
10
8
6
4
2
0
Baseline
1
2
3
Level
B
MCAv LF ((cm/s)²)
10
8
6
4
2
0
Baseline
1
2
3
Level
C
MAP VLF (mmHg²)
12
10
8
6
4
2
0
Baseline
1
2
3
Level
D
MCAv VLF ((cm/s)²)
16
14
12
10
8
6
4
2
0
Baseline
1
2
3
Level

FIGURE 5


A
LBNP
Blood Loss
LF Coherence
1
0.8
0.6
0.4
0.2
0
Baseline
1
2
3
Level
B
LF Phase (degrees)
60
50
40
30
20
10
0
Baseline
1
2
3
Level
C
LF Gain (cm/s/mmHg)
1.2
1
0.8
0.6
0.4
Baseline
1
2
3
Level

Articles in PresS. Am J Physiol Heart Circ Physiol (September 14, 2015). doi:10.1152/ajpheart.00435.2015

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^{o} 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 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/m^2) 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, $p \leq 0.001$), SV (LBNP 54.1 ± 3.3 BL 70.5 ± 2.7 mL, $p \leq 0.001$) 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, $p \leq 0.001$) versus BL. Overall, 45 mmHg of LBNP caused greater changes in hemodynamic parameters than 1000 mL of BL.

249

250 *Effects of LBNP and BL on Hemoconcentration*

251 As we expected, several markers indicated that LBNP caused hemoconcentration, while
252 BL induced hemodilution (Table 2). After LBNP there was an increase in hemoglobin (14.2 ±
253 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
254 decrease in estimated plasma volume (59 ±0.8 to 56 ± 0.9 %, $p \leq 0.001$) compared to baseline
255 values. BL induced a decrease in hemoglobin (14.3 ± 0.4 to 14.0 ± 0.4 g/dL, $p = 0.006$) and
256 hematocrit (41 ± 0.8 to 40 ± 0.9 %, $p = 0.006$) and an increase in estimated plasma volume (59 ±
257 0.9 to 61 ± 1.1 %, $p = 0.004$) compared to baseline values. At protocol termination, hemoglobin
258 ($p \leq 0.001$) and hematocrit ($p \leq 0.001$) were lower in BL versus LBNP and estimated plasma
259 volume ($p \leq 0.001$) was greater in BL when compared to LBNP.

260 *Effects of LBNP and BL on Standard Laboratory Coagulation Tests*

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

264 *Effects of LBNP and BL on TEG values*

265 At protocol termination, R times were quicker versus baseline for both LBNP and BL
266 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
267 hoc test $p = 0.037$ and $p = 0.039$, Figure 2) and these relative changes were not different from
268 each other. Regression line slopes produced from the relationship between TEG measures and
269 CVP fell on similar trajectories during LBNP and BL, except for the slope of α angle vs. CVP (-
270 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, $p \leq 0.001$ and $p = 0.002$) and norepinephrine (LBNP 148 ± 20 to 354 ± 44 BL 155 ± 22 to 211 ± 29 pg/mL, $p \leq 0.001$ 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 ($p = 0.003$).

DISCUSSION

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 β_2-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.

		Baseline	Termination
CVP (mmHg)			
	LBNP	7.3 ± 0.6	-0.2 ± 0.6*
	BL	6.1 ± 0.6†	1.8 ± 0.8*†
HR (bpm)			
	LBNP	60 ± 2.5	80 ± 5.1*
	BL	60 ± 2.8	67 ± 2.6*†
MAP (mmHg)			
	LBNP	93.5 ± 2.3	84.5 ± 4.7*
	BL	91.8 ± 1.9	87.0 ± 2.7
SV (mL)			
	LBNP	83.2 ± 2.7	54.1 ± 3.3*
	BL	89.5 ± 2.7†	70.5 ± 2.7*†
CO (L/min)			
	LBNP	5.0 ± 0.3	4.1 ± 0.1*
	BL	5.3 ± 0.3†	4.7 ± 0.2*†

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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Table 2. Effects of LBNP and BL on complete blood counts.

		Baseline	Termination
Hgb (g/dL)			
	LBNP	14.2 ± 0.4	14.7 ± 0.4*
	BL	14.3 ± 0.4	14.0 ± 0.4*†
Hct (%)			
	LBNP	41 ± 0.8	42 ± 0.8*
	BL	41 ± 0.8	40 ± 0.9*†
RBC (*10^12/L)			
	LBNP	4.8 ± 0.1	5.0 ± 0.1*
	BL	4.8 ± 0.1	4.7 ± 0.1*†
Plasma volume (%)			
	LBNP	59 ± 0.8	56 ± 0.9*
	BL	59 ± 0.9	61 ± 1.1*†
Platelet count (*10^9/L)			
	LBNP	194 ± 7	212 ± 10*
	BL	186 ± 9	200 ± 11*

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 ($P < 0.05$).

Table 3. Effects of LBNP and BL on standard coagulation tests.

		Baseline	Termination
PT (s)			
	LBNP	12.2 ± 0.2	12.0 ± 0.1*
	BL	12.1 ± 0.2	12.2 ± 0.3
APTT (s)			
	LBNP	32.2 ± 0.7	31.0 ± 0.8*
	BL	32.6 ± 0.9	32.4 ± 0.9

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.

		Baseline	Termination
LY30 (%)			
	LBNP	1.6 ± 0.4	3.6 ± 1.7
	BL	2.3 ± 1.1	3.1 ± 1.6
LY60 (%)			
	LBNP	5.6 ± 1.1	7.5 ± 2.5
	BL	6.1 ± 1.9	7.4 ± 2.5

LBNP = lower body negative pressure; BL = blood loss.
Values are means ± standard error, n = 12.

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Table 5. Effects of LBNP and BL on catecholamine levels.

	Baseline	Termination
Norephineprine (pg/mL)		
LBNP	148 ± 20	354 ± 44*
BL	155 ± 22	211 ± 29*†
Epinephrine (pg/mL)		
LBNP	53 ± 7	144 ± 30*
BL	49 ± 7	103 ± 19*

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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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

1. **Boulanger L, Joshi AV, Tortella BJ, Menzin J, Caloyeras JP, and Russell MW.** Excess mortality, length of stay, and costs associated with serious hemorrhage among trauma patients: findings from the National Trauma Data Bank. *Am Surg* 73: 1269-1274, 2007.

2. **Cannon WB and Gray H.** Factors affecting the coagulation ime of blood. 2. The hastening or retarding of coagulation by adrenalin injections. . *Am J Physiol* 34: 232-242, 1914.

3. **Chatpun S and Cabrales P.** Effects on cardiac function of a novel low viscosity plasma expander based on polyethylene glycol conjugated albumin. *Minerva anestesiologica* 77: 704-714, 2011.

4. **Cooke WH, Ryan KL, and Convertino VA.** Lower body negative pressure as a model to study progression to acute hemorrhagic shock in humans. *Journal of applied physiology (Bethesda, Md : 1985)* 96: 1249-1261, 2004.

5. **Cvirn G, Schlagenhauf A, Leschnik B, Koestenberger M, Roessler A, Jantscher A, Vrecko K, Juergens G, Hinghofer-Szalkay H, and Goswami N.** Coagulation changes during presyncope and recovery. *PloS one* 7: e42221, 2012.

6. **Dill DB and Costill DL.** Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247-248, 1974.

7. **Drobin D and Hahn RG.** Volume kinetics of Ringer's solution in hypovolemic volunteers. *Anesthesiology* 90: 81-91, 1999.

8. **Eastridge BJ, Hardin M, Cantrell J, Oetjen-Gerdes L, Zubko T, Mallak C, Wade CE, Simmons J, Mace J, Mabry R, Bolenbaucher R, and Blackbourne LH.** Died of wounds on the battlefield: causation and implications for improving combat casualty care. *J Trauma* 71: S4-8, 2011.

9. **Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, Mallett O, Zubko T, Oetjen-Gerdes L, Rasmussen TE, Butler FK, Kotwal RS, Holcomb JB, Wade C, Champion H, Lawnick M, Moores L, and Blackbourne LH.** Death on the battlefield (2001-2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg* 73: S431-437, 2012.

10. **Feng DL, Murillo J, Jadhav P, McKenna C, Gebara OC, Lipinska I, Muller JE, and Tofler GH.** Upright posture and maximal exercise increase platelet aggregability and prostacyclin production in healthy male subjects. *British journal of sports medicine* 33: 401-404, 1999.

11. **Gauer OH, Henry JP, and Sieker HO.** Changes in central venous pressure after moderate hemorrhage and transfusion in man. *Circ Res* 4: 79-84, 1956.

12. **Hagan RD, Diaz FJ, and Horvath SM.** Plasma volume changes with movement to supine and standing positions. *Journal of applied physiology: respiratory, environmental and exercise physiology* 45: 414-417, 1978.

13. **Harrison MH, Graveney MJ, and Cochrane L.** Some sources of error in the calculation of relative change in plasma volume. *Europ J Appl Physiol* 50: 13-21, 1982.

14. **Henry JP, Gauer OH, and Sieker HO.** The effect of moderate changes in blood volume on left and right atrial pressures. *Circ Res* 4: 91-94, 1956.

15. **Hinojosa-Laborde C, Shade RE, Muniz GW, Bauer C, Goei KA, Pidcoke HF, Chung KK, Cap AP, and Convertino VA.** Validation of lower body negative pressure as an experimental model of hemorrhage. *J Appl Physiol (1985)* 116: 406-415, 2014.

16. **Hirsch AT, Levenson DJ, Cutler SS, Dzau VJ, and Creager MA.** Regional vascular responses to prolonged lower body negative pressure in normal subjects. *Am J Physiol* 257: H219-225, 1989.

17. **Jen CJ and McIntire LV.** Characteristics of shear-induced aggregation in whole blood. *The Journal of laboratory and clinical medicine* 103: 115-124, 1984.

18. **Johnson BD, van Helmond N, Curry TB, van Buskirk CM, Convertino VA, and Joyner MJ.** Reductions in central venous pressure by lower body negative pressure or blood loss elicit similar hemodynamic responses. *J Appl Physiol (1985)* 117: 131-141, 2014.

19. **Libre EP, Cowan DH, Watkins SP, Jr., and Shulman NR.** Relationships between spleen, platelets and factor 8 levels. *Blood* 31: 358-368, 1968.

20. **Martini WZ, Cortez DS, Dubick MA, Park MS, and Holcomb JB.** Thrombelastography is better than PT, aPTT, and activated clotting time in detecting clinically relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. *The Journal of trauma* 65: 535-543, 2008.

21. **Masoud M, Sarig G, Brenner B, and Jacob G.** Orthostatic hypercoagulability: a novel physiological mechanism to activate the coagulation system. *Hypertension* 51: 1545-1551, 2008.

22. **Norsk P, Bonde-Petersen F, and Warberg J.** Influence of central venous pressure change on plasma vasopressin in humans. *J Appl Physiol (1985)* 61: 1352-1357, 1986.

23. **Park MS, Martini WZ, Dubick MA, Salinas J, Butenas S, Kheirabadi BS, Pusateri AE, Vos JA, Guymon CH, Wolf SE, Mann KG, and Holcomb JB.** Thromboelastography as a better indicator of hypercoagulable state after injury than prothrombin time or activated partial thromboplastin time. *The Journal of trauma* 67: 266-275; discussion 275-266, 2009.

24. **Rea RF, Hamdan M, Clary MP, Randels MJ, Dayton PJ, and Strauss RG.** Comparison of muscle sympathetic responses to hemorrhage and lower body negative pressure in humans. *J Appl Physiol (1985)* 70: 1401-1405, 1991.

25. **Retzlaff JA, Tauxe WN, Kiely JM, and Stroebel CF.** Erythrocyte volume, plasma volume, and lean body mass in adult men and women. *Blood* 33: 649-661, 1969.

26. **Rickards CA, Johnson BD, Harvey RE, Convertino VA, Joyner MJ, and Barnes JN.** Cerebral blood velocity regulation during progressive blood loss compared to lower body negative pressure in humans. *J Appl Physiol (1985)*: jap 00127 02015, 2015.

27. **Riddez L, Johnson L, and Hahn RG.** Central and regional hemodynamics during crystalloid fluid therapy after uncontrolled intra-abdominal bleeding. *The Journal of trauma* 44: 433-439, 1998.

28. **Ruttmann TG, Roche AM, Gasson J, and James MF.** The effects of a one unit blood donation on auto-haemodilution and coagulation. *Anaesthesia and intensive care* 31: 40-43, 2003.

29. **Sander-Jensen K, Mehlsen J, Stadeager C, Christensen NJ, Fahrenkrug J, Schwartz TW, Warberg J, and Bie P.** Increase in vagal activity during hypotensive lower-body negative pressure in humans. *Am J Physiol* 255: R149-156, 1988.

30. **Tuman KJ, Spiess BD, McCarthy RJ, and Ivankovich AD.** Effects of progressive blood loss on coagulation as measured by thrombelastography. *Anesthesia and analgesia* 66: 856-863, 1987.

31. **van Hoeyweghen R, Hanson J, Stewart MJ, Dethune L, Davies I, Little RA, Horan MA, and Kirkman E.** Cardiovascular response to graded lower body negative pressure in young and elderly man. *Exp Physiol* 86: 427-435, 2001.

32. **von Kanel R and Dimsdale JE.** Effects of sympathetic activation by adrenergic infusions on hemostasis in vivo. *European journal of haematology* 65: 357-369, 2000.

33. **Vosburgh CH and Richards AN.** An experimental study of the sugar content and extravascular coagulation of the blood after administration of adrenalin. *American Journal of Physiology* 9: 35-51, 1903.

34. **Ward KR, Tiba MH, Ryan KL, Filho IP, Rickards CA, Witten T, Soller BR, Ludwig DA, and Convertino VA.** Oxygen transport characterization of a human model of progressive hemorrhage. *Resuscitation* 81: 987-993, 2010.

35. **Wesseling KH, Jansen JR, Settels JJ, and Schreuder JJ.** Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *Journal of applied physiology (Bethesda, Md : 1985)* 74: 2566-2573, 1993.

36. **Winther K, Hillegass W, Tofler GH, Jimenez A, Brezinski DA, Schafer AI, Loscalzo J, Williams GH, and Muller JE.** Effects on platelet aggregation and fibrinolytic activity during upright posture and exercise in healthy men. *The American journal of cardiology* 70: 1051-1055, 1992.

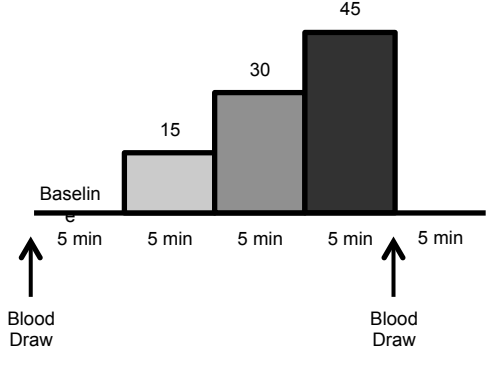
37. **Zaar M, Fedyk CG, Pidcoke HF, Scherer MR, Ryan KL, Rickards CA, Hinojosa-Laborde C, Convertino VA, and Cap AP.** Platelet activation after presyncope by lower body negative pressure in humans. *PLoS One* 9: e116174, 2014.

38. **Zaar M, Johansson PI, Nielsen LB, Crandall CG, Shibasaki M, Hilsted L, and Secher NH.** Early activation of the coagulation system during lower body negative pressure. *Clinical physiology and functional imaging* 29: 427-430, 2009.

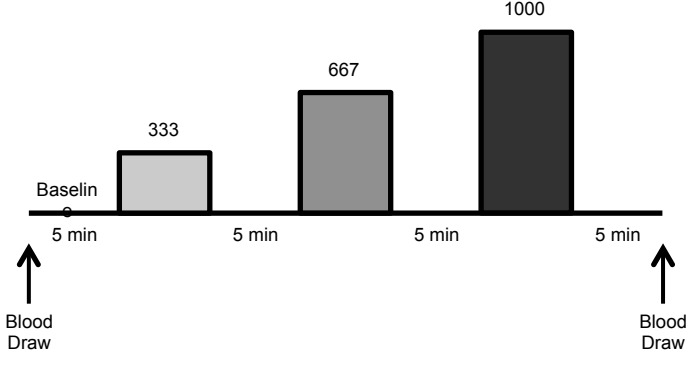
39. **Zaar M, Morkeberg J, Pott FC, Johansson PI, and Secher NH.** Coagulation competence and fluid recruitment after moderate blood loss in young men. *Blood Coagul Fibrinolysis* 25: 592-596, 2014.

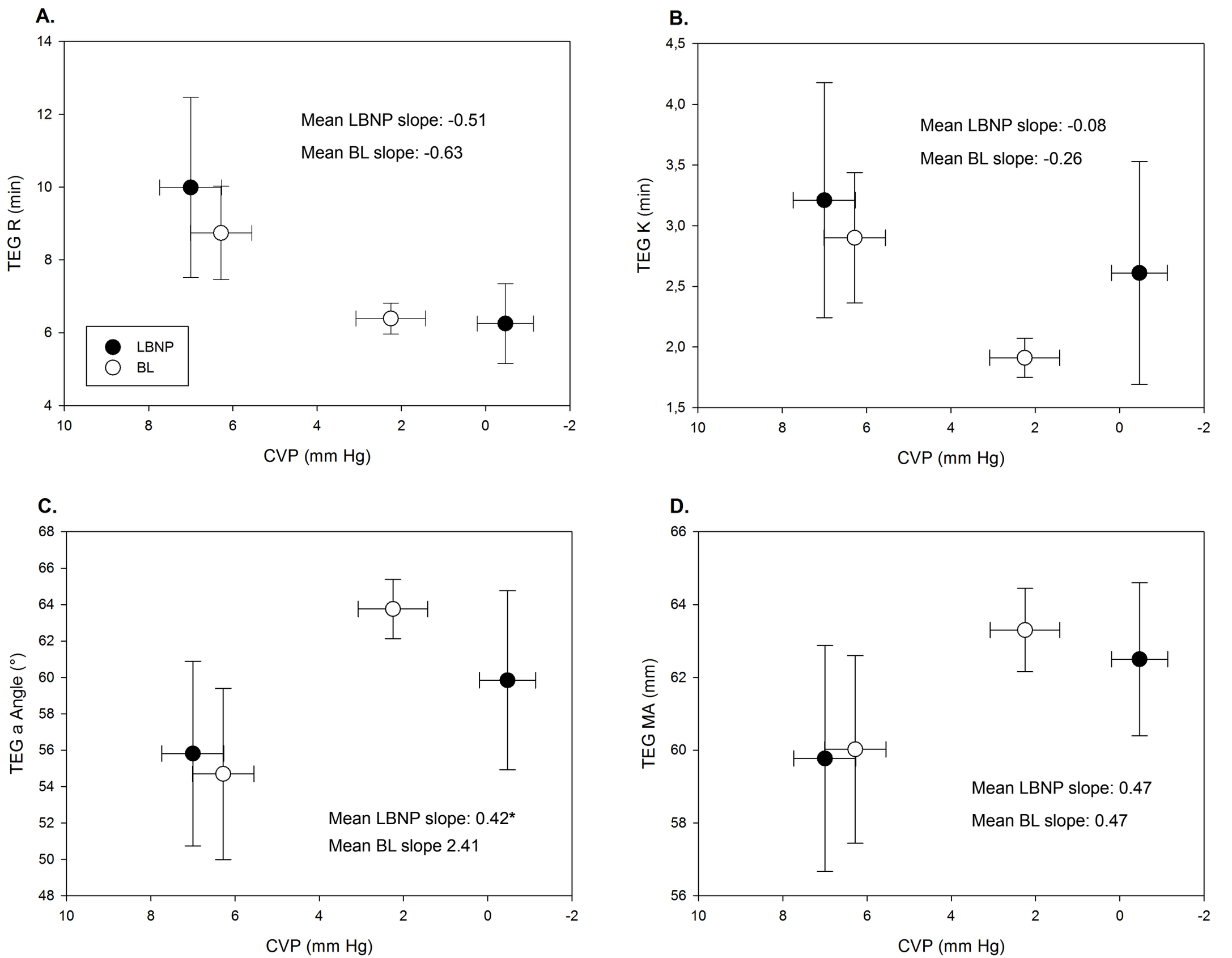
Randomized first protocol
45-75 minutes rest
in between protocols

LBNP (mmHg)



BL (total in mL)




A.
TEG R (min)
CVP (mm Hg)
Mean LBNP slope: -0.51
Mean BL slope: -0.63
LBNP
BL
B.
TEG K (min)
CVP (mm Hg)
Mean LBNP slope: -0.08
Mean BL slope: -0.26
C.
TEG a Angle (°)
CVP (mm Hg)
Mean LBNP slope: 0.42*
Mean BL slope 2.41
D.
TEG MA (mm)
CVP (mm Hg)
Mean LBNP slope: 0.47
Mean BL slope: 0.47