Coagulation changes during lower body negative pressure and blood loss in humans

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1Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota; 2Department of Physiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, New York; and 4U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas

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van Helmond N, Johnson BD, Curry TB, Cap AP, Convertino VA, Joyner MJ. Coagulation changes during lower body negative pressure and blood loss in humans. Am J Physiol Heart Circ Physiol 309: H1591–H1597, 2015. First published September 9, 2015; doi:10.1152/ajpheart.00435.2015.—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 (TEG) in 12 men who performed a LBNP and BL protocol in randomized order. LBNP consisted of 5-min stages at 0, −15, −30, and −45 mmHg of suction. BL included 5 min at baseline and following three stages of 333 ml of blood removal (up to 1,000 ml total). Arterial blood draws were performed at baseline and after the last stage of each protocol. We found that LBNP to −45 mmHg is a greater central hypovolemic stimulus versus 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 whether the slopes of these regression lines fell on similar trajectories for each protocol. Mean regression line slopes for coagulation markers versus 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 min; both P < 0.05). Our results indicate that LBNP models the general changes in coagulation markers observed during BL.

blood coagulation; hemorrhage; lower body negative pressure; blood coagulation tests; humans; central hypovolemia

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

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 noninvasive 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. Despite 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; Body mass index (BMI), 26.7 ± 0.5 kg/m²] participated in this study, which was approved by the Institutional Review Board. Before participation, all subjects provided written informed consent after all procedures and study risks were fully explained. Subjects were nonobese (BMI < 30), nonsmokers, and 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; Shetton, CT; 240 kcal) 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 Fig. 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 whether changes in coagulation markers, obtained from our previous investigations (18, 26), were similar across a broad range of central venous pressure (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 min before initiating the first protocol (≥30 min following invasive instrumentation). After the first protocol, subjects rested quietly for 45–75 min in the supine position. A longer duration was needed after the BL protocol to allow for blood reinfusion. 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 presyncope or syncope.

LBNP Protocol

Subjects laid in a LBNP chamber sealed at the iliac crest. The LBNP protocol was based on the first three stages of the protocol frequently used by the U.S. Army Institute of Surgical Research (4) (Fig. 1). Following a 5-min baseline period, the protocol commenced and consisted of 5-min 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 anticoagulant 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-min baseline period, 3 aliquots of 333 ml of blood were removed. A 5-min 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 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 before 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). Arterial blood pressure was measured beat-by-beat by a brachial artery catheter. 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, Irvine, CA) 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, hematocrit, red blood cell count, and platelet count. Total blood volume at baseline (BV0) was estimated according to Retzlaff et al. (25) using the following equation: 

\[ BV_0 = 31.9 \times \text{height (cm)} + 26.3 \times \text{weight (kg)} - 2,402. \]

Changes in blood volume and the estimated percent 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-hematocrit.

Hemostatic activity of arterial blood

Prothrombin time and activated partial thrombin time. Arterial blood was drawn into 3-ml sodium citrate tubes. Samples were centrifuged for 10 min at 3,000 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).

Whole blood thromboelastography. Thromboelastography (TEG) was performed on 1.5 ml of citrated whole arterial blood using a TEG 5000 device (Haemositex, Braintree, MA) within 4 min 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 thromboelas-
togram reaches 20 mm; α angle, the angle between the line in the
middle of the TEG tracing and the line tangental to the developing
body of the TEG tracing, which is reflective of the rate of fibrin
polymerization; maximum amplitude, expressing the maximum
strength in millimeters of the final clot; and lysis 30 and lysis 60,
which reflect fibrinolysis and are expressed as the percent decrease
in amplitude at 30 and 60 min, respectively, after maximum amplitude.

**Catecholamines**

 Plasma epinephrine and norepinephrine concentrations were deter-
dined from 4.5 ml of arterial blood using HPLC after prior alumina
eextraction (ESA Coulochem III; Dionex, Sunnyvale, CA).

**Data and Statistical Analysis**

Data were collected and analyzed offline using signal processing
software (WinDaq; DATAZ Instruments, Akron, OH). Hemodynamic
data were analyzed and averaged over the last 2 min 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 (Ab-
solute 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 HR and SV. Protocol (LBNP/
BL) × time (baseline/protocol termination) repeated-measures
ANOVA was used to determine whether 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. With use of the 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 whether the slopes of these
regression lines fell on similar trajectories between the two protocols.
Group data are presented as means ± SE. P values are reported.

**RESULTS**

Of the 12 subjects, two 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 presyncope
symptoms or syncope. Data obtained from the final completed
stage were used for these subjects. The mean time for 1,000 ml
of blood removal was 1,402 ± 157 s (~43 ml/min). The mean
hemodynamic values obtained during both protocols are pre-
sented in Table 1 and are reported elsewhere (18). The mean
TEG coagulation values across the range of CVP during LBNP
and BL are displayed in Fig. 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 termina-

tion are displayed in Tables 3 and 4. The mean catechol-
amine concentrations are presented in Table 5.

**Table 1. Changes in hemodynamic variables with LBNP and BL**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
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<tbody>
<tr>
<td>Central venous pressure, mmHg</td>
<td></td>
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<tr>
<td>LBNP</td>
<td>7.3 ± 0.6</td>
<td>–0.2 ± 0.6*</td>
</tr>
<tr>
<td>BL</td>
<td>6.1 ± 0.6†</td>
<td>1.8 ± 0.8†</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
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<tr>
<td>LBNP</td>
<td>60 ± 2.5</td>
<td>80 ± 5.1*</td>
</tr>
<tr>
<td>BL</td>
<td>60 ± 2.8</td>
<td>67 ± 2.6†</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>93.5 ± 2.3</td>
<td>84.5 ± 4.7*</td>
</tr>
<tr>
<td>BL</td>
<td>91.8 ± 1.9</td>
<td>87.0 ± 2.7</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td></td>
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<tr>
<td>LBNP</td>
<td>83.2 ± 2.7</td>
<td>54.1 ± 3.3*</td>
</tr>
<tr>
<td>BL</td>
<td>89.5 ± 2.7†</td>
<td>70.5 ± 2.7† †</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>5.0 ± 0.3</td>
<td>4.1 ± 0.1*</td>
</tr>
<tr>
<td>BL</td>
<td>5.3 ± 0.3†</td>
<td>4.7 ± 0.2†</td>
</tr>
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</table>

Values are means ± SE; n = 12. LBNP, lower body negative pressure; BL, blood loss. *Different from baseline (P < 0.05); †different vs. LBNP.

**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 cardiac output (CO) (LBNP, 5.0 ± 0.3; BL, 5.3 ± 0.3 l/min; P = 0.045) were slightly higher. At protocol termination, CVP (LBNP, −0.2 ± 0.6; BL, 1.8 ± 0.8 mmHg; P < 0.001), SV (LBNP, 54.1 ± 3.3; BL, 70.5 ± 2.7 ml; P < 0.001), and CO (LBNP, 4.1 ± 0.1; BL, 4.7 ± 0.2 l/min; P = 0.002) were lower during LBNP, and HR
was higher (LBNP, 80 ± 5.1; BL, 67 ± 2.6 beats/min; P < 0.001) versus BL. Overall, 45 mmHg of LBNP caused greater changes in hemodynamic parameters than 1,000 ml of BL.

**Effects of LBNP and BL on Hemoconcentration**

As we expected, several markers indicated that LBNP
causd hemoconcentration, whereas BL induced hemodilu-
tion (Table 2). After LBNP there was an increase in hemo-
globin (14.2 ± 0.4 to 14.7 ± 0.4 g/dl; P = 0.003) and hematocrit (41.8 ± 0.8 to 42.8 ± 0.8%; P = 0.001) and a decrease
in estimated plasma volume (59 ± 8 to 56 ± 9%; P = 0.001) compared with baseline values. BL induced a decrease
in hemoglobin (14.3 ± 0.4 to 14.0 ± 0.4 g/dl; P = 0.006) and hematocrit (41 ± 0.8 to 40 ± 0.9%; P = 0.006) and an increase in estimated plasma volume (59 ± 9 to 61 ± 11%; P = 0.004) compared with baseline values. At protocol termi-
nation, hematoglobin (P = 0.001) and hematocrit (P = 0.001)
were lower in BL versus LBNP and estimated plasma volume
(P = 0.001) was greater in BL when compared with LBNP.

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

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

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Effects of LBNP and BL on TEG Values

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

Effects of LBNP and BL on Catecholamine Levels

Epinephrine (LBNP, 53 ± 7 to 144 ± 30; BL, 49 ± 7 to 103 ± 19 pg/ml; P = 0.001 and P = 0.002) and norepinephrine (LBNP, 148 ± 20 to 354 ± 44; BL, 155 ± 22 to 211 ± 29 pg/ml; P ≤ 0.001 and P = 0.043) concentrations were both elevated at protocol termination in both LBNP and BL protocols (Table 5). Norepinephrine levels were higher during LBNP versus BL at protocol termination (P = 0.003).

DISCUSSION

The general results of this study indicate that BL and LBNP induce similar coagulation response trajectories across a wide range of conditions.
Table 4. Effects of LBNP and BL on clot lysis measures

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Termination</th>
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<tbody>
<tr>
<td>Lysis 30%</td>
<td>1.6 ± 0.4</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td>LBNP</td>
<td>2.3 ± 1.1</td>
<td>3.1 ± 1.6</td>
</tr>
<tr>
<td>Lysis 60%</td>
<td>5.6 ± 1.1</td>
<td>7.5 ± 2.5</td>
</tr>
<tr>
<td>LBNP</td>
<td>6.1 ± 1.9</td>
<td>7.4 ± 2.5</td>
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Values are means ± SE; n = 12.

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. (37) demonstrated a reduction in time to fibrin formation after LBNP to presyncope demonstrated by shortened R-time. 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 1,000 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 three separate 333-ml aliquots, whereas Zaar et al. (39) used two 450 ml aliquots to protocol completion when compared with 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 with LBNP. This discrepancy is primarily due to the differences in CVP achieved at the end of each protocol, since α angle was not different between protocols (Fig. 2). Contrary to a recent study that found increased lysis 60 (37) after LBNP, we did not find any differences in TEG measured fibrinolysis (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 with 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 whether 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, SV, and CO values as well as higher HR and norepinephrine values when compared with 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 min during the LBNP protocol and ~45 min during the BL protocol. This could introduce a difficulty in interpreting the results if there were a time effect on coagulation during the BL protocol. This could introduce a difficulty in interpreting the results if there were a time effect on coagulation variables. We found that subjects who performed LBNP first had slightly lower CVP (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 1,000 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.

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DISCLAIMER

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of the Army or the U.S. Department of Defense.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.v.H., B.D.J., T.B.C., and M.J.J. performed experiments; N.v.H. and B.D.J. analyzed data; N.v.H., B.D.J., T.B.C., A.P.C., V.A.C., and M.J.J. interpreted results of experiments; N.v.H. prepared figures; N.v.H. drafted manuscript; B.D.J., T.B.C., A.P.C., V.A.C., and M.J.J. edited and revised manuscript; N.v.H., B.D.J., T.B.C., A.P.C., V.A.C., and M.J.J. approved final version of manuscript; B.D.J., T.B.C., A.P.C., V.A.C., and M.J.J. conception and design of research.


18. Johnson BD, van Helmond N, Curry TB, van Buskirk CM, Conver-...