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 a 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.

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; Shelton, 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 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: 

\[ \text{BV}_0 = 31.9 \times \text{height (cm)} + 26.3 \times \text{weight (kg)} - 2,402. \]

Changes in blood volume and the estimated percent change in plasma volume from pre- to post-LBNP and from pre- to post-BL (%ΔPV) 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 (Haemonetics, 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 thromboelastograph reaches 20 mm; α, 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, 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 determined from 4.5 ml of arterial blood using HPLC after prior alumina extraction (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 (Absolute Aliens, Oy, Finland) by selecting the area under the arterial 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 presented 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 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 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, 1.9 ± 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 caused hemoconcentration, whereas BL induced hemodilution (Table 2). After LBNP there was an increase in hemoglobin (14.2 ± 0.4 to 14.7 ± 0.4 g/dl; P = 0.003) and hematocrit (41.0 ± 0.8% to 42.8 ± 0.8%; P = 0.01) and a decrease in estimated plasma volume (59.8 ± 0.8 to 56.9 ± 0.9%; 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 ± 0.8% to 40.0 ± 0.9%; P = 0.006) and an increase in estimated plasma volume (59.9 ± 0.9 to 61.1 ± 1.1%; P = 0.004) compared with baseline values. At protocol termination, hematocrit (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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**Table 1. Changes in hemodynamic variables with LBNP and BL**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Termination</th>
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</thead>
<tbody>
<tr>
<td>Central venous pressure, mmHg</td>
<td>7.3 ± 0.6</td>
<td>−0.2 ± 0.6*</td>
</tr>
<tr>
<td>LBNP</td>
<td>6.1 ± 0.6†</td>
<td>1.8 ± 0.8‡</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>60 ± 2.5</td>
<td>80 ± 5.1*</td>
</tr>
<tr>
<td>LBNP</td>
<td>60 ± 2.8</td>
<td>67 ± 2.6‡</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>93.5 ± 2.3</td>
<td>84.5 ± 4.7*</td>
</tr>
<tr>
<td>LBNP</td>
<td>91.8 ± 1.9</td>
<td>87.0 ± 2.7</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>83.2 ± 2.7</td>
<td>51.4 ± 2.3*</td>
</tr>
<tr>
<td>LBNP</td>
<td>89.5 ± 2.7†</td>
<td>70.5 ± 2.7†</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>5.0 ± 0.3</td>
<td>4.1 ± 0.1*</td>
</tr>
<tr>
<td>LBNP</td>
<td>5.3 ± 0.3†</td>
<td>4.7 ± 0.2‡</td>
</tr>
</tbody>
</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 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 \( \alpha \) 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; \( P \leq 0.001 \) 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

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

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td>14.2 ± 0.4</td>
<td>14.7 ± 0.4*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41 ± 0.8</td>
<td>42 ± 0.8*</td>
</tr>
<tr>
<td>Red blood cell count, *10^12/l</td>
<td>4.8 ± 0.1</td>
<td>5.0 ± 0.1*</td>
</tr>
<tr>
<td>Plasma volume, %</td>
<td>59 ± 0.8</td>
<td>56 ± 0.9*</td>
</tr>
<tr>
<td>Platelet count, *10^9/l</td>
<td>194 ± 7</td>
<td>212 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 12 \). *Different from baseline (\( P < 0.05 \)); †different from LBNP (\( P < 0.05 \)).

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

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time, s</td>
<td>12.2 ± 0.2</td>
<td>12.0 ± 0.1*</td>
</tr>
<tr>
<td>Activated partial thrombin time, s</td>
<td>32.2 ± 0.7</td>
<td>31.0 ± 0.8*</td>
</tr>
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</table>

Values are means ± SE; \( n = 12 \). *Different from baseline (\( P < 0.05 \)).
Table 4. Effects of LBNP and BL on clot lysis measures

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis 30, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>1.6 ± 0.4</td>
<td>3.6 ± 1.7</td>
</tr>
<tr>
<td>BL</td>
<td>2.3 ± 1.1</td>
<td>3.1 ± 1.6</td>
</tr>
<tr>
<td>Lysis 60, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBNP</td>
<td>5.6 ± 1.1</td>
<td>7.5 ± 2.5</td>
</tr>
<tr>
<td>BL</td>
<td>6.1 ± 1.9</td>
<td>7.4 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12.

Table 5. Effects of LBNP and BL on catecholamine levels

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

Values are means ± SE; n = 12. *Different from baseline (P < 0.05); †different from LBNP (P < 0.05).
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 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. Fifth, 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 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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GRANTS

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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


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15. tino VA, Joyner MJ. Reductions in central venous pressure by lower body negative pressure or blood loss elicit similar hemodynamic re-


