ABSTRACT

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Introduction: Tolerance to both actual and simulated hemorrhage varies between individuals. Low frequency (~0.1 Hz) oscillations in mean arterial pressure (MAP) and brain blood flow (indexed via middle cerebral artery velocity, MCAv), may play a role in tolerance to reduced central blood volume; subjects with high tolerance to simulated hemorrhage induced via application of lower body negative pressure (LBNP) exhibit greater low frequency power in MAP and MCAv compared to low tolerant subjects. The mechanism for this association has not been explored. We hypothesized that inducing low frequency oscillations in arterial pressure and cerebral blood flow would attenuate reductions in cerebral blood flow and oxygenation during simulated hemorrhage. **Methods:** 14 subjects (11M/3F) were exposed to oscillatory (0.1 Hz, 0.05 Hz) and non-oscillatory (0 Hz) LBNP profiles with an average chamber pressure of -60 mmHg. Each profile was separated by a 5-min recovery. Measurements included arterial pressure and stroke volume via finger photoplethysmography, MCAv via transcranial Doppler ultrasound, and cerebral oxygenation of the frontal lobe (ScO_2) via near infrared spectroscopy. **Results:** No differences were observed between profiles for reductions in MAP ($P=0.60$) and MCAv ($P=0.90$). The reduction in ScO_2, however, was attenuated ($P=0.04$) during the oscillatory profiles compared to the 0 Hz profile. A similar attenuation was observed in stroke volume ($P<0.001$). Importantly, tolerance was higher during the oscillatory profiles ($P=0.03$). **Discussion:** In partial support of our hypothesis, cerebral oxygenation was protected during the

oscillatory profiles. While MCAv was similar between conditions, the oscillatory pattern of cerebral blood flow may elicit a shear-stress induced vasodilation, so assessment of velocity may mask an increase in flow. Importantly, more subjects were able to tolerate the oscillatory profiles compared to the static 0 Hz profile, despite similar arterial pressure responses. These findings emphasize the potential importance of hemodynamic oscillations in maintaining perfusion and oxygenation of cerebral tissue during hemorrhagic stress.

PULSATILE PERFUSION THERAPY: A NOVEL APPROACH FOR IMPROVING CEREBRAL BLOOD FLOW AND OXYGENATION UNDER SIMULATED HEMORRHAGIC STRESS

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PULSATILE PERFUSION THERAPY: A NOVEL APPROACH FOR IMPROVING CEREBRAL BLOOD FLOW AND OXYGENATION UNDER SIMULATED HEMORRHAGIC STRESS

PRACTICUM REPORT

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CHAPTER I. BACKGROUND AND LITERATURE

Hemorrhage accounts for 30-40% of deaths resulting from traumatic injury in the civilian setting (9), and 90% of potentially survivable deaths on the battlefield (4). During hemorrhage, blood is preferentially shunted to vital organs to ensure tissue survival under this stress (5). As the brain is one of the most vital and metabolically active organs of the body (25), physiological mechanisms are activated to reduce any disruption of blood flow and oxygen/nutrient supply to this organ, such as the shunting of blood to vital organs like the brain, cerebral autoregulation and vasodilation, and increased oxygen extraction. Maintaining blood flow and oxygenation to the brain is paramount to survival of neural tissues, and critical in reducing long-term complications associated with survival from hemorrhagic injuries.

Lower body negative pressure (LBNP) has been shown to be an appropriate model for studying the physiological responses to the early "pre-shock" stages of hemorrhage in healthy, conscious human subjects (7, 8, 26, 27). Both blood loss and LBNP elicit similar responses in key hemodynamic parameters, including heart rate, stroke volume, cardiac output, arterial pressure, and cerebral blood velocity (7, 8, 22). Typically, LBNP is applied by stepwise reductions in chamber pressure until the subject reaches "pre-syncope", which is often defined as a systolic blood pressure ≤80 mmHg and/or the subject experiences symptoms such as dizziness, visual disturbances, nausea, or other subjective sensations of impending syncope. In studies using maximal LBNP to presyncope, it has been demonstrated that there is a continuum of tolerance among healthy human subjects (10, 12, 23), which has been attributed, in part, to protection of absolute blood flow (12, 14) in either the anterior (12, 23) or posterior circulations

(10) of the brain. However, accumulating evidence suggests that the pattern of cerebral blood flow during hemorrhagic stress may also be important in increasing tolerance.

Recent evidence indicates that low frequency oscillations (within the 0.04-0.15 Hz range) in brain blood flow and arterial pressure are associated with increased tolerance to central hypovolemia (16, 23). The observation of oscillations in blood pressure during hemodynamic challenges such as hemorrhage and orthostatic stress were first published over 60 years ago. In studies on hemorrhaged dogs with varying magnitudes of blood loss, a low frequency oscillatory pattern in blood pressure was observed, and was attributed to a baroreflex-mediated mechanism (6, 18). These low frequency oscillations in arterial pressure are also evident in humans subjected to progressive orthostatic stress induced by graded head-up tilt (2). While the study of arterial pressure oscillations has been extensive, only recently has this phenomenon been linked to tolerance to central hypovolemia simulating hemorrhage. Increased tolerance to central hypovolemia occurs both in subjects who exhibit endogenous low frequency oscillations (23), and when low frequency oscillations are forced by slow paced breathing at 6 breaths/min (0.1 Hz) (16). Rickards et al. reported that subjects with high tolerance to LBNP exhibited higher low frequency amplitude in both cerebral blood flow (as measured by middle cerebral artery velocity (MCAv) and mean arterial pressure (MAP)) compared with low tolerant subjects (23). Similarly, when breathing rate is paced at 0.1 Hz, Lucas et al. reported increases in tolerance to the central hypovolemic challenge of head-up tilt plus LBNP, along with an attenuated rate of decline in MCAv and MAP compared to spontaneous breathing (16).

While the association between low frequency oscillations and tolerance has been established, the mechanism/s underlying this relationship is not clear. One possible mechanism is shear stress-induced vasodilation of the cerebral arteries elicited by oscillatory flow and the

subsequent release of endothelial-derived mediators such as nitric oxide (NO). In support of this hypothesis, Adams et al. demonstrated that following 1 hour of 3 Hz periodic accelerations in piglets, the concentration of nitrite, a stable metabolite of NO, was higher relative to both baseline and the control condition of non-pulsatile flow (1). NO (indexed by measurement of nitrate and nitrite), was also elevated in dogs who received pulsatile flow at 2 Hz for 30-min compared to the non-pulsatile flow control group (20).

In the current study we seek to address the knowledge gap concerning the mechanisms mediating improved tolerance to central hypovolemia associated with low frequency hemodynamic oscillations. We hypothesize that inducing low frequency oscillations in arterial pressure and cerebral blood flow will increase cerebral blood flow and oxygenation during simulated hemorrhagic stress. We also hypothesize that the shear stress-mediated vasodilator, NO, will increase with oscillatory perfusion.

CHAPTER II: RESEARCH PROJECT

Specific Aims

In the current study, we aim to assess the role of low frequency oscillations in arterial pressure and cerebral blood flow on cerebral blood flow and oxygenation during a simulated hemorrhagic stress. We also aim to assess the possible role these oscillations play in increasing shear-stress and subsequent vasodilation as a means of improving blood flow to the brain.

Significance

Hemorrhage is a leading cause of death from traumatic injury in both the military and civilian settings (4, 9), and poses a significant challenge to vital organ perfusion and oxygenation. Under conditions of massive blood loss, reflex cardiovascular mechanisms result in the shunting of blood from non-vital tissues (such as peripheral skeletal muscle and viscera) towards the heart and brain. As the brain can only tolerate the subsequent ischemia and hypoxia for a short period of time, maintaining adequate perfusion and oxygenation is paramount to organ viability. Tolerance to simulated hemorrhagic stress is associated with increased low frequency oscillations in arterial pressure and cerebral blood flow (23). Understanding the mechanisms underlying the effect of low frequency oscillations on cerebral perfusion and oxygenation, may provide insight into developing potential therapeutic interventions for use in hemorrhaging patients.

Materials and Methods

Subjects. Young, healthy subjects were recruited to participate in this study conducted at the University of North Texas Health Science Center (UNTHSC) in Fort Worth, TX. The experimental protocol was reviewed and approved by the Institutional Review Board at UNTHSC (2016-049). Subjects participated in two sessions in the laboratory, a familiarization session and an experimental session. Upon arrival for the familiarization session, subjects were briefed regarding the aims of the research study, the experimental protocol, and associated risks, and given opportunities to ask any questions. Informed, written consent was then obtained from each subject. Subjects completed a health history questionnaire, and height, weight, sex, and age were recorded. Female subjects took a urine pregnancy test to ensure they were not pregnant. This was followed by a seated and standing 12-lead ECG and blood pressure assessment. Following these medical screening assessments, subjects were then placed in the LBNP chamber (VUV Analytics, Austin, Texas) while it was turned on to familiarize them with the sensation of the experimental protocol.

At least 24 hours following the familiarization session, subjects returned to the laboratory for the experimental session. Female subjects were tested during the first four days of their menstrual cycle corresponding to the early follicular/low hormone phase. For 24 hours prior to experimentation, subjects refrained from caffeine, alcohol, dietary supplements, medication, and exercise. Upon arrival to the laboratory, subject height, weight, and age were again recorded, and female subjects underwent a urine pregnancy test to ensure they were not pregnant.

Instrumentation. All subjects were placed in the LBNP chamber in the supine position with their iliac crest aligned with the opening of the chamber; they were sealed into the chamber with

heavy-duty plastic and a neoprene band around the waist. Subjects were instrumented for continuous ECG recording in a standard lead II configuration (shielded leads, cable and amplifier, AD Instruments, Bella Vista, NSW, Australia). A photoplethysmography blood pressure monitor (Finometer, Finapres Medical Systems, Amsterdam, Netherlands) was attached via a finger cuff for continuous measurement of arterial pressure, and calculation of stroke volume. An oral/nasal cannula or face mask was used for continuous recording of end-tidal gases ($etCO_2$ and etO_2) and calculation of respiration rate via a gas analyzer (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW, Australia). Cerebral blood velocity was measured in the middle cerebral artery (MCA) and posterior cerebral artery (PCA) through the temporal windows with transcranial Doppler (TCD) ultrasound probes (2 MHz; ST3, Spencer Technologies, Seattle, WA) placed on either side of the head. Cerebral oxygenation (ScO_2) was measured by placing a near infrared spectroscopy (NIRS) sensor (OxiplexTS, ISS, Champaign-Urbana, IL) over the frontal lobe. Efforts were made to measure MCAv and cerebral oxygenation on the same side. An indwelling venous catheter was placed in an antecubital fossa vein for blood sampling. Seven 10 ml blood samples were taken over the course of the experiment to include baseline, the end of each of the 3 oscillatory profiles, and the end of each recovery period (figure 1).

Protocol. Following instrumentation, baseline measurements of respiration rate were collected for 2-min. Subjects were then coached to breath in time with a metronome as follows: (a) if spontaneous respiration rate was 10 breaths per min or greater, the metronome was set at the subject's spontaneous rate; (b) if spontaneous respiration rate was below 10 breaths per min, the metronome was set to pace breathing at 10 breaths per min. Pacing the breathing at a rate of $\geq$10 breaths per minute ensured breathing rate did not influence low frequency oscillations in MAP

and MCAv (i.e., 10 breaths per min is a frequency of 0.17 Hz). After successfully pacing breathing, a 5-min baseline period began. One of three oscillatory LBNP (OLBNP) profiles was then used to assess the role of arterial pressure oscillations on cerebral perfusion and cerebral tissue oxygenation during central hypovolemia. A 5-min recovery period intervened between each profile, with a 10-min recovery at the end of the experiment. The order of the profiles was randomized and counter-balanced between subjects. The three profiles were as follows: 1) a 0 Hz (static) hypovolemic profile with the LBNP chamber pressure progressively reduced to -60 mmHg over 1-min and held at that pressure for a further 9-min period, 2) a 0.1 Hz hypovolemic profile with chamber pressure progressively reduced to -60 mmHg for 1-min after which pressure oscillated between -30 mmHg for 5-s and -90 mmHg for 5-s over 9-min (amplitude of 60 mmHg; 54 cycles over 9-min), and 3) a 0.05 Hz hypovolemic profile with chamber pressure progressively reduced to -60 mmHg for 1-min after which pressure oscillated between -30 mmHg for 10-s and -90 mmHg for 10-s over 9-min (amplitude of 60 mmHg; 27 cycles over 9-min). Subjects were continuously monitored during the entire protocol. LBNP was released if systolic arterial pressure fell below 80 mmHg, or the subject reported symptoms such as visual disturbances, dizziness, light-headedness, or nausea at any time.

Blood Sample Collection & Analysis. Whole blood samples were collected into a chilled syringe, then placed into appropriate tubes (EDTA for norepinephrine samples, citrate for nitrite samples); EDTA tubes were treated with glutathione (1.23 mg glutathione/1 ml whole blood) as a preservative. Samples for assessment of nitrite were centrifuged immediately at 4^{o}C for 5-min at 2000 RPM. Samples for assessment of norepinephrine were centrifuged at 4^{o}C for 15-min at 1500 RPM. The plasma for both samples was extracted and placed in Eppendorf tubes, then snap

frozen in liquid nitrogen, and stored in a -80^{o}C freezer for subsequent analysis. Plasma extracted from the blood samples was analyzed for plasma nitrite via ozone based chemiluminescence in the NO Metabolomics Facility at the University of Pittsburgh (PI: Dr. Sruti Shiva). One measurement was made for each sample, according to standard procedures. Norepinephrine was measured in duplicate with an enzyme linked immunosorbent assay (BA E-6200, Rocky Mountain Diagnostics, Colorado Springs, CO). Duplicate samples with a coefficient of variation less than 15% were included in the final analysis.

Data Analysis. Continuous waveform recordings of ECG, arterial pressure, stroke volume, MCAv, PCAv, cerebral oxygenation, and end-tidal gases were sampled and recorded at 1000 Hz (PowerLab/Labchart, AD Instruments, Bella Vista, NSW, Australia) for later analysis using specialized software (WinCPRS, Absolute Aliens, Turku, Finland). Baseline measurements were averaged over the entire 5-min period. The time frame used for analysis of the static and oscillatory LBNP profiles started once the chamber pressure reached -60 mmHg until the end of oscillations. If a subject tolerated the entire profile, this was 9-min. If a subject did not tolerate the entire profile, the time until LBNP termination was analyzed (i.e., < 9-min). The final 1-min of each recovery period was used for analysis.

R-waves were detected from the ECG signal and used to determine heart rate. Systolic and diastolic arterial pressure and systolic and diastolic cerebral blood velocities were then marked from the Finometer and Doppler tracings. MAP and mean MCAv and PCAv were calculated as the area under the arterial pressure and cerebral blood velocity waveforms with the WinCPRS software. Stroke volume was recorded directly from the Finometer via PowerLab/LabChart. Cardiac output was subsequently calculated as heart rate multiplied by

stroke volume, and total peripheral resistance was then calculated as MAP divided by cardiac output. Integrated cardiac baroreflex sensitivity was assessed by dividing the change in heart rate from baseline by the change in MAP from baseline (19).

Oscillatory patterns of arterial blood pressures and cerebral blood velocities were determined using fast Fourier power spectral analysis as previously described (23). Data were made equidistant by interpolating linearly and resampling at 5 Hz. Data were then passed through a low-pass impulse response filter with a cutoff frequency of 0.5 Hz. A minimum of 5-min of data was used for fast Fourier transformation with a Hanning window to obtain power spectra. The bands used for frequency analysis were 0.04-0.07 Hz, 0.07-0.15 Hz, corresponding with the very low (VLF), and low (LF) frequencies. Spectral power was expressed as the integrated area with the VLF and LF ranges. Coherence in the LF and VLF were calculated between MAP and mean MCAv and PCAv by dividing the cross-spectral densities of the two signals by the product of the individual autospectra. Transfer functions were considered valid and averaged only when coherences values were ≥ 0.5 (29).

Statistical analysis. All data were analyzed using one-way repeated measures ANOVAs followed by Holm-Sidak post hoc tests comparing 0.1 Hz and 0.05 Hz oscillatory profiles to the 0 Hz profile. One-way repeated measures ANOVAs was also used to analyze the baseline and recovery periods between each LBNP profile, followed by Holm-Sidak post hoc tests comparing each recovery period to baseline. Unless otherwise stated, all data are presented as mean ± standard error (SE), and exact P values are reported for all comparisons.

Results

Eighteen subjects were recruited to participate in this study. Four subjects were excluded due to technical difficulty in obtaining key outcome measurements (N=2), self-exclusion due to anxiety from the experimental procedures (N=1), and investigator withdrawal due to irregularity in the ECG recordings (N=1). As a result, 14 subjects completed the entire protocol and were included in the final analysis (11 male, 3 female; age, 26.2 ± 2.8 y; height, 175.6 ± 8.5 cm; weight, 78.3 ± 15.8 kg; mean ± SD).

The average chamber pressure during each profile was: 1) 0 Hz, -61.8 ± 0.7 mmHg; 2) 0.1 Hz, -57.6 ± 0.5 mmHg; 3) 0.05 Hz, -57.9 ± 0.6 mmHg. Tolerance to the two oscillatory profiles was higher compared to the static profile (0 Hz: 424.3 ± 39.1 s; 0.1 Hz: 502.9 ± 22.6 s; 0.05 Hz: 515.9 ± 24.3 s; P=0.03; figure 2). This higher tolerance was accompanied by an attenuated decrease in ScO_2 during the oscillatory profiles compared to the static profile, despite similar reductions in mean MCAv across all profiles (Table 1; figure 3). The reduction in PCAv was similar between the 0.1 Hz condition and the 0 Hz condition, but was attenuated during the 0.05 Hz condition compared to 0 Hz (figure 3). Due to difficulties in finding and maintaining TCD signals in some subjects, 13 MCAv recordings and 5 PCAv recordings were used for the final analysis. Respiration rate was successfully paced at greater than 10 breaths per min for all profiles, and while $etCO_2$ decreased during each of the profiles, it was not different between profiles (table 1). Decreases in stroke volume were attenuated during the oscillatory profiles compared with the static 0 Hz profile, resulting in an attenuated increase in heart rate for these conditions (figure 4). These differential stroke volume and heart rate responses between profiles resulted in similar reductions in cardiac output across the 3 profiles (P=0.56; figure 4). Similarly

there were no differences observed in the reduction of mean arterial pressure (P=0.60) or increase in total peripheral resistance (P=0.58) between profiles (figure 4).

Heart rate was slightly lower in each recovery period compared with baseline (P=0.08); whereas, systolic and mean arterial pressures were higher in the recovery periods compared with baseline (table 2). $EtCO_2$ was only lower than baseline during the second recovery period (table 2). Only the first and second recovery periods were included in this analysis vs. baseline because the final 10-min recovery period was at the end of the experiment, so did not impact responses to an LBNP profile.

Blood samples were successfully collected in a subset of subjects for the analysis of plasma concentrations of norepinephrine (N=11) and nitrite (N=7). Plasma concentrations of norepinephrine mirrored the response of heart rate and were lower during the oscillatory profiles compared to 0 Hz (figure 4), but did not return to baseline levels during recovery (table 2). Plasma concentrations of nitrite were not different between conditions (P=0.53), although there was very high variability in the responses between subjects (figure 5).

Frequency domain analysis confirmed that oscillating the LBNP chamber pressure resulted in an increase in oscillations in MAP, MCAv, and PCAv at the corresponding frequency (table 2). Transfer function analysis revealed that MAP-MCAv coherence was lower in the LF range during 0.05 Hz oscillations, but higher in the VLF range during 0.05 Hz oscillations; however, no difference in coherence was observed for MAP-PCAv between conditions (table 3). Due to low coherence for most subjects (<0.5), gain was not assessed for MAP-MCAv VLF, and MAP-PCAv LF and VLF.

Discussion

In this study we sought to assess the effects of low frequency oscillations in arterial pressure on cerebral blood flow and oxygenation during simulated hemorrhage. The key findings of our study are: 1) inducing low frequency oscillations at 0.05 Hz and 0.1 Hz via OLBNP increased tolerance to central hypovolemia, 2) this increase in tolerance with OLBNP was associated with an attenuation in the decrease in cerebral oxygenation but not in cerebral blood velocity in the MCA, 3) increased tolerance was associated with an attenuated decrease in PCAv during the 0.05 Hz oscillations only, 4) differences in the stroke volume and heart rate responses between profiles resulted in similar cardiac output and arterial pressure responses between conditions, and 5) responses of circulating nitric oxide, indexed by plasma nitrite, were highly variable between subjects for each oscillatory profile.

In previous studies using LBNP or LBNP plus head-up tilt to presyncope, oscillations in arterial pressure and cerebral blood velocity (spontaneous or induced) were associated with greater tolerance to this stress (16, 23, 24). In 2011, Rickards et al. reported that subjects who were high tolerant to presyncopal LBNP had higher amplitude in spontaneously occurring low frequency arterial pressure and MCAv oscillations compared with low tolerant subjects (23). In comparison, Lucas et al. had subjects breath at 0.1 Hz (6 breaths/min) to induce low frequency oscillations in arterial pressure and MCAv, which resulted in higher tolerance to combined head-up tilt and LBNP compared with the spontaneous breathing condition (16). In the present study, we were also able to induce oscillations in arterial pressure and cerebral blood velocity via OLBNP, and demonstrated a similar increase in tolerance to central hypovolemia. Using an external source to induce hemodynamic oscillations, such as OLBNP, may allow for isolation of the mechanism underlying the improvement in tolerance to central hypovolemia, without the

confounding influence of respiratory-driven oscillations.

The finding that low frequency oscillations protected against reductions in cerebral oxygenation during central hypovolemia, despite similar reductions in MCAv is intriguing. As the brain is sensitive to ischemia and hypoxia, developing interventions that maintain cerebral oxygenation during a hypovolemic challenge may be beneficial within the clinical setting. In our study, measurements of cerebral oxygenation were taken over the frontal lobe with near infrared spectroscopy. The MCA, along with the anterior cerebral artery, supplies blood to the anterior regions of the brain (i.e. the frontal lobe). No difference was observed in blood velocity through the MCA between conditions, so we assume there was no difference in delivery of oxygen to the frontal lobe. The sample volume measured via NIRS for assessment of cerebral oxygenation is derived predominantly from the venous circulation (75%; 20% arterial; 5% capillary) (17), and is calculated as the quotient of oxygenated hemoglobin concentration over total hemoglobin (oxygenated plus deoxygenated hemoglobin concentrations). As we observed attenuated decreases in oxygenated hemoglobin concentration and overall cerebral oxygenation with the 0.1 Hz and 0.05 Hz OLBNP profiles (see table 1), the oscillatory conditions appear to be modifying the amount of oxygen extracted from the arterial blood into the tissues compared to the non-oscillatory condition. While speculative, this may reflect a decreased metabolic demand in the neural tissues during oscillations. However, it is important to note the inherent limitation of measuring velocity as an index of cerebral blood flow via transcranial Doppler ultrasound because of the effects on interpretation of oxygen delivery. As this measurement does not account for vessel diameter, potential increases in diameter, and therefore flow and oxygen delivery, may be occurring with increased oscillations, but this effect is masked with assessment of velocity only. Further studies will seek to address this limitation with measurements of actual

flow through the extracranial arteries (e.g., internal carotid artery, vertebral artery) which supply the intracranial circulation.

Studies that have sought to improve tolerance to LBNP through maintaining or increasing MCAv by either clamping or increasing $etCO_2$ by inhalation of hypercapnic gas, have shown a dissociation between responses in MCAv and tolerance (13, 15). This disconnect between MCAv and tolerance is also observed in studies comparing subjects who are high or low tolerant to LBNP, where both groups show decreases in MCAv despite differences in tolerance (10, 23). Similarly, no difference was observed in MCAv responses between profiles in our study. However, we did observe protection of blood velocity in the PCA during the 0.05 Hz profile, which was associated with more subjects completing this OLBNP profile (0.05 Hz: 12/13 subjects vs. 0 Hz: 7/14 subjects, and 0.1 Hz: 11/14 subjects,). The mechanisms underlying why oscillatory blood pressure and flow might improve posterior circulation requires further investigation, but our finding lends support to a number of recent studies assessing the role of regional cerebral blood flow on responses to central hypovolemia. Kay & Rickards (10) demonstrated that subjects with high tolerance to central hypovolemia and a delay in the onset of presyncope, maintained blood flow to the brain stem (indexed by PCAv), despite similar reductions in anterior cerebral blood flow (indexed by MCAv). Further evidence of differences between the regional control of blood flow to the brain has been demonstrated during graded LBNP, with a protection of flow to the posterior circulation via the vertebral arteries in comparison to anterior flow through the internal carotid arteries (21).

During hypovolemia, the baroreflexes are activated in order to maintain arterial pressure and perfusion of the vital organs. Integrated cardiac baroreflex sensitivity was not different across the three LBNP profiles, and while MAP and cardiac output decreased, these reductions

were also not different between conditions. This suggests that each OLBNP profile induced a similar cardiovascular challenge, and appropriate compensatory mechanisms were engaged. Interestingly, however, the mechanisms underlying these compensatory responses were different for the static vs. oscillatory conditions. The reduction in stroke volume was attenuated for both the 0.1 Hz and 0.05 Hz oscillations compared to the non-oscillatory 0 Hz condition; this may be due to improved venous return during the upstroke of the oscillations (i.e., during the -30 mmHg LBNP phase). The attenuated decrease in stroke volume with both oscillatory profiles (0.1 Hz and 0.05 Hz) resulted in lower circulating norepinephrine, and a reduced heart rate response. In comparison, greater stroke volume decreases during the 0 Hz profile led to higher circulating norepinephrine and an increased heart rate response. Taken together, these findings suggest a role of low frequency hemodynamic oscillations in reducing the cardiovascular burden in maintaining blood flow to vital organs during hemorrhagic stress.

Plasma concentrations of nitrite were used as a marker of circulating nitric oxide in response to a presumed increase in shear-stress induced from the oscillations in blood flow (11). While we hypothesized that low frequency oscillations would increase plasma concentrations of nitrite, there were no differences in between conditions. When individual responses are considered, however, there was high inter-subject variability, therefore making it difficult to interpret the possible role of shear-stress induced vasodilation with oscillations. As this analysis was only performed on a subset of subjects (N=7), additional data is needed to better understand these responses.

Methodological Considerations. In addition to the limitation of measuring cerebral blood velocity and not flow, there are other methodological considerations that should be discussed.

Our experimental design included all OLBNP conditions (0, 0.05, and 0.1 Hz) within the same experimental session. While this could result in one condition affecting the cardiovascular responses to the next, the order of the conditions was randomized to control for any confounding order effects. In fact, of the 14 subjects included in the analysis, 5 completed the 0 Hz profile first, 4 completed the 0.05 Hz profile first, and 5 completed the 0.1 Hz profile first. It is also important to note that the time for recovery was only five minutes between each profile. This amount of time allowed for most of the hemodynamic responses to return to approximately baseline values (table 3); however, norepinephrine concentrations in the plasma remained elevated compared to baseline. This limitation will be addressed in future studies by conducting each OLBNP protocol on a different day.

Although the rate of breathing was paced at each subject's spontaneous rate during each protocol, the depth of breathing was not controlled, and $etCO_2$ decreased between 5.6-7.1 mmHg from baseline during all conditions (see table 1). This reduction in arterial CO_2 may have reduced the diameter of the blood vessels being measured, resulting in changes in blood flow that may not be detected via assessment of velocity via TCD. Recent studies have shown a decrease in MCA diameter during hypocapnia, but only with decreases in $etCO_2$ greater than occurred in our study (3, 28). Importantly, the magnitude of hypocapnia was similar between all conditions allowing us to make appropriate comparisons for cerebral blood velocity between groups.

Future Directions. The results from the current study provide information concerning hemodynamic responses to induced low frequency oscillations; however, we did not address the possible effects of low frequency oscillations on inflammation or oxidative stress. In the future, our studies will include measurements of inflammatory and oxidative stress markers to elucidate

whether low frequency oscillations in blood flow attenuate these responses. The current study was also limited to a single level of LBNP (-60 mmHg) with or without oscillations, and not all subjects reached pre-syncope. As such, this protocol was not a true test of tolerance to LBNP for all subjects. In the future, we will design a pre-syncopal LBNP protocol with and without oscillations to assess tolerance in every subject. Using this approach will also allow us to assess tolerance to LBNP with and without oscillations in other populations of interest such as older adults. Further investigations will also include a larger sample of males and females to understand the role of sex in these responses to oscillatory perfusion.

SUMMARY AND CONCLUSIONS

We assessed the effects of low frequency oscillations on cerebral blood velocity and oxygenation during simulated hemorrhagic stress. Inducing these oscillations during central hypovolemia resulted in a protection of frontal lobe cerebral oxygenation, but with no protection of arterial pressure or blood velocity in the anterior cerebral circulation. Importantly, inducing low frequency oscillations in arterial pressure and cerebral blood velocity was associated with increased tolerance to this simulated hemorrhagic stress.

Table 1. Cardiovascular, cerebrovascular, and respiratory responses during each oscillatory lower body negative pressure (OLBNP) profile.

	0 Hz LBNP	**0.1 Hz OLBNP**	**0.05 Hz OLBNP**	**ANOVA P-value**
Cardiovascular				
Heart Rate (beats/min)	92.0 ± 3.5	78.1 ± 3.0 *	76.5 ± 3.5 *	<0.001
Systolic Arterial Pressure (mmHg)	103.1 ± 2.8	106.1 ± 2.1	105.7 ± 1.8	0.11
Diastolic Arterial Pressure (mmHg)	67.0 ± 1.7	65.6 ± 1.4	65.7 ± 0.9	0.58
Mean Arterial Pressure (mmHg)	79.8 ± 2.0	80.1 ± 1.6	80.3 ± 1.1	0.68
Baroreflex Sensitivity (bpm/mmHg)	5.9 ± 1.8	6.0 ± 3.4	4.2 ± 6.0	0.78
Cerebrovascular				
ScO_2 (%)	65.6 ± 3.0	67.1 ± 3.0 †	66.7 ± 3.4 †	0.03
Deoxygenated Hemoglobin (µM)	15.4 ± 1.1	14.9 ± 1.0	16.7 ± 2.2	0.49
Oxygenated Hemoglobin (µM)	30.7 ± 2.8	31.9 ± 2.7 †	33.0 ± 2.9 †	0.03
Mean MCAv (cm/s)	45.0 ± 3.4	45.5 ± 3.6	47.1 ± 3.6	0.78
Mean PCAv (cm/s)	33.7 ± 4.6	31.0 ± 4.4	37.1 ± 5.8 †	0.07
Respiratory				
$etCO_2$ (mmHg)	29.9 ± 2.0	31.1 ± 1.9	29.6 ± 2.2	0.48
Respiratory rate (breaths/min)	14.3 ± 1.0	14.6 ± 1.1	15.2 ± 1.1	0.53

Data were analyzed with one-way repeated measures ANOVA followed by Holm-Sidak post hoc tests. Values are reported as mean ± SE. * $P<0.001$ vs. 0 Hz; † $P\leq0.06$ vs. 0 Hz.

Table 2. Cardiovascular, cerebrovascular, and respiratory responses for baseline and during the recovery periods for each oscillatory lower body negative pressure (OLBNP) profile.

	Baseline	**Recovery 1**	**Recovery 2**	**ANOVA P-value**
Cardiovascular				
Heart Rate (beats/min)	63.4 ± 2.5	59.8 ± 3.4 †	60.1 ± 3.4 †	0.08
Systolic Arterial Pressure (mmHg)	122.5 ± 2.3	128.1 ± 3.5 †	127.7 ± 3.4 †	0.03
Diastolic Arterial Pressure (mmHg)	67.7 ± 1.4	67.3 ± 3.9	71.7 ± 1.2	0.37
Mean Arterial Pressure (mmHg)	87.4 ± 1.7	93.0 ± 2.1 *	91.8 ± 2.1 †	0.009
Cerebrovascular				
ScO_2 (%)	69.2 ± 2.9	69.4 ± 3.0	69.3 ± 3.1	0.95
Mean MCAv (cm/s)	51.7 ± 3.3	52.9 ± 3.7	52.8 ± 3.7	0.62
Mean PCAv (cm/s)	41.2 ± 5.1	40.1 ± 4.5	39.5 ± 4.2	0.67
Respiratory				
$etCO_2$ (mmHg)	36.7 ± 0.9	35.5 ± 1.5	33.8 ± 1.8 †	0.08
Respiratory rate (breaths/min)	14.7 ± 1.0	14.3 ± 0.9	15.2 ± 1.1	0.31
Blood Samples				
Norepinephrine (pg/mL)	477.0 ± 75.6	725.9 ± 120.2 †	673.9 ± 102.7 †	0.02

Data were analyzed with one-way repeated measures ANOVA followed by Holm-Sidak post hoc tests. Values are reported as mean ± SE. * $P \leq 0.007$ vs. Baseline; † $P \leq 0.09$ vs. Baseline.

Table 3. Frequency analysis of arterial pressure and cerebral blood velocity (MCAv and PCAv) in the low frequency and very low frequency ranges.

	Low Frequency (0.07 - 0.15 Hz)				**Very Low Frequency (0.04 – 0.07 Hz)**			
	0 Hz	**0.1 Hz**	**0.05 Hz**	**ANOVA P-Value**	**0 Hz**	**0.1 Hz**	**0.05 Hz**	**ANOVA P-Value**
MAP Power ($mmHg^2$)	4.3 ± 0.8	9.5 ± 2.1 †	2.6 ± 0.4	0.008	3.7 ± 1.0	2.9 ± 0.6	24.3 ± 4.5 *	<0.001
MCAv Power $(cm/s)^2$	2.0 ± 0.7	4.6 ± 1.0 †	1.2 ± 0.3	0.003	0.8 ± 0.2	0.8 ± 0.2	2.3 ± 0.5 *	0.003
PCAv Power $(cm/s)^2$	1.1 ± 0.8	2.1 ± 0.8	1.4 ± 0.6	0.64	0.3 ± 0.1	0.4 ± 0.2	1.7 ± 0.4 †	0.01
MAP-MCAv Coherence	0.68 ± 0.06	0.69 ± 0.04	0.55 ± 0.07 †	0.03	0.39 ± 0.04	0.31 ± 0.04	0.65 ± 0.08 *	<0.001
MAP-MCAv Gain ($cm/s \cdot mmHg^{-1}$)	0.59 ± 0.07	0.58 ± 0.07	0.64 ± 0.07	0.28	-	-	-	-
MAP-PCAv Coherence	0.62 ± 0.10	0.65 ± 0.12	0.44 ± 0.18	0.12	0.47 ± 0.06	0.36 ± 0.10	0.53 ± 0.18	0.39
MAP-PCAv Gain ($cm/s \cdot mmHg^{-1}$)	-	-	-	-	-	-	-	-

Data were analyzed with one-way repeated measures ANOVA followed by Holm-Sidak post hoc tests. Values are reported as mean ± SE. * $P \leq 0.008$ vs 0 Hz; † $P \leq 0.08$ vs 0 Hz.

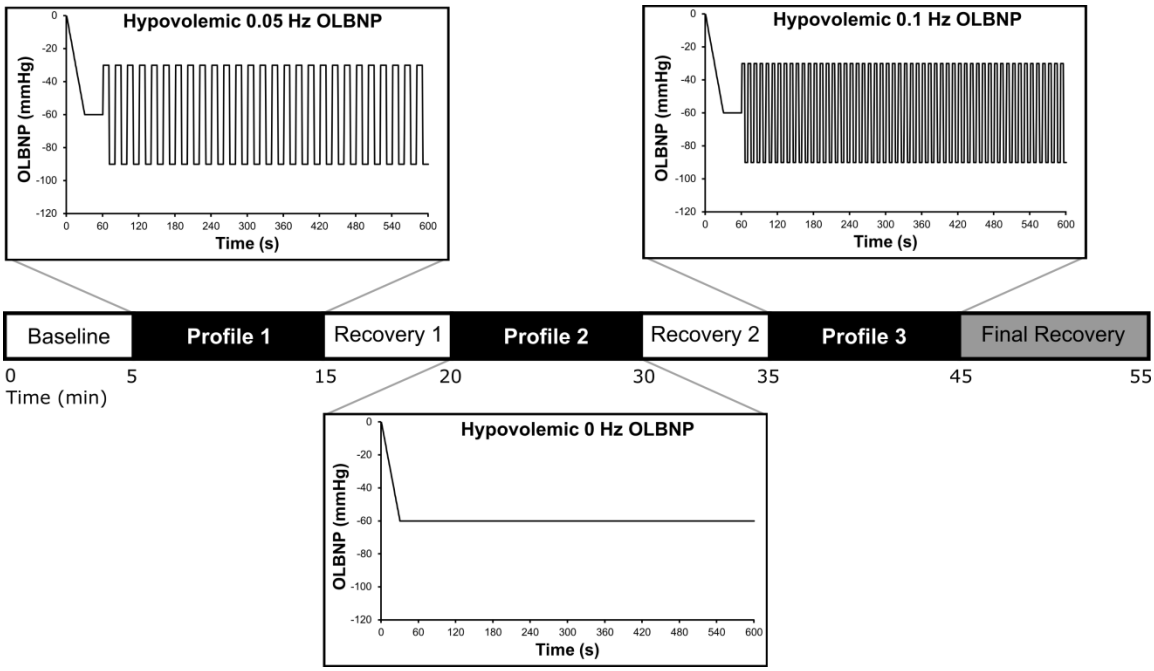

Figure 1. A representative experimental timeline. Each profile was 10-min in duration, including a 1-min transition from 0 to -60 mmHg LBNP. Each recovery period was 5-min in duration, and 10-min at the end of the experimental protocol. The order of profiles was randomized and counterbalanced.

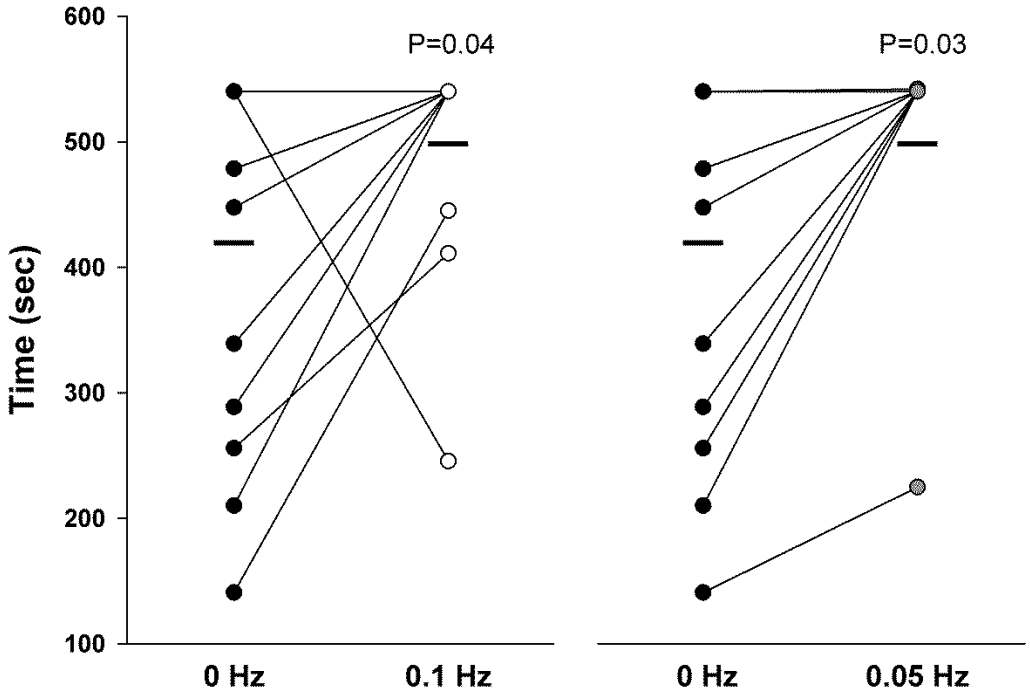

Figure 2. Tolerance to each oscillatory profile (time). Tolerance was higher for both the 0.1 Hz and 0.05 Hz oscillatory profiles compared to the static 0 Hz profile. Individual subject responses, and mean responses (solid bars) are presented. Data were compared by one-way repeated measures ANOVA with Holm-Sidak post hoc comparisons vs. the 0 Hz profile.

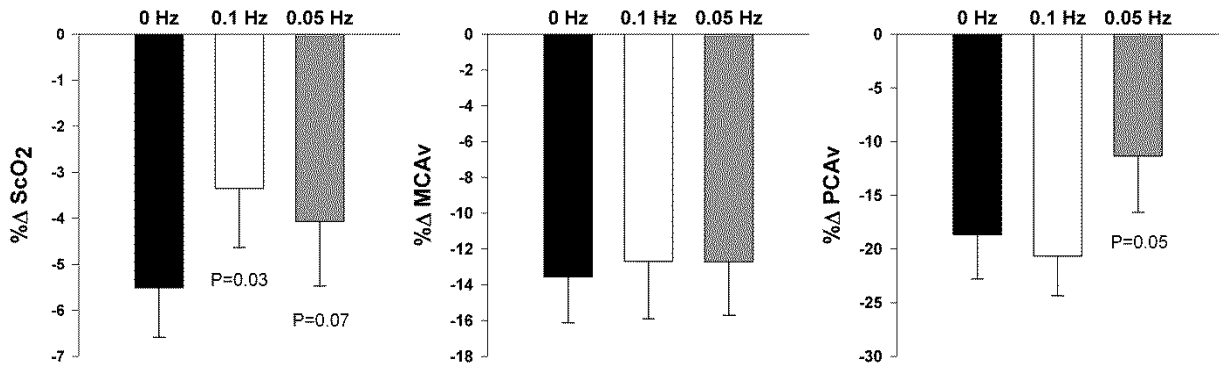

Figure 3. Cerebral oxygenation and cerebral blood velocity responses during oscillatory lower body negative pressure (OLBNP). Cerebral oxygenation (ScO_2), middle cerebral artery velocity (MCAv), and posterior cerebral artery (PCAv) data were analyzed with one-way repeated measures ANOVA followed by Holm-Sidak post hoc tests. Values are reported as mean ± SE.

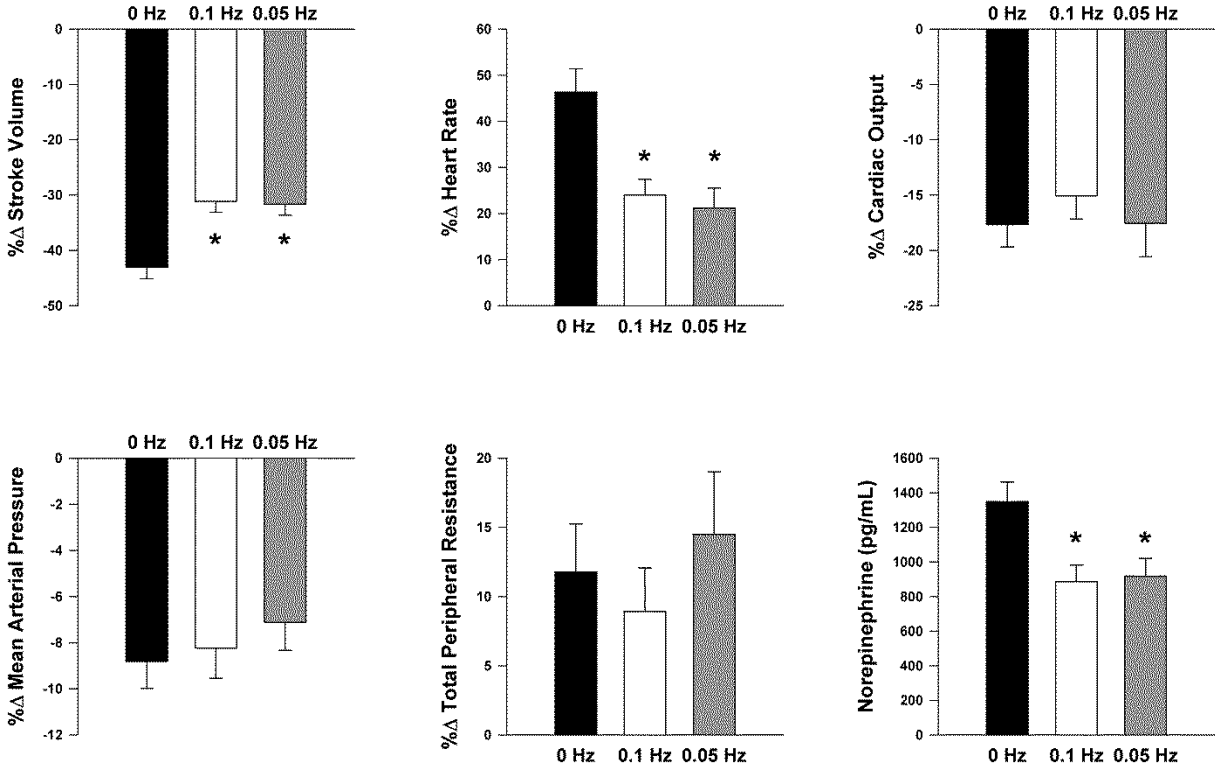

Figure 4. Hemodynamic and plasma norepinephrine responses to oscillatory lower body negative pressure (OLBNP). Data were analyzed with one-way repeated measures ANOVA followed by Holm-Sidak post hoc tests. Values are reported as mean ± SE. * P<0.001 vs. 0 Hz.

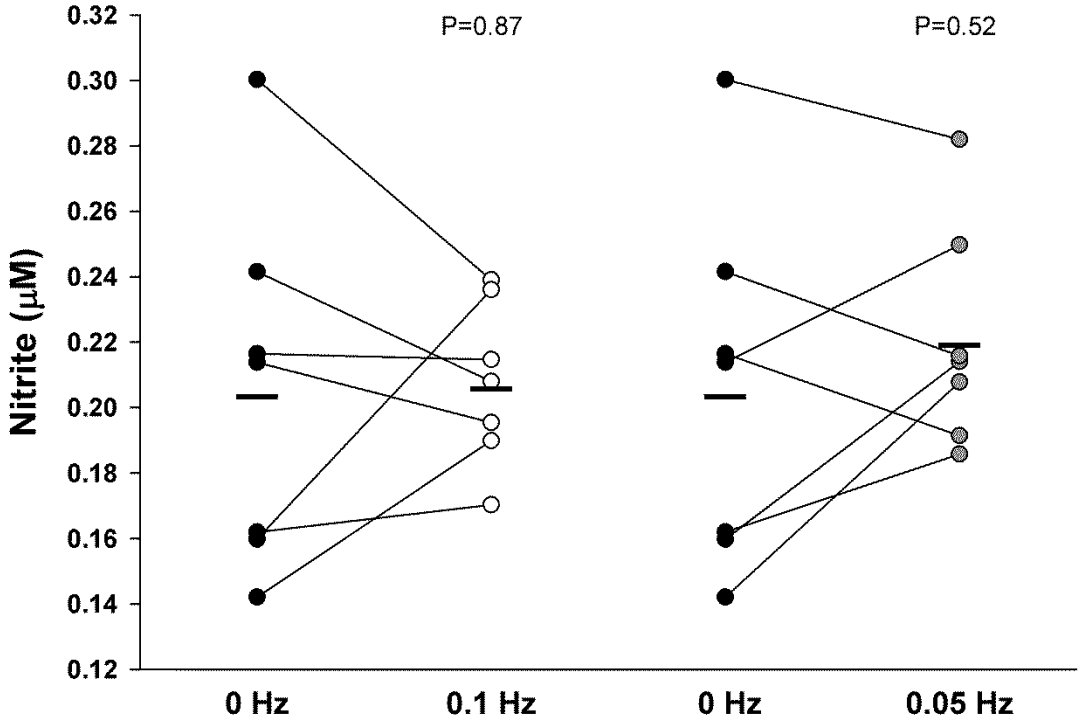

Figure 5. Individual and group mean (—)plasma nitrite concentration responses for each oscillatory lower body negative pressure (OLBNP) profile vs. 0 Hz. Data were analyzed with one-way repeated measures ANOVA followed by Holm-Sidak post hoc tests.

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