Title: The Role of Cerebral Oxygenation and Regional Cerebral Blood Flow on Tolerance to Central Hypovolemia

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Running Head: Cerebral blood flow and oxygenation and LBNP tolerance

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ABSTRACT:
Tolerance to central hypovolemia is highly variable, and accumulating evidence suggests that protection of anterior cerebral blood flow (CBF) is not an underlying mechanism. We hypothesized that individuals with high tolerance to central hypovolemia would exhibit protection of cerebral oxygenation (ScO₂), and prolonged preservation of CBF in the posterior versus anterior cerebral circulation.

Eighteen subjects (7M/11F) completed a presyncopal-limited lower body negative pressure (LBNP) protocol (3 mmHg/min onset rate). ScO₂ (via near-infrared spectroscopy), middle cerebral artery velocity (MCAv), posterior cerebral artery velocity (PCAv) (both via transcranial Doppler ultrasound), and arterial pressure (via finger photoplethysmography) were measured continuously. Subjects who completed ≥70 mmHg LBNP were classified as high tolerant (HT; N=7), and low tolerant (LT; N=11) if they completed ≤60 mmHg LBNP. The minimum difference in LBNP tolerance between groups was 193 s (LT = 1243 ± 185 s vs. HT = 1996 ± 212 s; P<0.001; Cohens $d=3.8$). Despite similar reductions in mean MCAv in both groups, ScO₂ decreased in LT subjects from -15 mmHg LBNP (P=0.002; Cohens $d=1.8$), but was maintained at baseline values until -75 mmHg LBNP in HT subjects (P<0.001; Cohens $d=2.2$); ScO₂ was lower at -30 and -45 mmHg LBNP in LT subjects (P≤0.02; Cohens $d≥1.1$). Similarly, mean PCAv decreased below baseline from -30 mmHg LBNP in LT subjects (P=0.004; Cohens $d=1.0$), but remained unchanged from baseline in HT subjects until -75 mmHg (P=0.006; Cohens $d=2.0$); PCAv was lower at -30 and -45 mmHg LBNP in LT subjects (P≤0.01; Cohens $d≥0.94$). Individuals with higher tolerance to central hypovolemia exhibit prolonged preservation of CBF in the posterior cerebral circulation, and sustained cerebral tissue oxygenation, both associated with a delay in the onset of presyncope.

Key Words: posterior cerebral artery; middle cerebral artery; lower body negative pressure
INTRODUCTION:
Hemorrhage due to trauma is one of the leading causes of morbidity and mortality worldwide in both the civilian and military settings (1, 2, 13, 22, 32). A major factor contributing to death and disability from severe blood loss is poor tissue perfusion and oxygenation of the vital organs (1, 13, 22). Prolonged cerebral hypoperfusion can lead to neuronal cell death, and if the patient survives, long-term cognitive impairment and physical disability (32). Understanding cerebral hemodynamic responses to blood loss is an essential target for improving survival to hemorrhagic injury, and developing effective therapeutic interventions (35). As there is considerable variability in survival time following hemorrhagic injuries (40) as well as tolerance to simulated hemorrhage (7, 15, 24, 26), it is crucial to determine the role of cerebral blood flow (CBF) and oxygenation on the ability to tolerate severe blood loss.

Lower body negative pressure (LBNP) has been extensively utilized as an experimental technique to induce physiologically significant central hypovolemia, and can be used to simulate pre-shock hemorrhage in humans (4, 9, 44, 51). Two recent studies reported comparable hemodynamic responses to LBNP and blood loss up to 1000 ml in humans (21, 36) and 25% loss of total blood volume in baboons (17). It is well established that during the initial stages of progressive central hypovolemia, reflex cardiovascular responses are initiated (e.g., tachycardia, peripheral vasoconstriction) (5, 6, 15, 19, 24, 38), protecting the vital organs from hypoperfusion. While traditionally protection of absolute CBF was thought to be essential in determining tolerance to central hypovolemia (24), recent studies have indicated a disconnect between tolerance to LBNP and protection of absolute flow (predominantly assessed by middle cerebral artery velocity (MCAv), an index of global cerebral blood flow) (20, 27, 28, 37). Most recently, Ogoh et al. suggested that flow in the posterior cerebral circulation (indexed by flow in the vertebral arteries) feeding the brain stem (specifically, the medulla oblongata) is more likely associated with tolerance to central hypovolemia than flow in the anterior cerebral circulation (indexed by flow in the internal carotid arteries) (33); responses between the two regions with central hypovolemia to presyncope, however, was not evaluated.
In addition to delivery of oxygen to the cerebral tissues, extraction of that oxygen from the blood may also play a crucial role in tolerance to central hypovolemia. Near-infrared spectroscopy (NIRS) is often used as a non-invasive method to measure cerebral oxygen saturation (ScO2) via assessment of oxy-(HbO2) and deoxy-hemoglobin (dHb) concentrations within the frontal cortex. NIRS measures oxygen saturation predominantly from venous blood (75%), with just 25% from arterial and capillary blood (30, 34). As such, following oxygen exchange within the capillaries, we and others (43) interpret a decrease in the HbO2 concentration and increase in the dHb concentration as an increase in cerebral oxygen extraction from the arterial blood supplying the tissue. Torella et al. reported that HbO2 decreased, while dHb increased in proportion to mild blood loss in humans (approx. 9-12% total blood volume) (47). While the magnitude of blood loss in this study was not sufficient to delineate the role of cerebral oxygen extraction on tolerance, Lewis et al. recently suggested that decreases in CBF would have minimal impact on tolerance to central hypovolemia due to compensatory increases in cerebral oxygen extraction (25); quantification of oxygen extraction during LBNP, however, was not reported.

Furthermore, in a study of fainters vs. non-fainters following withdrawal of 500 ml of blood plus head-up tilt (3), the fainters had decreased oxygen extraction in the cerebral tissues (positive oxygenation index, i.e., HbO2 – dHb), while the non-fainters had increased oxygen extraction (negative oxygenation index), suggesting that increased tolerance may be due to increased cerebral oxygen extraction. The onset of presyncope is thought to be due to a mismatch between oxygen supply and demand in the brain (3, 25), but this hypothesis has not been explored in relation to tolerance to maximal central hypovolemia.

By applying LBNP continuously (3 mmHg/min decompression rate) to induce significant central hypovolemia to presyncope in healthy, conscious humans, we assessed if; 1) maintenance of ScO2 and/or increased oxygen extraction plays a role in determining tolerance to this stress; and; 2) differences between perfusion of the anterior and posterior regions of the brain were related to tolerance to central hypovolemia. We hypothesized that individuals with higher tolerance to central
hypovolemia would have protection of ScO2, and prolonged preservation of CBF in the posterior versus anterior cerebral circulation, thus delaying the onset of presyncope.

**METHODS:**

**Subjects**

Thirty-four healthy, normotensive, non-smoking subjects volunteered 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. Prior to approval to participate in the study, each subject completed an orientation session, where a medical history was obtained and physical exam was performed, including seated and standing ECG and blood pressure measurements. Females underwent a urine pregnancy test and were excluded if pregnant; the pregnancy test was repeated immediately prior to experimentation. Subjects were given a verbal briefing and written description of all the measurements and risks associated with the experiment, and were made familiar with the laboratory, personnel, procedures, and monitoring equipment. Each subject gave written informed consent to participate in this study. Because of the potential effects on vascular volume and cerebrovascular and baroreflex function, subjects were asked to refrain from exercise, stimulants that might alter autonomic function (e.g., caffeine and cold medications including ephedrine, diphenhydramine), alcohol, prescription or non-prescription drugs, and herbal medications for 24 hours prior to the orientation and experimental sessions. Subjects were also instructed to remain hydrated (*ad libitum* water consumption) and maintain their normal sleep pattern. Experiments were conducted at the same time of day (morning) to avoid potential effects of circadian rhythm on the study outcomes, in a temperature controlled laboratory (22-24°C).

**Instrumentation**

Subjects were placed in the supine position with their lower body inside a LBNP chamber (VUV Analytics, Austin, TX) and positioned on a bicycle seat to ensure they did not move during chamber decompression. Durable plastic and a neoprene band were wrapped around the subject’s waist to
create an airtight seal with the LBNP chamber; the seal was in line with the subject's iliac crest. All subjects were instrumented for the continuous measurement of heart rate (HR) via a standard lead II ECG (shielded leads, cable and amplifier, AD Instruments, Bella Vista, NSW, Australia), and beat-to-beat arterial pressure and stroke volume (SV) via infrared finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). Respiration rate and end tidal CO₂ (etCO₂) were measured on a breath-by-breath basis through a facemask via capnography (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW, Australia). Cerebral blood velocity was recorded from the middle cerebral artery (MCAv) and posterior cerebral artery (PCAv) via transcranial Doppler (TCD) ultrasound (2 MHz probes; ST3, Spencer Technologies, Seattle, WA). HbO₂, dHb, total hemoglobin concentration (THC; HbO₂ + dHb) and ScO₂ [(HbO₂/THC)*100] were measured or calculated from the frontal cortex via near-infrared spectroscopy (NIRS, OxiplexTS, ISS Inc., Champaign-Urbana, IL). Efforts were made to ensure both MCAv and cerebral oxygenation measurements were made on same side of the head within each subject.

Protocol
Each subject was exposed to LBNP to the point of maximal tolerance (i.e., presyncope). The protocol consisted of a 5-min baseline followed by continuous application (ramp-profile) of negative pressure at a decompression rate of 3 mmHg/min until the onset of presyncope (23), determined by one or more of the following criteria: 1) instantaneous systolic arterial pressure (SAP) below 80 mmHg; 2) sudden relative bradycardia, and/or; 3) voluntary subject termination due to subjective presyncopal symptoms such as gray-out, nausea, sweating, dizziness, blurred vision or general discomfort. The chamber pressure was released immediately at the onset of hemodynamic decompensation or upon reaching -100 mmHg LBNP. Release of the chamber pressure occurred within seconds, and pre-syncopal symptoms generally resolved within 30-60 seconds. Following LBNP termination, subjects remained in the chamber for a 10-min recovery period.

Data analysis:
All continuous waveform data (e.g., ECG, arterial blood pressure, SV, MCAv, PCAv, ScO2, THC, etCO2) were collected at 1000 Hz (LabChart, AD Instruments, Bella Vista, NSW, Australia) and analyzed offline via specialized software (WinCPRS, Absolute Aliens, Turku, Finland). R-waves that were generated from the ECG signal were detected to determine the timing of each cardiac cycle. Beat-to-beat SAP and diastolic arterial pressures (DAP) were then detected from the continuous arterial pressure tracing. Systolic and diastolic cerebral blood velocities were also detected and marked from the continuous MCAv and PCAv tracings. MAP, mean MCAv, and mean PCAv were automatically calculated as the area under the arterial pressure and cerebral blood velocity waveforms via the WinCPRS software.

Since there is variable tolerance to central hypovolemia (15, 24, 26), subjects became presynopal at different levels of LBNP. As such, subjects were classified as high tolerant (HT) if they made it to -70 mmHg LBNP or greater, and low tolerant (LT) if they made it to -60 mmHg LBNP or less. If the LBNP protocol was terminated between -61 and -69 mmHg LBNP, these subjects were excluded from all subsequent analysis to ensure there was a definitive separation between groups.

**Statistical Analysis:**

Physiological responses were compared between the HT and LT groups at 15 mmHg intervals up to -45 mmHg LBNP, as this was the last common maximal level of LBNP for the majority of LT subjects. HT subject data is also presented up to -75 mmHg LBNP since they were able to tolerate longer periods of LBNP. All variables were calculated from the final 4-min of each 15 mmHg interval of LBNP, yielding data points approximating responses at baseline (0 mmHg), -15 mmHg (Range: -6 to -15 mmHg), -30 mmHg (Range: -21 to -30 mmHg), -45 mmHg (Range: -36 to -45 mmHg), -60 mmHg (Range: -51 to -60), and -75 mmHg (-66 to -75 mmHg) LBNP. In addition, to compare physiological responses between the HT and LT subjects at presyncope, the final 1-min (PS-1) immediately prior to presyncope was assessed for each subject. Absolute and percentage change from baseline values are reported for the key variables of interest.
The physiological responses to LBNP up to -45 mmHg were analyzed using a two-way (LBNP level and tolerance) repeated measures analysis of variance (ANOVA) followed by Tukey post-hoc tests. For the HT group only, physiological responses up to -75 mmHg LBNP were also analyzed via a one-way ANOVA, followed by Tukey post-hoc tests. The -75 mmHg LBNP level was used for this analysis as 6 of the 7 HT subjects reached this level of LBNP. Unpaired t-tests were used to compare the HT vs. LT group responses at baseline and at the PS-1 min time point or Mann-Whitney tests were run on data that was not normally distributed (dHb at PS-1 only). A Fisher Exact test was used to compare the ratio of males to females between the HT and LT groups. All data are presented as mean ± SE (unless otherwise stated). Effect size was calculated using Cohen’s d. A critical alpha level of 0.05 is used for all comparisons, and exact P-values are also reported where appropriate.

**RESULTS:**

**LBNP tolerance:**

Of the 34 subjects who participated in this study, data were analyzed and included from 27 subjects who 1) reached the maximal LBNP pressure (-100 mmHg), or; 2) had a minimum instantaneous SAP < 80 mmHg, or; 3) exhibited subjective pre-syncopal symptoms combined with mean SAP < 100 mmHg for the 1-min prior to presyncope and/or minimum SAP ≤ 90 mmHg within the 1-min prior to presyncope. Of the 27 subjects who reached these thresholds, 7 subjects reached presyncope between -61 and -69 mmHg LBNP, therefore are not included in this particular data set (these subjects are included in other independent analyses conducted on this data set, including Kay & Rickards (23)). Of the remaining 20 subjects, 2 were excluded from subsequent analyses as they exhibited a resting SAP > 140 mmHg. The remaining 18 subjects (11 male, 7 female; age, 26 ± 3 yrs; height, 172 ± 9 cm; weight, 74 ± 15 kg; means ± SD) were allocated to the HT (N = 7) or LT (N = 11) groups based on the level of LBNP they reached prior to presyncope, as previously described (i.e., HT ≥ 70 mmHg LBNP; LT ≤ 60 mmHg LBNP). By design, the *minimum* difference in LBNP tolerance between the HT and LT groups was 193 s (LT = 1243 ± 185 s vs. HT = 1996 ± 212 s; P < 0.001; Cohen’s $d$=3.8). Of the 18
subjects included in the final analysis, the LBNP protocol was terminated at or before -45 mmHg LBNP for 4 subjects, between -45 and -60 mmHg LBNP for 7 subjects, between -70 and -90 mmHg LBNP for 5 subjects, between -90 and -99 mmHg LBNP for 1 subject, and one subject completed the protocol to -100 mmHg LBNP. Data in Tables 1 and 2 compares the baseline characteristics of each group.

**Cardiovascular Responses to LBNP:**

Data are presented as means ± SE at 15 mmHg intervals of LBNP. Both groups experienced progressive reductions in SV from baseline, reaching 20-30% below baseline by -45 mmHg of LBNP (P<0.001, Cohen's $d=2.7$; Figure 1); the SV reduction in the LT group was greater at -30 and -45 mmHg LBNP (P≤0.006; Cohen's $d=0.96$) versus the HT group. However, at the final 1-min prior to presyncope (PS-1 min), SV decreased by 63 ± 4% in the HT group compared to 42 ± 4% in the LT group (P = 0.004; Cohen's $d=1.7$). In response to these reductions in SV, compensatory increases in HR occurred in both the HT and LT groups (Figure 2A), with the HT group exhibiting a greater maximal HR response at the final 1-min prior to presyncope (PS-1 min) compared to the LT group (HT: 132 ± 5 beats/min vs. LT: 92 ± 6 beats/min; P<0.001, Cohen's $d=2.3$). MAP was maintained at baseline levels up to -30 mmHg, then fell below baseline at -45 mmHg LBNP in both the HT and LT groups (P≤0.04, Cohen's $d=0.67$; Figure 2B); by presyncope MAP fell to similar levels in both groups (P = 0.92, Cohen's $d=0.05$).

Mean PCAv decreased below baseline from -30 mmHg LBNP in the LT subjects (P=0.013, Cohen's $d=1.0$), but remained unchanged in the HT subjects up to -45 mmHg (P≥0.86, Cohen's $d=0.32$; Figure 3B), only beginning to fall below baseline at -75 mmHg (P=0.007, Cohen's $d=1.98$). By presyncope, mean PCAv had decreased by the same magnitude in both HT and LT groups (P=0.77, Cohen's $d=0.15$), and to similar absolute values (P=0.71, Cohen's $d=0.23$; Table 2). In comparison, a decrease (%Δ) in mean MCAv from baseline was observed in the LT group by -30 mmHg and by -45 mmHg in the HT group (P ≤ 0.05, Cohen's $d=0.89$; Figure 3A). Despite similar reductions in MCAv in both groups, however, there was an immediate and progressive decrease in ScO$_2$ in the LT group only from...
-15 mmHg LBNP (P = 0.004, Cohen’s d=1.8), but no change in ScO2 for the HT group at any level of LBNP up to -60 mmHg; ScO2 only began to fall below baseline from -75 mmHg LBNP in HT subjects (P <0.001, Cohen’s d=2.2) (Figure 4A). At presyncope, the HT group exhibited greater reductions in MCAv (HT: -35 ± 4% vs. LT: -23 ± 4%, P = 0.07, Cohen’s d=1.0), and lower absolute MCAv (P=0.06, Cohen’s d=1.2; table 2).

Cerebral HbO2 and dHb responses are presented in figures 4B and 4C. HbO2 was maintained throughout LBNP up to and including -75 mmHg LBNP in the HT group, while it started immediately decreasing at -15 mmHg LBNP for the LT group and continued to decrease until presyncope. At presyncope, HbO2 had decreased by approximately 10% in both groups (P = 0.86, Cohen’s d=0.09). Cerebral dHb progressively increased for both groups, from -15 mmHg LBNP for the LT group (P ≤ 0.02, Cohen’s d=1.4), and from -60 mmHg LBNP for the HT group (P ≤ 0.001, Cohen’s d=1.9). At presyncope, the increase in dHb in the HT was greater than the LT group (P = 0.03, Cohen’s d=1.5). Absolute data for the measured variables at each level of LBNP and at presyncope are presented in table 2.

The respiratory responses to LBNP are presented in Table 2. Respiration rate for both HT and LT subjects was maintained at baseline levels within groups throughout LBNP, but was higher overall in the LT group throughout LBNP up to -45 mmHg (Group main effect P = 0.01). EtCO2 progressively decreased from baseline in both groups, with a difference between groups only evident at -45 mmHg LBNP (P = 0.01, Cohen’s d=1.2). There were no differences at presyncope between the HT and LT groups for either respiratory rate or etCO2.

**DISCUSSION:**
In this study we examined the role of regional cerebral blood flow and oxygenation on tolerance to central hypovolemia elicited by continuous application of LBNP to presyncope. The key findings of this study demonstrate that individuals with high tolerance to central hypovolemia, 1) exhibit prolonged
protection of cerebral tissue oxygen saturation in the frontal lobe despite early reductions in cerebral
blood flow (i.e., delivery); 2) show similar reductions in anterior CBF (indexed by mean MCAv) as low
tolerant subjects up to -45 mmHg LBNP, but a greater reduction in anterior CBF at presyncope, and; 3)
have protection of posterior CBF (indexed by mean PCAv) at sub-maximal levels of LBNP. Our data
support the hypothesis that individuals with higher tolerance to central hypovolemia appear to have
prolonged preservation of CBF in the posterior versus anterior cerebral circulation, and a delayed
mismatch in oxygen delivery-demand, resulting in sustained cerebral tissue oxygenation. Combined,
these two responses were associated with a delay in the onset of presyncope.

Regional cerebral blood flow:

To date, studies assessing CBF responses to maximal central hypovolemia to presyncope have
focused primarily on the MCA as a marker of global cerebral blood flow. There is growing evidence,
however, that protection of cerebral blood flow through the MCA is not necessarily associated with
tolerance to central hypovolemia (20, 27, 28, 37); the findings from the current study support this
concept as MCAv responses between HT and LT subjects were similar up to the last common level of
LBNP, despite LT subjects reaching presyncope at this time point. The present study is one of very few
to report CBF responses within the posterior cerebral circulation during central hypovolemia elicited by
LBNP, including the PCA or vertebral arteries (VA) (12, 33). Autonomic and respiratory control centers
are located within the medulla oblongata in the brainstem, which receives blood and oxygen supply
through these posterior cerebral arteries (45). As such, disruption of posterior cerebral flow may be
related to the symptoms and hemodynamic dysfunction associated with presyncope during central
hypovolemia, such as LBNP or hemorrhage (33). Deegan et al. reported similar responses between
mean MCAv and blood flow in the VA during head-up tilt plus LBNP to presyncope, although they
combined all 18 subjects into a single group and did not assess potential differences between
individuals with varying tolerance to this stress (12). Most recently, Ogoh et al. examined blood flow
responses in the VA feeding the posterior cerebral circulation and internal carotid arteries (ICA) feeding
the anterior cerebral circulation up to sub-maximal LBNP of -50 mmHg (33). Based on the significant,
Although weak, association between the fall in ICA flow and the magnitude of LBNP (r=0.29; P=0.029) versus no change in VA flow with LBNP (r=0.167; P=0.22), these investigators postulated that cerebral perfusion of the posterior regions of the brain would only decrease with severe orthostatic stress, so may be associated with tolerance to central hypovolemia. We were able to explicitly test this hypothesis by exposing all of our subjects to maximal levels of presyncopal limited LBNP, and found that mean PCAv was protected in HT subjects up to -60 mmHg LBNP, but decreased progressively in LT subjects.

At presyncope, the reduction in mean PCAv was similar between both groups and was coincident with subjective presyncopal symptoms (e.g., dizziness, lightheadedness, blurred vision, and nausea) in most subjects, indicating that hypoperfusion of the posterior regions of the brain is, indeed, associated with tolerance to maximal central hypovolemia.

**Cerebral oxygen saturation and extraction:**

NIRS is often used to measure oxygen saturation in the cerebral tissues, measuring a mixed sample volume of arterial (20%), capillary (5%), and venous blood (75%) (30, 48). At the last common level of LBNP between groups (i.e., -45 mmHg LBNP), the LT subjects had a lower ScO₂ than the HT group.

At presyncope, the HT group had an 8% (Range: 3.5-14.8%) reduction in ScO₂ compared with a 6% (Range: 0.9-14.3%) reduction in the LT group. While previous studies have shown that a 10-15% reduction in ScO₂ is associated with presyncope (3, 16, 29), we have demonstrated that much smaller reductions in ScO₂ can be associated with presyncope.

The brain compensates for reductions in cerebral blood flow by increasing oxygen extraction (determined via assessment of cerebral arterial venous oxygen difference) (25, 31). Reducing the cerebral blood flow reserve (by indomethacin) prior to exposure to maximal LBNP does not change tolerance, suggesting that increases in oxygen extraction would compensate for the decrease in oxygen delivery (25); oxygen extraction was not assessed during LBNP to directly test this hypothesis.

While Glaister et al. reported a decrease in HbO₂ and an increase in dHb during LBNP to presyncope (14), our data are the first, to our knowledge, to demonstrate differential responses of HbO₂ and dHb...
within HT and LT subjects. With a decrease in HbO₂ and an increase in dHb on the venous side of the
circulation (i.e., NIRS sample volume is 75% venous blood), we interpret this as an increase in
extraction of oxygen from the blood into the tissues. Our data show that the LT group had an immediate
and progressive increase in oxygen extraction, evidenced by an immediate decrease in HbO₂ and
increase in dHb beginning at -15 mmHg LBNP. This increased oxygen extraction was accompanied by
a reduction in oxygen delivery (i.e., decreased mean MCAv). In contrast, despite comparable
reductions in oxygen delivery as the LT subjects (evidenced by similar decreases in mean MCAv), the
HT group exhibited maintenance of HbO₂ throughout LBNP, suggesting constant oxygen extraction.
Interestingly, despite greater reductions in SV (P=0.004) and mean MCAv (P=0.07) at presyncope in
the HT group, and the small and highly variable reductions in ScO₂, both the HT and LT groups
experienced the same maximal reduction in HbO₂ (approx. 10%). This finding indicates that reductions
in cerebral tissue HbO₂ concentration may be a more accurate indicator of impending presyncope than
cerebral oxygen saturation or CBF through the anterior circulation (i.e., mean MCAv).

There are a number of reasons that may account for the apparent increase in oxygen extraction,
including a reduction in oxygen delivery via decreases in CBF and/or hypoxia, and/or increased
metabolic demand. A reduction in CBF would increase oxygen extraction (25); with less oxygen
available to the tissues, extraction would need to increase to compensate for decreased delivery and to
meet metabolic demand. Interestingly in our study, despite both groups experiencing the same
magnitude of mean MCAv reductions up to -45 mmHg LBNP, the HT subjects were able to maintain
stable tissue oxygen saturation and oxygen extraction (evidenced by constant HbO₂). The LT group
started to increase oxygen extraction with as little as a 4% reduction in MCAv (at -15 mmHg), while the
HT group had constant oxygen extraction until their MCAv decreased from baseline by ~35% (at
presyncope). This finding suggests that HT subjects may have more efficient utilization of oxygen until
a critical threshold of delivery (i.e., CBF) is reached, at which time, increases in extraction occurred with
further reductions in flow.
A reduction in the partial pressure of oxygen and/or oxygen saturation, either due to hypoxia or impaired gas exchange, would also reduce oxygen delivery, eliciting an increase in oxygen extraction to meet metabolic demand. However, arterial oxygen content should be similar between groups as 1) experiments were conducted in a normoxic testing environment; 2) we assume hemoglobin concentration would increase in both groups based on similar reductions in central blood volume and subsequent fluid extravasation (17, 21), and; 3) central hypovolemia elicited by LBNP stress does not induce any changes in arterial oxygen saturation or PaO₂ (21, 50).

Finally, psychological stress/anxiety and increased neuronal activity may also play a role in the increased oxygen extraction observed in the LT subjects, and a subsequent oxygen demand and supply mismatch at earlier levels of LBNP. We did not systematically assess anxiety or psychological stress in our subjects; measurements of subjective stress levels and/or stress hormones such as cortisol could further elucidate this effect on tolerance to LBNP. Overall, the HT group only increased oxygen extraction once CBF decreased 35% from baseline, thus contributing to the capacity to tolerate a greater magnitude of central hypovolemia and delay the onset of presyncopal symptoms.

**Perspectives and Significance:**

Reduced metabolic demand and/or increased oxygen efficiency in the HT group contributes to the delay in the onset of presyncope. This may have important clinical implications for the monitoring of cerebral perfusion and oxygenation in patients, where increased metabolic demand elicited by anxiety, stress and/or pain may increase cerebral oxygen extraction and reduce tolerance to hypovolemic stress such as traumatic hemorrhage, and orthostasis. Under these conditions, the magnitude of hypovolemia that may induce unconsciousness will depend on individual cerebral metabolic demand; at presyncope, HT subjects had a 63% reduction in SV, compared to a 42% reduction in the LT group. In addition, these data demonstrate the important role of HbO₂ on ScO₂ calculations, the measurement most often reported when using cerebral NIRS monitoring devices. The HT group had stable ScO₂ due to stable HbO₂, whereas the LT group had progressively decreasing HbO₂, which decreased ScO₂.
Despite differences in the onset of increasing oxygen extraction during LBNP, we can conclude that an overall 10-11% reduction in HbO$_2$ is associated with an oxygen supply and demand mismatch, and the subsequent appearance of presyncopal symptoms, regardless of whether subjects were HT or LT. Therefore, it may be more applicable to use the measure of HbO$_2$ as a reference point for impending presyncope as opposed to ScO$_2$. Similarly, the finding that reduced PCAv distinguished LT from HT subjects from an early level of central hypovolemia suggests that investigation of the posterior cerebral circulation may provide important insight into the mechanisms of tolerance to hemorrhage, and could be a target for interventions to increase tolerance to this stress. These findings from healthy subjects in the current study require further investigation in clinical populations with orthostatic intolerance.

**Methodological Considerations:**

While application of LBNP does not mimic all of the responses observed in traumatic hemorrhage (e.g., tissue trauma, pain, metabolic responses such as acidosis), this technique allows us to isolate the physiological responses of central hypovolemia without these confounding factors. In addition, although LBNP does not elicit blood cell loss as seen in actual hemorrhage, it does mimic some of the cardiovascular and cerebral blood flow responses elicited by hemorrhage (17, 21, 36).

We interpret the reduction in NIRS-derived HbO$_2$ and increase in dHb with LBNP as an increase in oxygen extraction at the level of the cerebral tissues within the frontal lobe. This interpretation should be experimentally quantified during LBNP with direct measures of oxygen extraction via arterial-jugular venous oxygen measurements across the brain, and direct assessment of CBF [e.g., via duplex Doppler ultrasound of the extracranial vessels (46)]. Additionally, NIRS is a non-invasive method to obtain measurements of ScO$_2$, and concentrations of HbO$_2$ and dHb within the cerebral tissue, but it also may be contaminated by changes in oxygenation of the skin (11, 18, 41, 42). For this study, we used a spatially resolved NIRS device with 4 emitters that were 2.0, 2.5, 3.0, and 3.5 cm from the detector, compared with only two emitters on many other NIRS devices. Theoretically, as each emitter distance samples from a different depth, via mathematical correction, measurements from the
extracranial sample volume (i.e., skin, muscle, and fat) can be removed from the final oxygen saturation measurements of the intracranial cerebral tissues. The sensor was also covered with black cloth to reduce contamination from room light, and all sinus cavities were avoided during sensor placement. Furthermore, while preparation of this sensor for use includes a calibration step, this is simply to ensure the known absorption and scattering co-efficients of the calibration block have been detected by the sensor. Systematic calibration against known oxy- and deoxy-hemoglobin concentrations and/or known oxygen saturations is not performed with this device, or any cerebral NIRS device as far as these authors are aware, and there is not a "gold standard" device for this purpose.

Finally, transcranial Doppler is used to assess CBF with the assumption that the diameter of the insonated vessel remains constant. MCA diameter does not change with mild sympathetic activation induced by LBNP up to -40mmHg (39), which may be comparable to our LT group, but not the HT group, as they reached levels of LBNP ≥ 70mmHg. During greater levels of LBNP, there is increased sympathetic nerve activity in the periphery (8), but it is unknown if cerebral sympathetic activity also increases, which could constrict the cerebral vessels. If this did occur, however, CBF would be further decreased relative to the observed reduction in velocity (i.e., we would be underestimating the reduction in flow). Recent studies (10, 49) using high resolution magnetic resonance imaging (MRI), also suggest that increases in etCO₂ ≥ 9 mmHg above baseline elicit MCA vasodilation, while decreases in etCO₂ ≥ 13 mmHg below baseline elicit MCA vasoconstriction. In the current study, etCO₂ in both groups only fell by ~9 mmHg below baseline, so MCA caliber should be constant. If however, there was a mild vasoconstrictive effect from this magnitude of hypocapnia, the observed decrease in velocity of blood flowing through the artery of interest would be attenuated, and the subsequent measure of velocity would underestimate the actual reduction in flow. The role of sympathetic activity and variations in arterial CO₂ on PCA caliber, however, is unknown.

Conclusions:
The novel findings of this study indicate that subjects with increased tolerance to central hypovolemia have prolonged maintenance of cerebral tissue oxygenation (despite reductions in cerebral flow within the anterior cerebral circulation), and protection of CBF within the posterior cerebral circulation. We postulate that high tolerant subjects maintain relatively constant oxygen metabolism in the brain, thus delaying the onset of presyncope. These findings suggests that the measurement of NIRS-derived oxy-hemoglobin and/or posterior cerebral blood flow may be more sensitive indicators for tracking the onset of presyncope than oxygen saturation or measures of anterior cerebral blood flow, and are targets for potential interventions to prolong tolerance to central hypovolemia in the clinical setting.
ADDITIONAL INFORMATION:

Author contributions: CAR is responsible for conception of the work, obtaining funding for the study, conducting experiments, supervising data analysis, drafting the work and revising it critically for important intellectual content, and final approval of the version to be published. VLK is responsible for conducting experiments, data analysis, drafting the work and revising it critically for important intellectual content, and final approval of the version to be published. Neither author has any conflicts of interest.

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**Table 1**: Demographics for subjects with high tolerance (HT) and low tolerance (LT) to LBNP at baseline.

<table>
<thead>
<tr>
<th></th>
<th>HT</th>
<th>LT</th>
<th>P-Value</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>7</td>
<td>11</td>
<td>-</td>
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<tr>
<td>LBNP Tolerance Time, s</td>
<td>1996 ± 212</td>
<td>1243 ± 185</td>
<td>&lt; 0.001</td>
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<tr>
<td>Sex, female/male</td>
<td>2/5</td>
<td>5/6</td>
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<tr>
<td>Age, yrs</td>
<td>26 ± 3</td>
<td>26 ± 3</td>
<td>0.80</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172 ± 10</td>
<td>171 ± 9</td>
<td>0.84</td>
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<tr>
<td>Weight, kg</td>
<td>70 ± 13</td>
<td>77 ± 16</td>
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<tr>
<td>Baseline HR, bpm</td>
<td>59.4 ± 2.8</td>
<td>60.4 ± 2.3</td>
<td>0.79</td>
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<tr>
<td>Baseline MAP, mmHg</td>
<td>96.5 ± 2.2</td>
<td>93.3 ± 2.2</td>
<td>0.34</td>
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</tbody>
</table>

Data are means ± SD for age, height, weight, and means ± SE for all other data. HT, high tolerance; LT, low tolerance; HR, heart rate; MAP, mean arterial pressure.
Table 2: Hemodynamic responses during progressive lower body negative pressure (LBNP) to presyncope in high tolerant (HT) and low tolerant (LT) groups.

<table>
<thead>
<tr>
<th>LBNP Level (mmHg)</th>
<th>ANOVA P-Values</th>
<th>SAP, mmHg</th>
<th>MAP, mmHg</th>
<th>CO, %Δ</th>
<th>TPR, %Δ</th>
<th>PCAv, cm/s</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LBNP</td>
<td>HT vs. LT</td>
<td>Interaction</td>
<td>PS 1-min</td>
<td>P-Value</td>
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<tr>
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<tr>
<td>75</td>
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</tbody>
</table>

**SAP, mmHg**

- **HT**: 129.9 ± 2.9 130.0 ± 2.6 126.4 ± 3.1 122.0 ± 4.3 * 116.2 ± 3.3 * 111.3 ± 2.2 * <0.001 0.04 0.78 95.4 ± 2.6
  - P = 0.16
- **LT**: 123.7 ± 2.0 124.5 ± 2.6 118.1 ± 1.7 *† 113.3 ± 2.7 * – – 99.6 ± 1.6

**DAP, mmHg**

- **HT**: 74.1 ± 2.1 74.0 ± 2.0 74.0 ± 2.2 73.7 ± 2.1 73.6 ± 2.1 74.7 ± 2.0 0.96 0.87 0.84 68.5 ± 2.7
  - P = 0.38
- **LT**: 73.0 ± 2.0 73.7 ± 1.8 74.2 ± 2.0 72.4 ± 2.8 – – 65.0 ± 2.5

**MAP, mmHg**

- **HT**: 96.5 ± 2.2 95.8 ± 2.0 94.6 ± 2.2 92.2 ± 2.3 * 89.6 ± 2.1 * 88.1 ± 1.9 * <0.001 0.37 0.84 78.4 ± 2.6
  - P = 0.92
- **LT**: 93.3 ± 2.2 93.8 ± 2.1 91.4 ± 1.9 87.9 ± 3.1 * – – 78.0 ± 2.4

**CO, %Δ**

- **HT**: – 1.6 ± 1.7 -2.4 ± 3.6 -7.4 ± 5.5 -10.3 ± 5.1 * -11.6 ± 5.5 * 0.04 0.81 0.32 -19.7 ± 6.1
  - P = 0.25
- **LT**: – 0.7 ± 1.4 -4.3 ± 2.6 -0.4 ± 3.7 – – -9.7 ± 5.5

**TPR, %Δ**

- **HT**: – -2.1 ± 1.5 1.4 ± 4.1 5.8 ± 7.4 6.1 ± 7.5 4.6 ± 7.6 0.53 0.82 0.33 5.9 ± 9.8
  - P=0.43
- **LT**: – 0.1 ± 1.4 3.2 ± 2.8 -1.7 ± 4.7 – – -3.4 ± 6.7

**PCAv, cm/s**

- **HT**: 40.2 ± 2.8 40.4 ± 2.6 40.5 ± 2.8 39.1 ± 2.5 38.9 ± 2.9 36.2 ± 2.4 * <0.001 0.07 0.04 30.2 ± 1.4
  - P = 0.71
<table>
<thead>
<tr>
<th></th>
<th>MCAv, cm/s</th>
<th>ScO2, %</th>
<th>HbO2, µM</th>
<th>dHb, µM</th>
<th>THC, µM</th>
<th>Respiratory Rate, breaths/min</th>
<th>etCO2, mmHg</th>
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<tr>
<td>HT</td>
<td>61.4 ± 4.8</td>
<td>66.2 ± 3.2</td>
<td>32.7 ± 3.2</td>
<td>16.5 ± 1.8</td>
<td>49.2 ± 3.7</td>
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<td></td>
<td>60.0 ± 5.2</td>
<td>65.8 ± 3.3</td>
<td>32.0 ± 3.2</td>
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<td>18.2 ± 1.5</td>
<td>48.3 ± 3.2</td>
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<td>33.2 ± 1.8</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.18</td>
<td>&lt;0.001</td>
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<tr>
<td>LT</td>
<td>0.97</td>
<td>0.06</td>
<td>0.06</td>
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<td>LT</td>
<td>48.7 ± 3.9</td>
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<td>28.9 ± 2.2</td>
<td>P = 0.70</td>
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<td>48.7 ± 3.9</td>
</tr>
</tbody>
</table>
Data are presented as absolute means ± SE. HT, high tolerance; LT, low tolerance; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance MCAv, middle cerebral artery velocity; ScO2, cerebral oxygen saturation; HbO2, oxygenated hemoglobin concentration; dHb, deoxygenated hemoglobin concentration; THC, total hemoglobin concentration; etCO2, end tidal carbon dioxide. PS-1 time point refers to the 1 minute prior to pre-syncope. *P ≤0.05 compared to baseline within a group. †P ≤0.04 between HT and LT groups.
FIGURE LEGENDS

Figure 1  Stroke volume (SV) % change from baseline was greater in the low tolerant (LT, dashed line, open circles) vs high tolerant (HT, solid line, closed circles) group at -30 and -45 mmHg lower body negative pressure (LBNP). The maximal reduction in SV over the 1-min prior to presyncope (PS-1) was greater in the HT (filled triangle) vs LT group (open triangle). *, denotes P≤0.02 compared with baseline. †, denotes P≤0.006 between groups. A two-way repeated measures ANOVA (within and between HT and LT groups up to -45 mmHg LBNP), one-way repeated measures ANOVA (within HT group), and unpaired t-test (between HT and LT groups at PS-1) were used for analysis. The P-values for each main effect and interaction for the 2-way ANOVA are presented.

Figure 2  Heart rate (Panel A) increased and mean arterial pressure (MAP, Panel B) decreased in both the high tolerant (HT, solid line, closed circles) and low tolerant (LT, dashed line, open circles) groups during lower body negative pressure (LBNP). Over the 1-min prior to presyncope (PS-1), HR was higher in the HT group (filled triangle) vs LT group (open triangle), but there was no difference in MAP. *, denotes P≤0.04 compared with baseline. †, denotes P=0.008 between groups. A two-way repeated measures ANOVA (within and between HT and LT groups up to -45 mmHg LBNP), one-way repeated measures ANOVA (within HT group), and unpaired t-test (between HT and LT groups at PS-1) were used for analysis. The P-values for each main effect and interaction for the 2-way ANOVA are presented.

Figure 3  Middle cerebral artery velocity (MCAv, Panel A) decreased in the high tolerant (HT, solid line, closed circles) and low tolerant (LT, dashed line, open circles) groups during lower body negative pressure (LBNP). Posterior cerebral artery velocity (PCAv, Panel B) was lower in the LT group vs. HT group at -30 and -45 mmHg LBNP, and did not change from baseline until -75 mmHg in the HT group. Over the 1-min prior to presyncope (PS-1), the % change in MCA was greater in the HT (filled triangle) vs LT (open triangle) group, but there was no difference in PCAv at this time point. *, denotes P≤0.05 compared with baseline. †, denotes P≤0.03 between groups. A two-way repeated measures ANOVA (within and between HT and LT groups up to -45 mmHg LBNP), one-way repeated measures ANOVA
(within HT group), and unpaired t-test (between HT and LT groups at PS-1) were used for analysis. The P-values for each main effect and interaction for the 2-way ANOVA are presented.

**Figure 4**  Percent change from baseline for cerebral oxygen saturation (ScO₂, **Panel A**), oxygenated hemoglobin (Oxy Hb, **Panel B**), and deoxygenated hemoglobin (Deoxy Hb, **Panel C**) in the high tolerant (HT, solid line, closed circles) vs low tolerant (LT, dashed line, open circles) group throughout lower body negative pressure (LBNP). The reductions in ScO₂ and Oxy Hb were greater in the LT vs. HT group at -30 and -45 mmHg LBNP, but similar between groups for the final 1-min prior to presyncope (PS-1), represented by filled (HT) and open (LT) triangles. Deoxy Hb only increased from early levels of LBNP in the LT group, and was higher at presyncope (PS-1) in the HT group. *, denotes P≤0.02 compared with baseline. †, denotes P≤0.05 between groups. A two-way repeated measures ANOVA (within and between HT and LT groups up to -45 mmHg LBNP), one-way repeated measures ANOVA (within HT group), and unpaired t-test or Mann-Whitney U test (for deoxy Hb) (between HT and LT groups at PS-1) were used for analysis. The P-values for each main effect and interaction for the 2-way ANOVA are presented.


FIGURE 1 – R1
(N=18)

LBNP: <0.001
HT/LT: 0.04
Interaction: 0.01

P= 0.004
FIGURE 2 – R1
(N=18)

LBNP: <0.001
HT/LT: 0.19
Interaction: <0.001

A

Heart Rate (bpm)

B

MAP (mmHg)

LBNP: <0.001
HT/LT: 0.37
Interaction: 0.84

P<0.001
P=0.92
FIGURE 3 – R1
(N=18)

LBNP: <0.001
HT/LT: 0.30
Interaction: 0.45

LBNP: <0.001
HT/LT: 0.04
Interaction: 0.03

MCAv ( %
 from baseline)

PCAv ( %
 from baseline)

LBNP (mmHg)

LBNP (mmHg)

P=0.77

P=0.07
FIGURE 4 – R1
(N=18)

A

LBNP: <0.001
HT/LT: 0.01
Interaction: 0.005

B

LBNP: <0.001
HT/LT: 0.03
Interaction: 0.004

C

LBNP: <0.001
HT/LT: 0.06
Interaction: 0.10

ScO$_2$ (%Δ from baseline)

Oxy Hb (%Δ)

Deoxy Hb (%Δ)

P=0.16

P=0.86

P=0.03