ABSTRACT

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Tolerance to central hypovolemia, including hemorrhage, is highly variable between individuals. The role of cerebral oxygenation and regional cerebral perfusion on tolerance to central hypovolemia has not been explored. Protection of posterior cerebral perfusion may be an important factor in tolerance, as the posterior circulation supplies blood to the autonomic and respiratory control centers in the brain stem. Additionally, despite the reduction in cerebral oxygen delivery with central hypovolemia via decreased flow, the role of compensatory increases in oxygen extraction and subsequent cerebral tissue oxygenation on tolerance have not been identified. The oscillatory pattern of cerebral blood flow has recently been identified as a contributing factor to improving tolerance to central hypovolemia, and may be more important than the protection of absolute flow. This finding was demonstrated when comparing high vs. low tolerant individuals, and in subjects who exhibited increased tolerance to central hypovolemia while breathing against inspiratory resistance. We hypothesized that healthy human subjects with naturally high tolerance to central hypovolemia, and subjects breathing against inspiratory resistance under hypovolemic stress would exhibit 1) protection of cerebral oxygen saturation (ScO_2); 2) prolonged preservation of cerebral blood flow in the posterior versus anterior cerebral circulation, and; 3) higher LF oscillations in cerebral blood flow.

The major findings from these investigations are: 1) subjects with high tolerance to central hypovolemia exhibited protection of ScO_2 and velocity in the posterior cerebral circulation; 2) LF oscillations did not play a role in the protection of ScO_2; 3) resistance breathing improved tolerance to central hypovolemia, but not via protection of ScO_2 or velocity in either the anterior or posterior cerebral circulation, and; 4) resistance breathing was associated with increased high frequency oscillatory power in arterial pressure, anterior and posterior cerebral blood velocity, and ScO_2.

We conclude that individuals with naturally high tolerance to central hypovolemia exhibit protection of cerebral tissue oxygenation and prolonged preservation of perfusion within the posterior cerebral circulation, but not in the anterior circulation, thus delaying the onset of presyncope. Improved tolerance to central hypovolemia via resistance breathing was not related to these mechanisms, but may have been associated with increased depth of breathing, subsequently decreasing intracranial pressure and increasing cerebral perfusion pressure.

THE ROLE OF CEREBRAL OXYGENATION AND PERFUSION ON TOLERANCE TO CENTRAL HYPOVOLEMIA

DISSERTATION

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By

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PEER REVIEWED PUBLICATIONS

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Kay VL, Rickards CA. *"Reproducibility of Continuous Ramp Lower Body Negative Pressure Protocol for Simulating Hemorrhage."* J. Appl. Physiol., In Preparation, 2015.

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ABSTRACTS & PRESENTATIONS

Rickards CA, **Kay VL**. *"Reproducibility of a continuous ramp lower body negative pressure (LBNP) protocol for simulated hemorrhage."* FASEB J 2015 29:800.7. Experimental Biology, April 2015. [Poster Presentation]

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ABBREVIATIONS:

ACA, anterior cerebral artery

CBF, cerebral blood flow

CO, cardiac output

CPP, cerebral perfusion pressure

CVR, cerebral vascular resistance

DAP, diastolic arterial pressure

dHb, deoxygenated hemoglobin

ECG, electrocardiogram

etCO_2, end tidal carbon dioxide

HbO_2, oxygenated hemoglobin

HCT, hematocrit

HF, high frequency

HR, heart rate

HT, high tolerant

HUT, head-up tilt

ICA, internal carotid artery

ICP, intracranial pressure

ITD, impedance threshold device

ITP, intrathoracic pressure

LBNP, lower body negative pressure

LF, low frequency

LT, low tolerant

MAP, mean arterial pressure

MCA, middle cerebral artery

MCAv, mean middle cerebral artery velocity

MRI, magnetic resonance imaging

MSNA, muscle sympathetic nerve activity

NIRS, near-infrared spectroscopy

$PaCO_2$, arterial partial pressure of carbon dioxide

PaO_2, arterial partial pressure of oxygen

PCA, posterior cerebral artery

PCAv, mean posterior cerebral artery velocity

PS, presyncope

RBC, red blood cell

SAP, systolic arterial pressure

ScO_2, cerebral oxygen saturation

SV, stroke volume

TCD, transcranial Doppler

THC, total hemoglobin concentration

TPR, total peripheral resistance

TTPS, time to presyncope

VA, vertebral artery

VLF, very low frequency

CHAPTER I

REVIEW OF RELATED LITERATURE

Hemorrhage is one of the most common injuries in trauma patients, and is a primary cause of death in both civilian and military trauma (6, 8, 78, 98, 103), with more than 60% of deaths occurring within 6 hours of injury (8, 109). Additionally, 80% of deaths suffered from traumatic hemorrhage on the battlefield are considered potentially survivable (32), with a majority of deaths occurring in the pre-hospital setting (32). A major factor contributing to death and disability from severe blood loss is hypotension and subsequent poor tissue perfusion and oxygenation, especially in the cerebral tissues, which leads to eventual neuronal cell death. In addition, prolonged cerebral hypoperfusion accounts for long-term complications, including cognitive impairment and physical disability (78), so is a primary target for improving survival from hemorrhagic injury.

Hemodynamic Responses to Hemorrhage:

Hemorrhage elicits a state of inadequate tissue perfusion due to loss of blood volume, and is one of the most common causes of hypovolemic shock (i.e., mismatched oxygen supply vs. demand) (35, 101). There are four main classes of hemorrhage that have been identified in conscious mammals, including humans, based on the volume of blood loss and subsequent physiological responses. Class I is associated with up to 15% total blood volume loss, equating

to ≤ 750 ml of blood. This class is often associated with volume loss during blood donation, and physiological responses such as blood pressure and heart rate remain relatively unchanged (35). Class II (15-30% total blood volume loss; 750-1500 ml of blood) is represented by the maintenance of arterial blood pressure due to a reflex sympathoexcitatory responses (99) to protect the vital organs from hypoperfusion. This class is often called the "compensatory stage" as the blood loss activates baroreflex-mediated sympathetic stimulation, resulting in increased heart rate and vasoconstriction of the peripheral vessels to promote shunting of blood to the vital organs such as the heart and brain. This stage is also characterized by decreased pulse pressure (due to the increase in diastolic pressure from increased vascular resistance), and increased respiration rate (1, 35). Once about one third of total blood volume is lost (Class III, 30-40%; 1500-2000 mL), cardiovascular reflexes can no longer adequately compensate to maintain arterial pressure; this class is the "decompensatory phase" of hemorrhage. Decompensation consists of sympatho-inhibition and/or uncoupling between arterial pressure and sympathetic activity (1, 35), resulting in decreased blood pressure, bradycardia (99), and a reduction in cerebral perfusion. Class IV, the most life-threatening of all the classes, is characterized by >40% loss of blood volume (>2000 mL), which represents severe hemorrhage (35), where profound hypotension elicits a robust reflex tachycardia and vasoconstriction leading to the inability to maintain microvascular perfusion (52). Reduced perfusion throughout the microvasculature leads to tissue ischemia, cellular dysfunction and, ultimately, death (51, 117).

Experimental Model of Hemorrhage - Lower Body Negative Pressure (LBNP):

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 (14, 26, 46, 95, 110, 118, 120). This method has also been widely used in aerospace investigations (14) and combat casualty care research (14, 81). Application of LBNP in conscious human subjects allows for the study of hemodynamic responses associated with blood loss, with a reduction in the risks and ethical considerations associated with actual experimental hemorrhage. With this technique, the subject lays supine within the LBNP chamber, and is sealed at the waist to create an airtight seal. Negative pressure is then applied within the chamber, creating a pressure gradient between the upper and lower body, resulting in the redistribution of blood from the upper body into the lower extremities, and inducing "central hypovolemia".

Cooke et al. estimated equivalent blood loss with the magnitude of LBNP, based on hemodynamic responses across multiple independent studies (26). LBNP of 10-20 mmHg was estimated to equate to mild blood loss (400-550 ml; ~10% total blood volume), 20-40 mmHg of LBNP equated to moderate blood loss (550-1000 ml, ~10-20% of total blood volume), and ≥40 mmHg of LBNP equated to severe blood loss (>1000 ml; >20% of total blood volume). Two recent studies extended upon these estimations with actual comparisons between LBNP and blood loss in human and non-human primate models. Hinojosa-Laborde et al. compared up to 25% of total blood volume loss in baboons with LBNP (46). Following the hemorrhage protocol, the magnitude of LBNP applied was designed to match the pulse pressure and central venous pressure responses elicited from the hemorrhage study (46). This design allowed for the assessment of specific levels of LBNP to match increments of blood loss; 6.25% total blood volume loss equated to -22 ± 6 mmHg LBNP, 12.5% total blood volume loss equated to -41 ± 7 mmHg LBNP, 18.75% total blood volume loss equated to -54 ± 10 mmHg LBNP, and 25% total blood volume loss equated to -71 ± 7 mmHg LBNP (46). Arterial pressure, heart rate (HR),

stroke volume (SV), cardiac output (CO), and vascular resistance responses were all comparable between hemorrhage and LBNP. A second study in conscious humans, conducted by Johnson et al., compared stepwise LBNP up to -45 mmHg LBNP (three -15 mmHg levels) to 3 incremental stages of blood loss totaling 1000 ml (55, 88). Although LBNP elicited greater central hypovolemia than hemorrhage, the trajectories of responses for CVP, SV, HR, total peripheral resistance (TPR), and middle cerebral artery velocity (MCAv) were similar between LBNP and blood loss (55, 88). Both of these studies concluded that LBNP elicits similar cardiovascular responses as actual blood loss, therefore validating the use of LBNP as a model for simulating hemorrhage (46, 55). Based upon the results from both of these studies, the estimations of blood loss induced by application of LBNP by Cooke et al. (26) were modestly underestimated.

As with any experimental technique, there are limitations to this approach. Application of LBNP does not mimic all of the responses observed in traumatic hemorrhage, including tissue trauma, pain, changes in hemoglobin and hematocrit, and metabolic responses such as acidosis. The major difference between LBNP and hemorrhage is the effect on hematocrit and hemoglobin. In particular, hematocrit, which is the fraction of whole blood volume comprising red blood cells (RBC), increases during LBNP (46, 57, 118), but decreases during hemorrhage (46, 55). LBNP induces a pressure gradient across the vasculature which essentially moves plasma volume out of the circulation into the interstitial space, causing edema in the lower extremities, and thus increasing the proportion of RBCs within the circulation (i.e., increased hematocrit). During hemorrhage, however, there is loss of whole blood, including RBCs, and movement of fluid from the extravascular space into the circulation in order to counteract the reduced volume of blood; hematocrit decreases, along with the oxygen carrying capacity of the blood (55). Despite these mechanistic differences, LBNP does have the benefit of allowing for

the isolation of physiological responses to central hypovolemia without additional confounding factors.

Conventionally, LBNP is applied in discrete, progressively decreasing steps, with each step lasting anywhere from 2 (65) to 12 minutes (15). A number of studies have reported reproducibility of both tolerance and hemodynamic responses [i.e., HR, systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP)] to stepwise LBNP at various time intervals from 3 days (66) up to 1 year (15). However, application of LBNP in this step-wise fashion may not accurately mimic actual volume loss (i.e., hemorrhage), as the cardiovascular system is able to compensate and stabilize when the negative pressure is held constant at each step. As such, an innovative methodological approach in this investigation is the implementation of a *ramp* LBNP protocol at a constant decompression rate of 3 mmHg/min, to more accurately simulate continuous bleeding. We know of only three previous studies that have utilized ramp LBNP (6, 24, 56), however, *the reproducibility of this approach is currently unknown, specifically, the time to presyncope (i.e., tolerance) and physiological responses, including regional cerebral blood flow and oxygenation* [Specific Aim 1].

Physiological Responses to Central Hypovolemia:

LBNP induces similar reflex physiological responses to actual blood loss as outlined previously. Although these physiological responses are known to occur in individuals in response to central blood loss, the underlying mechanisms, the onset and duration of these responses, and subsequent tolerance to hypovolemia vary between individuals. Consequently, there is considerable variability in survival time following hemorrhagic injuries (9, 105, 106), and in tolerance to LBNP (40, 64, 66). Previous studies have elucidated various factors that

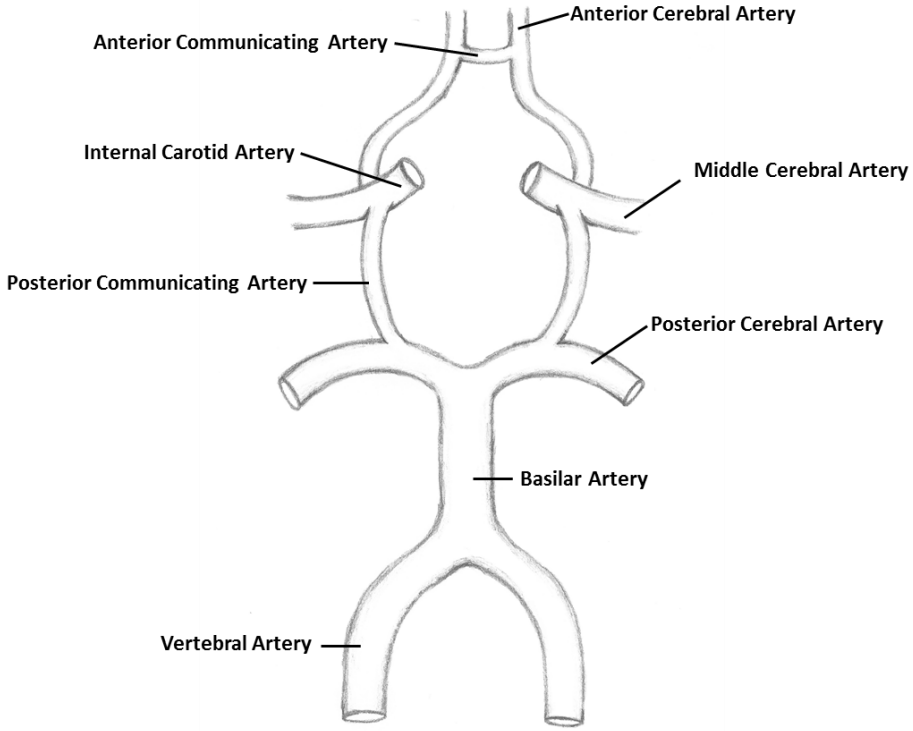
contribute to protection against central hypovolemia, including (a) increased in the release of vasoactive substances (18, 22, 40, 97), e.g., epinephrine, norepinephrine, vasopressin; (b) enhanced sympatho-excitation and vagal withdrawal (18, 40), evidenced by increased peripheral vasoconstriction (48, 64, 112, 118) and tachycardia (21, 40); (c) subsequent protection of CO (64); and (d) maintenance of cerebral autoregulation (69, 114). It is crucial to determine the physiological mechanisms responsible for increased tolerance to severe blood loss, as they may be prime targets for interventions to increase tolerance, and subsequently, improve patient outcomes.

Cerebral Blood Flow and Oxygenation Responses to Central Hypovolemia

1. Cerebral Blood Flow

Perfusion and oxygenation of the cerebral tissues is essential for maintaining consciousness during periods of decreased central blood volume. The brain is a complex vital organ, requiring ~15% of total resting CO, and total cerebral oxygen uptake accounts for ~20% of basal metabolic rate (91). Under conditions of hypotension such as hemorrhage, the proportion of blood directed to the brain increases (33, 58, 80), and is redirected to areas related to control of the cardiovascular and respiratory systems (12). The brain is supplied by two main sets of extracranial arteries, the left and right internal carotid arteries (ICA) and the left and right vertebral arteries (VA), which anastomose within the cranium giving rise to arteries that form the Circle of Willis (Figure 1). The collateral nature of the Circle of Willis within the brain allows for constant cerebral tissue perfusion even when a major feeding vessel is blocked (31). The three major vessels (one on each on the left and right sides) in the intracranium that constitute the Circle of Willis are the middle cerebral artery (MCA), the posterior cerebral artery (PCA), and

the anterior cerebral artery (ACA); Figure 1. The MCA is the largest of the three major intracranial vessels, and supplies blood to the frontal, temporal, and parietal lobes. These regions of the brain are important for motor function, cognition, language/speech, and processing sensory information (29). The vertebral arteries fuse to form the basilar arteries when then feed into the posterior cerebral arteries. The posterior cerebral arteries then supply blood to the cerebellum, brainstem, and occipital lobe, all of which are important for coordination, and vital physiological functions such as sympathetic control, respiration, and visual processing (111).



Complex interrelated regulatory mechanisms control cerebral blood flow (CBF), many of which are challenged under conditions of hypovolemic hypotension. Cerebral tissue is very sensitive to changes in oxygen and nutrient supply (primarily glucose), therefore these regulatory mechanisms aid in the prevention of tissue hypoxia and ultimately, neuronal cell death. During acute hypotension, the baroreflex response increases peripheral systemic sympathetic activity,

resulting in a redistribution of blood to the vital organs, such as the heart and brain. Two main factors affect cerebral blood flow: cerebral perfusion pressure [CPP = MAP – intracranial pressure (ICP)] and cerebral vascular resistance (CVR): CBF=CPP/CVR. As ICP remains fairly constant under most physiological conditions (4), CPP is primarily determined by MAP (85). As such, theoretically, as MAP decreases with hypovolemia, so would the resultant CBF. However, the brain also has the intrinsic ability to maintain *relatively* constant cerebral blood flow over perfusion pressures ranging from approximately 60-160 mmHg (62, 63), a mechanism termed "cerebral autoregulation", by modifying CVR.

While the baroreflex and cerebral autoregulation can maintain MAP and CBF with a hypovolemic stimulus such as hemorrhage, other factors challenge the maintenance of CBF well before the lower limit of cerebral autoregulation is reached. For example, arterial blood gases, sympathetic stimulation, and cerebral metabolism also play key roles in the regulation of the cerebral circulation. Even very small reductions in the partial pressure of arterial carbon dioxide ($PaCO_2$) cause vasoconstriction and a subsequent decrease in CBF, while large reductions in the partial pressure of arterial oxygen (PaO_2) are required before causing vasodilation and increases in CBF. As the cerebral circulation is more sensitive to changes in $PaCO_2$ compared to PaO_2 [CBF only increases under conditions of severe hypoxia (i.e., PaO_2 <60 mmHg) (3)], reductions in CBF induced by central hypovolemia, such as LBNP, are mainly effected by reductions in $PaCO_2$ under normoxic conditions. Willie et al. recently demonstrated differences in the sensitivity of changes in CBF in the extracranial feeding vessels, the ICA and VA, during acute changes in arterial blood gases (119). The VAs feeding the posterior circulation had greater reactivity to hypocapnia than the ICAs feeding the anterior circulation. Autonomic and respiratory control centers are located within the medulla oblongata in the brainstem, which

receives blood and oxygen supply through these posterior cerebral circulation (111). As such, disruption of posterior CBF with hypocapnia may be associated with presyncopal symptoms and hemodynamic dysfunction elicited with central hypovolemia, such as LBNP or hemorrhage.

The role of peripheral autonomic activity during hypovolemia is well known, eliciting reflex increases in HR and systemic vasoconstriction. The role of sympathetic activity on the intracranial vessels in humans, however, is less clear (45, 79, 113). By use of direct nerve recording in the superior cervical ganglion, Cassaglia et al. demonstrated, in lambs, increases in cerebral sympathetic activity (i.e., increased CVR) in response to hypertension (via adrenaline, phenylephrine, or angiotensin II); however, there was no change in sympathetic activity due to a sodium nitroprusside induced drop in MAP by 48% (11). These investigators suggested that increased sympathetic activity during hypertension served as a mechanism to protect the cerebral microcirculation from a dangerous increase in flow. Conversely, a hypotensive stress such as hemorrhage should theoretically decrease CVR, allowing for the maintenance of cerebral perfusion. As the hypotension from hemorrhage induces sympathetic activation, this stress has been suggested to shift the autoregulatory curve to the right (84), subsequently rendering CBF more susceptible to changes in MAP at higher pressures.

Local metabolic demand also plays a crucial role in regulating CBF. The brain is a highly metabolic organ that requires a constant and stable supply of nutrients (i.e., glucose and oxygen) via blood flow. During a hypovolemic stress, when CBF is reduced, there is a potential mismatch between supply and demand of oxygen within the cerebral tissues. To compensate for this mismatch, the cerebral tissues extract more oxygen from the reduced blood supply (65). During physiologically and psychologically stressful events, such as hemorrhage, pain and anxiety may also play a detrimental role in increasing metabolic demand of the tissues, which further

increases oxygen demand. As such, with progressive loss of blood, and increasing metabolic demand of the cerebral tissues, when the oxygen supply can no longer match demand, cognitive impairment and neuronal cell death can result (39, 41).

Transcranial Doppler (TCD) is one of the most common methods for measuring cerebral blood velocity of the intracranial vessels in humans is. A Doppler probe emits ultrasound waves through the skull, and the sound waves are then reflected off the red blood cells within the intracranial vessels back to the transducer (70, 86). The difference in frequency between the transmitted and reflected sound waves is called the "Doppler Shift", which is proportional to the velocity of blood within the insonated vessel (70, 86). Depending on the vessel of interest, the TCD probe is placed in regions of the skull that have thinner bone, termed "acoustic windows". The sound is emitted from the probe at an optimal frequency of 2 MHz; higher frequencies cannot penetrate the skull (30). The transtemporal window, located over the temporal bone of the skull, is used to measure velocities of blood within the ACA, MCA, and PCA. This window is unique in that it is a naturally thin area of the skull with a particular foramina that allows access to the intracranial vessels via TCD.

It is important to note that TCD is used to assess CBF with the assumption that the diameter of the insonated intracranial vessel remains constant. Traditionally, the intracranial cerebral arteries have been seen as conduit vessels that are not altered by variations in perfusion pressure, sympathetic activity, or arterial blood gases. Serrador et al. demonstrated that MCA diameter remains unchanged down to -40 mmHg LBNP using magnetic resonance imaging (MRI) (102). It is unknown, however, if cerebral vasoconstriction occurs at higher levels of LBNP to the point of presyncope. Recently, two studies utilizing higher resolution MRI have challenged the assumption of constant intracranial vessel diameter during acute changes in

$PaCO_2$ (27, 115). Hypercapnia ≥ 9 mmHg above baseline elicits MCA vasodilation, while hypocapnia to ≥ 13 mmHg below baseline elicits MCA vasoconstriction (27, 115). According to Poiseuille's Law, where flow is determined by the fourth power of vessel radius, very small changes in vessel radius can have a large effect on flow. Due to the non-invasive nature of TCD in human cerebral blood flow studies, and the difficulty in directly measuring vessel caliber, it is important to consider the possibility of changing diameters on CBF within these intracranial vessels, and interpretation of the measured parameter of velocity. If diameters of insonated intracranial vessels are dynamic, and change with sympathetic activation or changes in arterial CO_2, a decrease in vessel diameter would result in an increase in velocity, and a subsequent over-estimation of CBF, and vice versa with increased vessel diameter.

While traditionally protection of absolute CBF was thought to be essential in determining tolerance to central hypovolemia (64), recent studies have identified a divergence between tolerance to LBNP and protection of absolute flow [predominantly assessed by MCAv, an index of global cerebral blood flow] (53, 67, 68, 89). Rickards et al. demonstrated that subjects who were either high or low tolerant to central hypovolemia had similar maximal reductions in MCAv at the point of presyncope (89). Similarly, Lucas et al. demonstrated that inhalation of a hypercapnic gas mixture increased cerebral blood velocity, but did not improve tolerance to LBNP (67). Most recently, Ogoh et al. investigated regional CBF under conditions of mild hypovolemia (induced by LBNP up to -50 mmHg), and showed that flow in the ICA was weakly correlated with reductions in central blood volume, while flow through the VA was unchanged. These investigators suggested that flow in the posterior cerebral circulation (indexed by flow in the VAs) 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 ICAs). The effect on tolerance (i.e., high vs. low tolerance), however, was not investigated with these regional differences in CBF. *As such, the role of regional cerebral perfusion on tolerance to central hypovolemia will be examined within this dissertation* [Specific Aim 2].

2. Cerebral Oxygenation

Near infrared spectroscopy (NIRS) is a non-invasive technology that has been used for over 35 years to measure tissue oxygen saturation using a modified version of the Lambert-Beer Law (75, 100). In particular, NIRS is often used to measure oxygen saturation in the cerebral tissues (ScO_2), measuring a mixed sample volume of arterial (20%), capillary (5%), and venous blood (75%) (73, 114). When photons penetrate tissue, their transmission depends on three main factors: reflectance, scattering, and absorption. The latter two factors work as a function of wavelength, which is determined by the molecular properties of the particular tissue being measured. As such, the NIRS probe utilized in the investigations described herein contains eight fiber optic light emitters in pairs at 2.0, 2.5, 3.0, and 3.5 cm from the detector. The emitters emit a certain wavelength of light that is absorbed into the tissue, or scattered; the scattered light is detected by a single detector. Light is emitted at 690 nm and 830 nm into the tissue with a frequency of 110 MHz. These specific wavelengths are used because they are within the near-infrared range that are most effectively transmitted through biological tissue, and absorbed by deoxygenated hemoglobin (dHb, measured at ~690 nm) and oxygenated hemoglobin (HbO_2, measured at 830 nm) (54). The depth of the measurement depends on the distance of the light source (i.e., emitter) from the detector. The most proximal emitter from the detector (2.0 cm) allows for sampling from the shallow tissue, while the most distal (3.5 cm) emitter measures

saturation from the deeper tissues. Theoretically, the use of eight emitters and four progressively increasing emitter-detector distances permits the mathematical exclusion of superficial measurements from skin, fat, and bone, thereby affording more accurate measurements of oxygen saturation from the deeper cerebral tissues (28, 47). ScO_2 is calculated from the HbO_2 and dHb concentrations measured in the frontal cortex by dividing HbO_2 by the total hemoglobin concentration (THC; HbO_2 + dHb). NIRS technology is, however, limited by the fact that ScO_2 can only be measured from the frontal cortex with most monitors, and is therefore not a global representation of oxygen saturation and utilization across all cerebral tissues (36, 73).

Cerebral oxygen saturation monitored with NIRS is often used in the clinical setting as a surrogate for CBF, as it is assumed that reductions in cerebral oxygenation are synonymous with reductions in CBF. Clinically, NIRS is extensively used for the monitoring of neonates (108), during carotid artery endarterectomy (94), head trauma (38), seizures (49), acute heart failure (5), and in the monitoring of blood loss (112). Under conditions of reduced perfusion, the cerebral tissue is very sensitive to changes in oxygen delivery therefore making NIRS-derived measurements of cerebral oxygenation a potentially important clinical monitoring tool. Previous studies have shown that during experimentally induced central hypovolemia via LBNP, CBF decreases with a concurrent decrease in cerebral oxygen saturation (37, 42, 50). However, these reductions are not directly proportional; a 30-50% reduction in CBF is associated with presyncope (64, 68, 89), while only a 10-15% reduction in ScO_2 is associated with presyncopal symptoms and impending loss of consciousness (13, 44, 72).

As perfusion and oxygenation of the cerebral tissues is crucial to maintaining consciousness during central hypovolemia, maintenance of cerebral oxygenation may play a key role in improved tolerance and delayed onset of presyncopal symptomology. While ScO_2

responses have been previously assessed during submaximal LBNP up to -50 mmHg (42), subjects were not taken to presyncope, so there was no examination of the effect of tolerance on ScO_2 responses. In addition to *delivery* of oxygen to the cerebral tissues via CBF, *extraction* of that oxygen may also play a crucial role in tolerance to central hypovolemia, particularly under conditions of reduced delivery. As previously mentioned, the NIRS measure of oxygenation is predominantly from venous blood (73, 114), therefore our lab interprets a decrease in HbO_2, along with an increase in dHb as an increased extraction of oxygen into the tissues. Lewis et al., recently demonstrated that the brain compensates for reductions in CBF, elicited by indomethacin or hypocapnia, by increasing oxygen extraction (determined directly via assessment of cerebral arterial-venous oxygen difference) (65). In addition, reducing the cerebral blood flow reserve (i.e., via indomethacin or hypocapnia) prior to exposure to maximal LBNP did not change tolerance, suggesting that increases in oxygen extraction compensated for the decrease in oxygen delivery; oxygen extraction was not assessed *during* LBNP to directly test this hypothesis (65). Torella et al. also reported increased oxygen extraction (indexed by a decrease in oxygenated hemoglobin in the cerebral venous blood sample measured via NIRS) during mild blood loss in humans (~12% total blood volume) (61). *Thus, presyncope may be associated with a mismatch in oxygen supply and demand in the cerebral tissues* (13, 65), *but this hypothesis has not been explored in relation to tolerance to central hypovolemia.*

3. Hemodynamic Oscillations with Central Hypovolemia:

Traditionally, protection of CBF has been thought to be essential in determining tolerance to central hypovolemia (64). However, recent studies have indicated that the pattern of cerebral blood flow (i.e., oscillations in flow) may be more important than the protection of absolute CBF

(67, 68, 89). These distinct oscillations were first described in arterial pressure following a 25% hemorrhage in dogs by Guyton and Harris (43). Other studies of actual and simulated hemorrhage in both animal and human models have demonstrated similar oscillatory patterns in arterial pressure (82, 83, 104, 125, 126). The mechanisms underlying these oscillations are dependent on the frequency of interest. For example, central hypovolemia has been shown to elicit increases in low frequency (LF; 0.04 - 0.15 Hz) oscillations in muscle sympathetic nerve activity (MSNA), arterial pressure and cerebral blood velocity (25, 34, 59). These oscillations occur spontaneously in this frequency range due to sympathetic modulation of arterial pressure via the baroreflex (25, 57, 93), with subsequent transfer to the cerebral circulation. A number of investigators have reported that increased LF oscillations in mean MCAv and MAP are associated with increased tolerance to central hypovolemia (68, 89). Zhang & Levine (124) postulated that these oscillatory patterns may increase shear stress on the cerebral vessels, such that release of vasodilators (i.e., nitric oxide and histamine) increase vessel diameter, increasing flow, and subsequently, maintain oxygen delivery to the cerebral tissues.

Respiration can also impact LF oscillations if breathing rate is within the range of interest, i.e., 2.4 to 9.0 breaths/min for the LF range of 0.04 and 0.15 Hz. Lucas et al. recently demonstrated this effect by exposing subjects to presyncopal-limited central hypovolemia via head-up tilt plus LBNP while having them breath at a fixed rate of 6 breaths/min (equivalent to 0.1 Hz in the LF range) vs. spontaneous breathing at 16-20 breaths/min (equivalent to 0.27 – 0.33 Hz in the HF range) (68). Similar reductions in absolute CBF were observed under both conditions; however, when breathing within the LF range (i.e., increased MAP and MCAv LF oscillations), subjects exhibited improved tolerance vs. the spontaneous breathing condition (68). In addition, Lucas et al. observed that subjects breathing within the LF range had greater tidal

volume than spontaneous breathing (68). These findings reinforce the notion that LF oscillations in arterial pressure and cerebral blood flow may be protective under conditions of central hypovolemia.

Pulsatile blood flow at other frequencies has also been shown to be protective under conditions of tissue hypoperfusion, such as stroke, cardiac arrest, and severe hemorrhage (2, 7, 96). Whole body periodic acceleration in the head-to-foot direction at frequencies of 3-6 Hz increases blood flow to the cerebral tissues due to release of vasodilators (i.e., nitric oxide, prostaglandins, and histamine) from *pulsatile* shear stress (2). In a hemorrhage model, periodic acceleration at a frequency of 3 Hz (180 cycles/sec) also delayed the onset of shock and preserved regional blood flow in the brainstem and brain cortex (7). In studies of cardiac bypass surgery, pulsatile perfusion at and above the cardiac frequency (≥ 1Hz) has also been shown to optimize microvascular perfusion (measured in the sublingual mucosa) in humans, and decreased neuronal cell damage in a dog model (96). *To date, however, there have been no studies examining the potential role of increased lower frequency oscillations (i.e., < 1 Hz) in cerebral blood flow on protection of both cerebral blood flow and oxygenation.*

Therapeutic Intervention for Prolonging the Therapeutic Window of Treatment

Prolonging the therapeutic window for treatment is imperative for survival from life-threatening events such as hemorrhage, cardiac arrest, and stroke. Optimizing perfusion and oxygenation of the cerebral tissues may prolong this therapeutic window for individuals under hypovolemic stress. As such, inspiratory resistance breathing (via an impedance threshold device, ITD) (90) has been a target interventional strategy for prolonging tolerance to central hypovolemia.

During normal inspiration, intrathoracic pressure (ITP) decreases which results in increased venous return, ventricular preload, and CO (17). The ITD has a rubber valve and spring mechanism that requires additional inspiratory effort (-7 cm.H_2O, "cracking pressure") before it opens to allow inflow of air. This additional inspiratory resistance enhances reductions in ITP upon inspiration, which then augments venous return to the heart (20), and also reduces ICP (121, 122). This amplified venous return and CO, increases arterial pressure, which subsequently reduces ICP and increases CPP (121, 122). Numerous studies have demonstrated that inspiratory resistance increases survival time from severe hemorrhage and cardiac arrest in swine (121-123), increases tolerance to central hypovolemia in healthy conscious humans (19, 20, 76, 90), may decrease adverse effects of traumatic brain injury (60) and improves blood pressure in hypotensive patients secondary to trauma (116).

Animal studies allow for actual controlled bleeding experiments along with invasive measurements of ICP and endotracheal pressures (a surrogate for ITP), which cannot be performed in a healthy human model of blood loss. Yannopoulos et al. performed a study in swine to examine the effects of resistance breathing under normovolemic and hypovolemic conditions (121). Normovolemic pigs spontaneously breathing through an ITD with a cracking pressure of -10 mmHg (~14 cm H_2O) for 15 minutes, showed increases in MAP, CPP, and right atrial pressure, along with decreases in ICP (121). Similarly, animals bled up to 50% of their total blood volume and subjected to resistance breathing [-10 mmHg (~14 cm H_2O) valve cracking pressure], showed decreases in ICP, and increases in MAP and CPP (121). Interestingly, the attenuated decrease in MAP and decrease in ICP were linearly correlated with blood loss and valve cracking pressure (i.e., the greater the blood loss and cracking pressure, the higher the MAP and lower the ICP) (121). Sigurdsson et al. bled pigs up to 50% of their total

blood volume, and compared a control vs. treatment group with resistance breathing for 90 minutes (107). This study demonstrated that resistance breathing decreased ICP and increased MAP compared to the control group, which subsequently improved short term survival (107). Investigators using animal models of hemorrhage have concluded that resistance breathing improves hemodynamics (71, 77, 122) and short-term survival rates (107) following central hypovolemia.

Current treatment of hypotension from all causes, including hemorrhage, is most often treated with fluid resuscitation. Metzger et al. compared normal saline fluid resuscitation (1 liter) with resistance breathing (-7 cm H_2O) in a swine model following hemorrhage (55% of total blood volume was removed) (77). Resistance breathing increased systolic blood pressure back to baseline levels, while conventional fluid resuscitation increased systolic blood pressure higher than baseline, which could potentially lead to clot disruption, hemodilution, and increased ICP (77), all of which are negative consequences with hemorrhagic injuries. In summary, resistance breathing following hemorrhage in animal studies maintains arterial pressure and CPP without compromising hemostasis.

A number of studies have also been conducted in humans demonstrating the benefits of resistance breathing under various conditions. Spontaneous breathing through an ITD increases SV, CO, and arterial pressure in healthy humans who are normotensive and normovolemic (17, 23). Furthermore, resistance breathing increases CBF in resting supine humans (23), but does not protect CBF under hypovolemic conditions (87, 90). Although CBF is not protected with resistance breathing, Rickards et al. demonstrated increased HF oscillations in cerebral blood flow velocity during an acute hypotensive orthostatic challenge induced by a squat-stand test (87). Convertino et al. also demonstrated the protective effects of resistance breathing during

head-up tilt (HUT) induced hypotension (i.e., 25% reduction in stroke volume), with attenuated reductions in SV and CO, and decreased TPR (16). Previous studies have also shown that LBNP tolerance increases with resistance breathing, associated with the protection of SV, CO, and arterial pressure (17, 23, 92). In this study with LBNP, although CBF was not protected via resistance breathing, LF oscillatory power of CBF was enhanced, thus delaying the reporting of presyncopal symptoms (90). This protective effect may be due to increases in CPP via reductions in ICP (121), and/or greater *induced* LF oscillations in cerebral blood flow causing the release of local vasodilators that could enhance tissue perfusion and oxygenation (89, 90). *The role of resistance breathing on cerebral oxygenation and posterior cerebral circulation with central hypovolemia has not been explored* [Specific Aim 3].

Since hemorrhagic shock is associated with poor survival (74), early recognition of blood loss, and early intervention is critical in order to minimize end organ dysfunction, morbidity, and mortality (10). *The studies described herein will investigate the role of* ***endogenous*** *oscillations* (Specific Aim 2B) *versus* ***induced*** *oscillations that are created using inspiratory resistance breathing* (Specific Aim 3B) *on cerebral oxygenation and regional cerebral perfusion under conditions of experimentally induced central hypovolemia.*

SPECIFIC AIMS

Our long-term goal is to delineate the physiological mechanisms underlying tolerance to central hypovolemia, focusing on regulation of cerebral perfusion and oxygenation. The **objectives** of this study are to determine 1) whether the protection of cerebral oxygenation (ScO_2) is a key determinant in tolerance to central hypovolemia, rather than protection of absolute cerebral blood flow; 2) if the oscillatory pattern of cerebral blood flow is important for the delivery and maintenance of oxygen to the cerebral tissues under conditions of reduced perfusion; and 3) if there is a difference in regional perfusion during central hypovolemia, with protection of the posterior circulation playing a role in delaying the onset of presyncope.

Specific Aim 1: Determine if tolerance time and physiological responses to a continuous ramp LBNP protocol are reproducible.

We hypothesized that tolerance time and physiological responses to *continuous* LBNP applied at a rate of 3 mmHg/min would be similar when examined by separate trials ≥4 weeks apart.

Specific Aim 2: Define the roles of cerebral oxygenation (ScO_2), the pattern of cerebral blood flow (CBF), and regional cerebral perfusion on tolerance to central hypovolemia.

Aim 2A: To test the hypothesis that tolerance to central hypovolemia is associated with protection of ScO_2, despite absolute reductions in cerebral blood flow.

Aim 2B: To test the hypothesis that protection of ScO_2 is associated with increased endogenous low frequency (LF) oscillations in CBF.

Aim 2C: To test the hypothesis that increased tolerance to central hypovolemia is associated with maintenance of posterior cerebral circulation.

Specific Aim 3: Explore the role of resistance breathing on cerebral oxygenation (ScO_2), the pattern of cerebral blood flow (CBF), and regional cerebral perfusion associated with improved tolerance to central hypovolemia.

Aim 3A: To test the hypothesis that inspiratory resistance breathing improves tolerance to central hypovolemia by maintaining ScO_2, despite reductions in CBF.

Aim 3B: To test the hypothesis that by *inducing* LF oscillations in CBF via inspiratory resistance breathing, tolerance to central hypovolemia is increased via maintenance of ScO_2.

Aim 3C: To test the hypothesis that inspiratory resistance breathing protects posterior cerebral circulation resulting in improved tolerance to central hypovolemia.

EXPERIMENTAL DESIGN

Healthy human subjects were recruited for each of the experiments described herein. Every subject was required to visit the laboratory a total of 4 times for one familiarization session, and three experimental sessions. Experimental sessions were performed at a minimum of 1-month intervals in order to avoid increased LBNP tolerance from multiple exposures, and to ensure all female subjects were tested within the early follicular phase (days 1-4) of their menstrual cycle. Due to the potential effect 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 each experiment. Subjects were also encouraged to stay hydrated the day before each study. Experiments were conducted at the same time of day (morning) in a temperature controlled laboratory (22-24^{0}C).

PROTOCOL:

<u>Day 1</u>: Familiarization

Subjects were evaluated to ensure they met all of the inclusion criteria for the study, including: aged between 18-45 years, non-smoking, normotensive (systolic blood pressure <140 mmHg; diastolic blood pressure <90 mmHg), normal 12-lead ECG, normal clinical results from a medical exam, body mass index (BMI) ≤30 unless athletic/muscular build, and all females underwent a urine pregnancy test to ensure they were not pregnant. Exclusion criteria were: subjects who smoked, other nicotine use, females who were pregnant or post-menopausal, use of prescription or non-prescription drugs (including anti-hypertensive medications, beta blockers,

bronchodilators), a history of hyperthyroidism, atherosclerosis, respiratory illness or pulmonary disease, or concussion, signs of cardiovascular or cerebrovascular abnormalities, known or suspected abdominal hernia, and BMI >30 unless athletic/muscular build. During the familiarization session, the subject was shown the monitoring equipment to be used during the protocol, and met the personnel running the experiments. Subjects also had the opportunity to lie in the LBNP chamber, which was turned on for 5 minutes so they could become familiar with the noise and pressure. Subjects also breathed through the ITD, to become familiar with the facemask and sensation of inspiratory resistance prior to participation in the experiment.

Prior to inclusion in the project, each subject was verbally briefed about the study, including all risks, and they provided voluntary, written informed consent to participate. In addition, each subject underwent a medical screening protocol (i.e., blood pressure, 12-lead ECG, and medical history) which was then reviewed and approved by a physician prior to inclusion in the study.

<u>Day 2</u>: "BASELINE" Maximal Lower Body Negative Pressure (LBNP) Protocol

This first experimental session was used to assess the subjects' baseline tolerance to a presyncopal-limited LBNP protocol. Upon arrival to the laboratory, subjects were encouraged to empty their bladder to ensure optimal comfort during the experiment and to avoid potential confounding effects on sympathetic nervous system activity. The subject was positioned inside of the LBNP chamber in the supine position, with their iliac crest in line with the opening of the chamber. Physiological measurements and instrumentation during the baseline experiment, and each subsequent experimental protocol included:

1) Heart rate via lead II electrocardiogram (ECG; shielded leads, cable and amplifier, AD Instruments, Bella Vista, NSW, Australia)
2) Non-invasive beat-to-beat arterial blood pressure and stroke volume via finger photoplethysmography (Finometer™, Finapres Medical Systems, Amsterdam, The Netherlands)
3) Cerebral blood velocity in the middle cerebral artery (MCAv) and posterior cerebral artery (PCAv) via transcranial Doppler (TCD) ultrasound (2 MHz probes; ST3, Spencer Technologies, Seattle, WA)
4) Cerebral oxygenation saturation (ScO_2), oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (dHb), and total hemoglobin concentration (THC; HbO_2 + dHb) of the frontal cortex via near-infrared spectroscopy (NIRS) (OxiplexTS, ISS Inc., Champaign-Urbana, IL)
5) Respiration rate and end-tidal carbon dioxide ($etCO_2$) via capnography (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW, Australia)
6) Impedance threshold device (ITD, ResQGARD7®, Advanced Circulatory Systems Inc., Roseville, MN) that was attached to a custom adapter (Hans Rudolph Inc., Shawnee, KS) on a facemask (7940 Series, Hans Rudolph Inc., Shawnee, KS)
7) Inspiratory and expiratory pressures were directly recorded from the facemask using a pressure transducer (Digimano 1000, Netech Corporation, Farmingdale, NY)
8) Catheterization of an antecubital vein for assessment of hematocrit from a whole blood sample

Following instrumentation and a 5-10 min stabilization period, 5-min of baseline measurements began. During this time, a blood sample was drawn for post-experimental assessment of hematocrit to ensure adequate hydration of all subjects (i.e., male: 40-55%; female: 35-50%). LBNP then commenced at a decompression rate of 3 mmHg/min. During LBNP, application of negative pressure to the lower body decreases venous return and preload, which in turn reduces stroke volume, cardiac output, and cerebral blood flow (14, 26, 95, 120). This stimulus leads to the eventual onset of presyncopal symptoms, defined by one or more of the following criteria: 1) a rapid fall in SAP >15 mmHg; 2) diminished SAP below 80 mmHg; 3) sudden, relative bradycardia; 4) 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. The chamber pressure was released 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. All instrumentation was then removed and the subject was helped out of the chamber.

Day 3 and 4: "CONTROL" or "ITD" LBNP Protocol (Randomized, Cross-over Design)

When subjects returned to the laboratory for their second LBNP exposure, they participated in either the control LBNP protocol **OR** the LBNP plus ITD protocol (randomized, cross-over design to account for the possible order effect of the ITD intervention protocol always following the control protocol).

1) **Control LBNP Protocol**:

The control protocol was identical to the baseline protocol described previously. Subjects were exposed to progressively increasing LBNP at a rate of 3 mmHg/min until the onset of presyncopal symptoms.

2) **LBNP + ITD Protocol**:

Subjects were exposed to progressively increasing LBNP at a rate of 3 mmHg/min, but 5-min prior to the pre-determined time of presyncope (determined from baseline protocol on day 1) or with a 30% reduction in stroke volume from baseline (whichever is first), the ITD was placed on the facemask, and subjects were instructed to breathe spontaneously at a rate and depth most comfortable to them. Application of LBNP continued at the same rate (3 mmHg/min) until the onset of presyncopal symptoms.

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CHAPTER II

Reproducibility of Continuous Ramp Lower Body Negative Pressure Protocol for Simulating Hemorrhage

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ABSTRACT:

Central hypovolemia elicited by application of lower body negative pressure (LBNP) has been used extensively to simulate hemorrhage in human subjects. Conventional LBNP protocols include progressive, discrete steps in pressure held for specific time intervals. The aim of this study was to assess the reproducibility of applying graded LBNP at a constant rate of progression until presyncope to replicate actual bleeding. During two trials, at least 4 weeks apart, progressive LBNP was applied at a rate of 3 mmHg/min in 18 healthy human subjects (12M; 6F) until the onset of presyncopal symptoms. Heart rate (HR), mean arterial pressure (MAP), stroke volume (SV), total peripheral resistance (TPR), mean middle cerebral artery velocity (MCAv), and cerebral oxygen saturation (ScO_2) were measured continuously. Time to presyncope (TTPS) and hemodynamic responses were compared between the two trials. TTPS (1649 ± 98 s vs. 1690 ± 88 s; P=0.47 (t-test); r=0.77) and the subsequent magnitude of central hypovolemia (%Δ SV -54 ± 4 % vs. -53 ± 4 %; P=0.55) were similar between trials. There were no statistically distinguishable differences between either baseline ($P \geq 0.17$) or presyncopal values for HR, MAP, TPR, mean MCAv, or ScO_2 ($P \geq 0.19$). The rate of change from baseline to presyncope for all hemodynamic responses was also similar between trials ($P \geq 0.12$). Continuous LBNP applied at a rate of 3 mmHg/min was reproducible in healthy human subjects, eliciting similar reductions in central blood volume and subsequent reflex hemodynamic responses.

INTRODUCTION:

Lower body negative pressure (LBNP) has been extensively utilized as an experimental technique to induce central hypovolemia and simulate hemorrhage in healthy, conscious humans (3, 7, 12, 21, 23-25). It is well known that the progressively increasing LBNP results in decreased venous return, SV, cardiac output (CO), and mean arterial pressure (MAP), stimulating sympathetically-mediated increases in HR and systemic vascular resistance (5, 7, 25). LBNP also results in reductions of mean middle cerebral artery velocity (MCAv) and cerebral oxygenation (ScO_2), which ultimately leads to presyncopal symptomology such as dizziness, nausea, and visual disturbances (8, 9, 11, 13, 17). Conventionally, LBNP is applied in discrete, progressively decreasing steps, with each step lasting anywhere from 2 (18) to 12 minutes (4). However, application of LBNP with this step-wise approach may not accurately mimic actual volume loss (i.e., hemorrhage), as the cardiovascular system is able to compensate and stabilize when the negative pressure is held constant. In order to more accurately simulate *continuous* bleeding, we have implemented a ramp pressure profile with application of negative pressure at a continuous decompression rate of 3 mmHg/min.

Ramp LBNP profiles have been utilized in very few studies to date (1, 6, 15). In these studies, continuous decompression elicited similar hemodynamic responses (i.e., reductions in MAP, SV, CO, MCAv, and increased HR) (1, 6, 15) as those observed during step-wise LBNP profiles, but the reproducibility of these responses has not been reported. In contrast, a number of studies have assessed the reproducibility of not only tolerance to LBNP, but also the hemodynamic responses (3, 14, 16, 20). These investigators concluded that tolerance and hemodynamic responses to step-wise LBNP was reproducible within subjects tested at varying time intervals from 3 days (20) up to 1 year (3). As ramp pressure profiles may be utilized as a method to assess hemodynamic responses associated with continuous bleeding, it is important to

determine the reproducibility of this experimental technique. Therefore, we tested the hypothesis that tolerance time and physiological responses to continuous LBNP applied at a rate of 3 mmHg/min would be reproducible in a cohort of young, healthy human subjects.

METHODS:

Subjects

Twenty-seven 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 electrocardiogram (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. All female subjects were tested in the early follicular phase of their menstrual cycle (days 1-4), determined by self-report. 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^{o}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 CO_2 ($etCO_2$) were measured on a breath-by-breath basis through a facemask (7940 Series, Hans Rudolph Inc., Shawnee, KS) 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). Oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (dHb), total hemoglobin concentration (THC; HbO_2 + dHb) and ScO_2 [(HbO_2/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 underwent two identical experimental sessions separated by at least one month, designated as Trial 1 and Trial 2. These repeated trials were part of a larger study reported elsewhere (Kay & Rickards, in review; Chapter 3), so 6 subjects were exposed to an additional LBNP protocol with an acute intervention in between Trials 1 and 2 described in the present investigation; Trial 2 was always conducted at least one month following this protocol. The maximal LBNP protocol consisted of a 5-min baseline period followed by continuous application of negative pressure at a decompression rate of 3 mmHg/min until the onset of presyncope, determined by one or more of the following criteria: 1) 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, ScO_2, THC, $etCO_2$) 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 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 and mean MCAv and PCAv were automatically calculated as the area under the arterial pressure and cerebral blood velocity waveforms via the WinCPRS software. CO was calculated as the product of HR and SV; cerebrovascular resistances (CVR) in the MCAv and PCAv were calculated as MAP divided by MCAv and PCAv, respectively. Total peripheral resistance (TPR) was calculated as MAP divided by CO.

Statistical Analysis

All variables were analyzed from the final 4-min of each 5 min interval of continuous LBNP. In addition, to compare physiological responses between Trial 1 vs. Trial 2 at presyncope, data was analyzed during the final 1-min prior to presyncope (PS-1). Pearson correlations were used to explore the relationship between time to presyncope (TTPS) between each trial, and all of the measured hemodynamic parameters during each LBNP exposure. Paired t-tests were also used to compare TTPS between trials, and the rates of change for all hemodynamic responses (per mmHg LBNP, and per min) between trials. Two-way repeated measures analyses of variance (ANOVAs) were used to compare baseline and presyncopal hemodynamic responses across trials, followed by Tukey *post-hoc* tests. Absolute and percentage changes from baseline values are reported for the key variables of interest. Outliers were identified via Grubb's test [the extreme studentized deviate (ESD) method] and removed from subsequent analysis. All data are presented as mean ± SE (unless otherwise stated), and exact P-values are reported for all comparisons.

RESULTS:

LBNP tolerance

Of the 27 subjects who completed both experimental trials, data was only analyzed and included for 18 subjects who reached true presyncope, defined as mean SAP < 100 mmHg for the 1-min prior to presyncope, and/or minimum SAP ≤ 90 mmHg within the 1-min prior to presyncope. One subject was identified as an outlier via the Grubb's test, and was excluded from analysis. There was no difference (P=0.47) in time to presyncope between Trial 1 (1649 ± 98 sec) and Trial 2 (1690 ± 88 sec; slope = 1.01; r=0.77) (Figure 1). Maximal LBNP pressures at presyncope were also linearly associated (slope=1.01; r=0.74) between Trial 1 (-68 ± 5 mmHg) and Trial 2 (-70 ± 4 mmHg; P=0.47). Of the 6 subjects who participated in an additional LBNP protocol in between Trials 1 and 2 (as previously described), tolerance was not systematically higher in subjects exposed to 3 vs. 2 LBNP protocols (P=0.46). The minimum time between Trial 1 and 2 was 30 days, the maximum time was 119 days, and the average time between trials was 57±7 days. In addition, each subject exhibited similar subjective presyncopal symptomology between trials (i.e., blurred vision, sweating, nausea, dizziness).

Cardiovascular Responses to LBNP:

There were no statistically distinguishable differences in HR, MAP, ScO_2, MCAv, PCAv and $etCO_2$ between trials at baseline (P≥0.17) or in the maximal responses at presyncope (P≥0.19; Table 1). As shown in Figure 2 subjects also exhibited similar relative reductions in SV (P=0.40), CO (P=0.34), and ScO_2 (Trial 1: -6.9 ± 1%; Trial 2: -6.7 ± 1%; P=0.72), and increases in TPR (P=0.40) at presyncope. As demonstrated in Figures 2-4, all hemodynamic measurements of interest followed similar trajectories throughout LBNP for both trials; these

responses consistently exhibited high linear associations for all physiological responses (Table 2). The data presented in Table 3 demonstrates that there were no statistically distinguishable differences in any of the measured parameters for rate of change per minute of the LBNP protocol ($P \geq 0.12$), or the rate of change per mmHg of LBNP ($P \geq 0.12$).

DISCUSSION:

In this study we examined the reproducibility of continuously applying LBNP to presyncope at a rate of 3 mmHg/min in a cohort of young, healthy human subjects. The key findings demonstrate that 1) TTPS is reproducible with application of ramp-LBNP at a decompression rate of 3 mmHg/min; 2) maximal SV reduction (50-55%) was similar between trials; and 3) all other reflex physiological responses were highly reproducible. As there were no statistically distinguishable differences between either baseline or presyncopal values for any of the hemodynamic parameters explored in this study, subjects appeared to be in a similar physiological state at rest, and the presyncopal state is represented by reproducible physiological responses.

While a number of studies have assessed the reproducibility of the traditional step-wise LBNP pressure profile (3, 14, 20), none, to our knowledge, have examined the reproducibility of a continuous ramp-LBNP pressure profile. Since the introduction of LBNP as a research tool in the 1960s (2, 22), many laboratories have adopted this technique for the investigation of physiological responses to variations in central blood volume, using both cross-sectional and interventional experimental designs. The majority of investigators utilize step LBNP protocols, but vary the profiles in terms of the magnitude and length (time) of each pressure step, and the termination point, which is generally limited by either the subject (i.e., pre-syncope or

discomfort), or the physical capability of the LBNP chamber (i.e., maximum pressure). In those investigations that have explored the reproducibility of step-LBNP, different pressure profiles have been used, and the time separation between repeated LBNP exposures has varied, from days up to a year. Howden et al. tested the reproducibility of a step-wise pressure profile in subjects who underwent LBNP to tolerance on 3 occasions, each separated by 72-120 hours (3-5 days) (14). These investigators demonstrated that there were no differences in HR, or arterial pressure (SAP, DAP) responses during maximal LBNP to presyncope ($P \geq 0.31$) when retesting subjects within 72-120 hours (14). However, there was a difference in tolerance, assessed via calculation of the LBNP tolerance index (LTI, ΔmmHg*min), or the cumulative stress index (CSI, mmHg*min); Trial 1 and 2 were identical, but Trial 2 to 3, and Trial 1 to 3 differed (14). Conversely, other investigators have shown that tolerance does not change between four LBNP exposures separated by at least 72 hours (TTPS in min, P=0.85) (20), or even up to 1 year (Year 1: 46.8 ± 1.0 min vs. Year 2: 46.6 ± 1.4 min; r=0.94) (3). Lightfoot et al. did demonstrate, however, that the reproducibility of LBNP tolerance improves with repeated exposures, and exposures closer in time; r=0.71 for test 1 vs. test 2, compared with r=0.97 for test 2 vs. test 3, and r=0.93 for test 3 vs. test 4 (20). The findings from our study show similar reproducibility using the ramp pressure profile (r=0.77), where tolerance to two repeated presyncopal-limited LBNP exposures (indexed by TTPS) was assessed in the same subjects separated by at least one month (Range: 30-119 days). In agreement with previous studies utilizing step LBNP protocols (3, 14, 20), we also demonstrated the reproducibility of HR, SAP, DAP, and MAP responses. The present study, however, is novel in also demonstrating the reproducibility of MCAv, PCAv, and ScO_2 responses, which has not been assessed during any LBNP protocol. Continued use of

ramp LBNP for investigation of cerebral blood velocity and oxygenation responses to experimental central hypovolemia is warranted based on these findings.

Adaptation to LBNP and variability in tolerance to central hypovolemia are important factors to consider when subjecting individuals to repeated exposures of presyncopal-limited LBNP. Multiple studies have shown that tolerance to LBNP is variable, such that subjects become presyncopal at different magnitudes of central hypovolemia (10, 17, 20). Although tolerance may vary from subject to subject, tolerance within an individual subject over multiple exposures of LBNP appears to be similar. The time between multiple LBNP exposures is also an important consideration when designing studies using this technique. Lightfoot et al. explored this factor by exposing subjects to repeated step-wise presyncopal-limited LBNP every day for 9 days, with no LBNP exposure on days 5 or 6 (19). LBNP tolerance progressively increased over the course of 9 daily LBNP exposures, by a maximum of 49%, and an increase in tolerance duration of 6.5 minutes; even with a 2 day break, LBNP tolerance remained at day 5 levels (19). Lightfoot et al. speculated that repeated exposures to a central hypovolemic stress may cause acute resetting of the baroreflex, allowing for more effective cardiovascular compensation during subsequent hypotensive stress, therefore leading to improved tolerance (19). These investigators also concluded that more than 2 days should intervene between repeated LBNP exposures, to avoid the risk of cardiovascular adaptation; we are confident that the minimum one month interval used in the present study was sufficient to avoid any physiological adaptation. Future studies could be performed to compare the reproducibility of ramp-LBNP with various lengths of time between exposures, and across more than two trials.

Conclusions:

The findings of this study indicate that ramp LBNP applied at an onset rate of 3 mmHg/min is reproducible in terms of tolerance time and hemodynamic responses, and therefore can be used as a reliable method for assessment of cardiovascular and cerebrovascular responses to central hypovolemia. The continuous nature of the decompression profile may more accurately simulate actual blood loss, although direct comparison of responses to actual hemorrhage versus continuous LBNP is required to adequately address this hypothesis.

Table 1: Comparison of physiological responses between baseline and presyncope (PS-1) within and between trials of presyncopal limited lower body negative pressure at a decompression rate of 3 mmHg/min.

	Trial 1		Trial 2		Trial 1 vs Trial 2 P-Values	
	Baseline	**PS-1**	**Baseline**	**PS-1**	**Baseline**	**PS-1**
HR (bpm)	60.8 ± 2.5	108.2 ± 6.7*	59.4 ± 1.4	106.3 ± 6.5*	0.70	0.60
MAP (mmHg)	99.0 ± 1.9	76.6 ± 1.6*	96.9 ± 1.6	77.2 ± 1.1*	0.17	0.66
SV (% Δ)	-	-54.0 ± 3.6*	-	-52.5 ± 3.6*	-	0.40
CO (% Δ)	-	-22.5 ± 3.7*	-	-20.7 ± 1.8*	-	0.34
TPR (% Δ)	-	4.2 ± 5.4	-	1.5 ± 2.9	-	0.40
$etCO_2$ (mmHg)	41.0 ± 1.1	28.4 ± 1.7*	41.7 ± 1.1	29.3 ± 1.9*	0.69	0.56
MCAv (cm/s)	64.4 ± 3.1	44.3 ± 2.6*	63.1 ± 3.5	47.0 ± 3.1*	0.50	0.19
PCAv (cm/s)	40.7 ± 2.4	30.8 ± 1.7*	41.1 ± 2.3	31.4 ± 2.2*	0.70	0.75
ScO_2 (%)	67.3 ± 1.7	62.7 ± 1.6*	67.0 ± 1.6	62.5 ± 1.6*	0.69	0.79

Data are presented as means ± SE. HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; $etCO_2$, end tidal carbon dioxide; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity, ScO_2, cerebral oxygen saturation. Baseline and presyncopal (PS-1) responses were compared between Trial 1 and Trial 2 (Trial 1 vs. Trial 2 P-Values). *: $P<0.001$ between baseline and PS-1 within a trial.

Table 2: Correlation data for physiological responses between Trial 1 and Trial 2 with continuous application of lower body negative pressure at a decompression rate of 3 mmHg/min.

Parameter	Slope	Correlation Coefficient (r)	P-Value
HR (beats/min)	1.07	0.99	P<0.001
MAP (mmHg)	1.16	0.99	P<0.001
SV (% Δ)	1.03	0.99	P<0.001
CO (% Δ)	1.12	0.98	P<0.001
TPR (% Δ)	0.85	0.83	P=0.02
etCO_2 (mmHg)	0.89	0.97	P<0.001
MCAv (cm/s)	1.09	0.99	P<0.001
PCAv (cm/s)	1.02	0.99	P<0.001
ScO_2 (% Δ)	1.06	0.98	P<0.001

Data are presented as means ± SE. HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; etCO_2, end tidal carbon dioxide; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity, ScO_2, cerebral oxygen saturation.

Table 3: Rate of change (per minute and per mmHg LBNP) from baseline to presyncope for all physiological variables for the two trials.

	Rate of Change (per min)			**Rate of Change (per mmHg)**		
Parameter	***Trial 1***	***Trial 2***	***P-Value***	***Trial 1***	***Trial 2***	***P-Value***
HR (bpm)	1.61 ± 0.15	1.56 ± 0.18	0.71	0.65 ± 0.06	0.63 ± 0.07	0.63
MAP (mmHg)	-0.85 ± 0.09	-0.73 ± 0.07	0.15	-0.36 ± 0.04	-0.30 ± 0.03	0.13
SV (% Δ)	-1.98 ± 0.11	-1.84 ± 0.09	0.19	-0.82 ± 0.05	-0.75 ± 0.04	0.16
CO (% Δ)	-0.86 ± 0.16	-0.75 ± 0.07	0.30	-0.36 ± 0.07	-0.31 ± 0.03	0.25
TPR (% Δ)	0.17 ± 0.22	0.03 ± 0.10	0.47	0.07 ± 0.09	0.01 ± 0.04	0.46
$etCO_2$ (mmHg)	-0.47 ± 0.06	-0.43 ± 0.05	0.61	-0.19 ± 0.03	-0.17 ± 0.02	0.53
MCAv (cm/s)	-0.72 ± 0.08	-0.56 ± 0.08	0.12	-0.30 ± 0.04	-0.23 ± 0.04	0.12
PCAv (cm/s)	-0.35 ± 0.08	-0.35 ± 0.01	0.99	-0.14 ± 0.04	-0.14 ± 0.01	0.92
ScO_2 (%)	-0.16 ± 0.02	-0.15 ± 0.02	0.64	-0.07 ± 0.01	0.06 ± 0.01	0.53

Data are presented as means ± SE. HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; $etCO_2$, end tidal carbon dioxide; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity, ScO_2, cerebral oxygen saturation.

FIGURE LEGENDS

Figure 1 **Panel A:** Correlation between time to presyncope for two trials of a ramp lower body negative pressure (LBNP) protocol (3 mmHg/min); solid line represents line of identity. **Panel B:** Mean time to presyncope for Trial 1(1649 ± 98 sec; filled bar) and Trial 2 (1690 ± 88 sec; open bar) (P=0.47).

Figure 2 Percent change from baseline responses for stroke volume (SV, **Panel A**), cardiac output (CO, **Panel B**), and total peripheral resistance (TPR, **Panel C**) to a presyncopal-limited lower body negative pressure (LBNP) protocol for Trial 1 and Trial 2.

Figure 3 Heart rate (HR, **Panel A**) and mean arterial pressure (MAP, **Panel B**) responses to a presyncopal-limited lower body negative pressure (LBNP) protocol for Trial 1 and Trial 2.

Figure 4 Mean middle cerebral artery velocity (MCAv, **Panel A**), mean posterior cerebral artery velocity (PCAv, **Panel B**), cerebral oxygen saturation (ScO_2, % change from baseline, **Panel C**), and end tidal carbon dioxide ($etCO_2$, **Panel D**) responses to a presyncopal-limited lower body negative pressure (LBNP) protocol for Trial 1 and Trial 2.

Figure 1

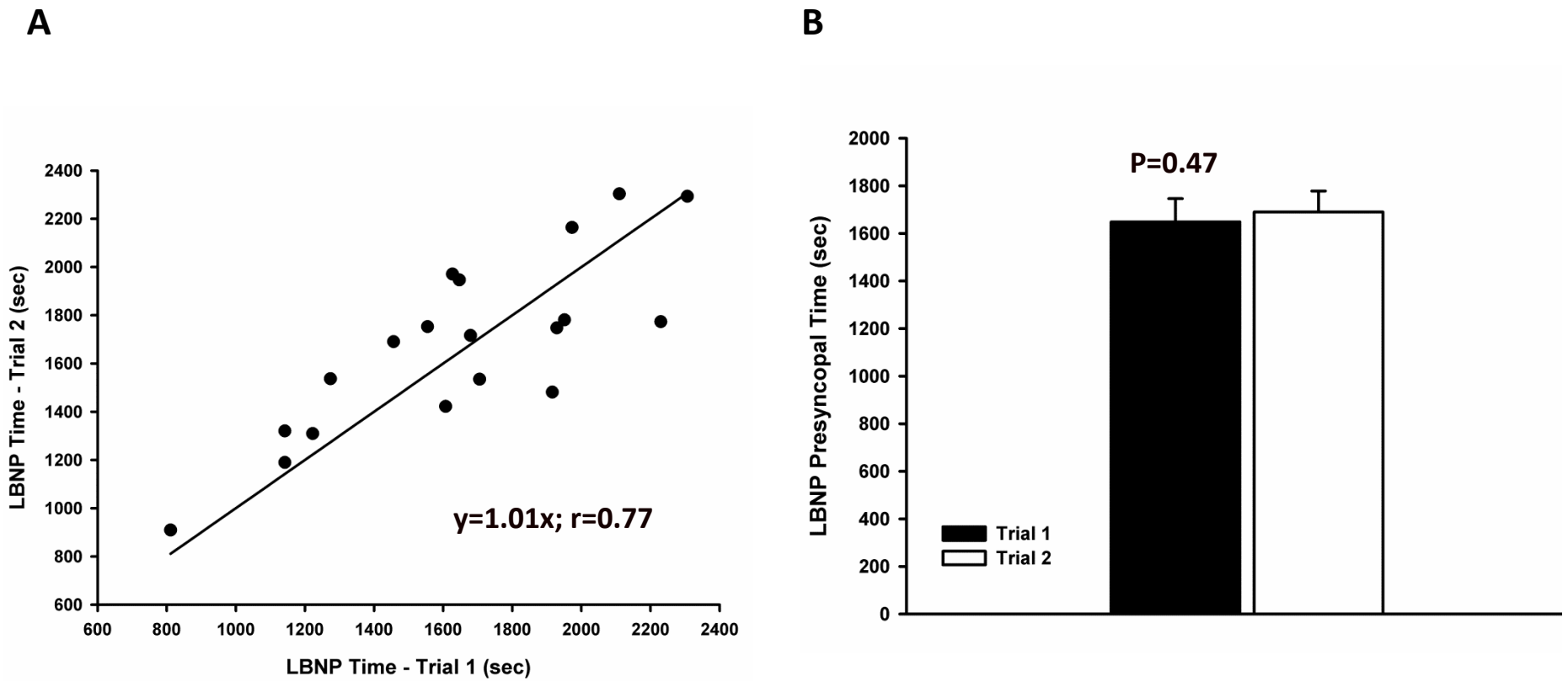

A
B
LBNP Time - Trial 2 (sec)
LBNP Time - Trial 1 (sec)
y=1.01x; r=0.77
LBNP Presyncopal Time (sec)
P=0.47
Trial 1
Trial 2

Figure 2

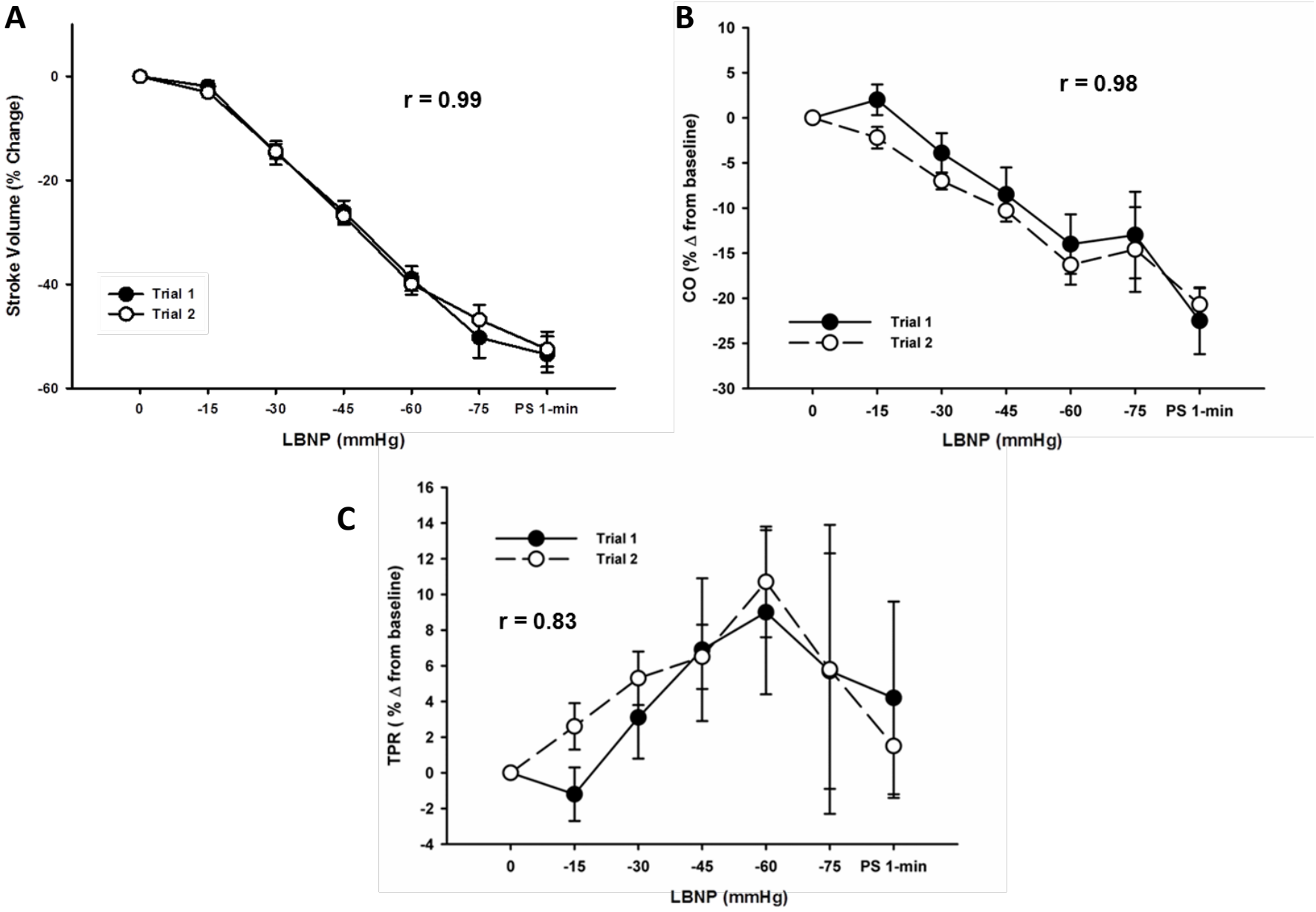

A
Stroke Volume (% Change)
0
-20
-40
-60
r = 0.99
Trial 1
Trial 2
0
-15
-30
-45
-60
-75
PS 1-min
LBNP (mmHg)
B
CO (% Δ from baseline)
10
5
0
-5
-10
-15
-20
-25
-30
r = 0.98
Trial 1
Trial 2
0
-15
-30
-45
-60
-75
PS 1-min
LBNP (mmHg)
C
TPR (% Δ from baseline)
16
14
12
10
8
6
4
2
0
-2
-4
Trial 1
Trial 2
r = 0.83
0
-15
-30
-45
-60
-75
PS 1-min
LBNP (mmHg)

Figure 3

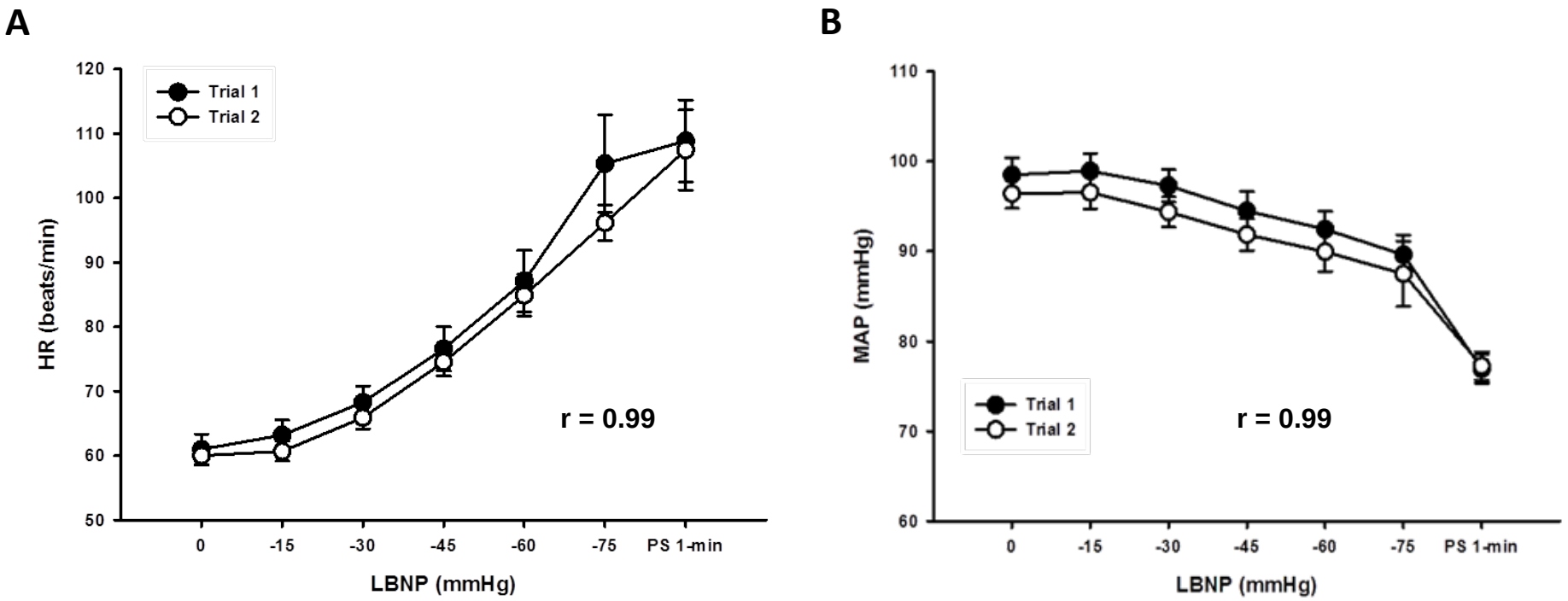
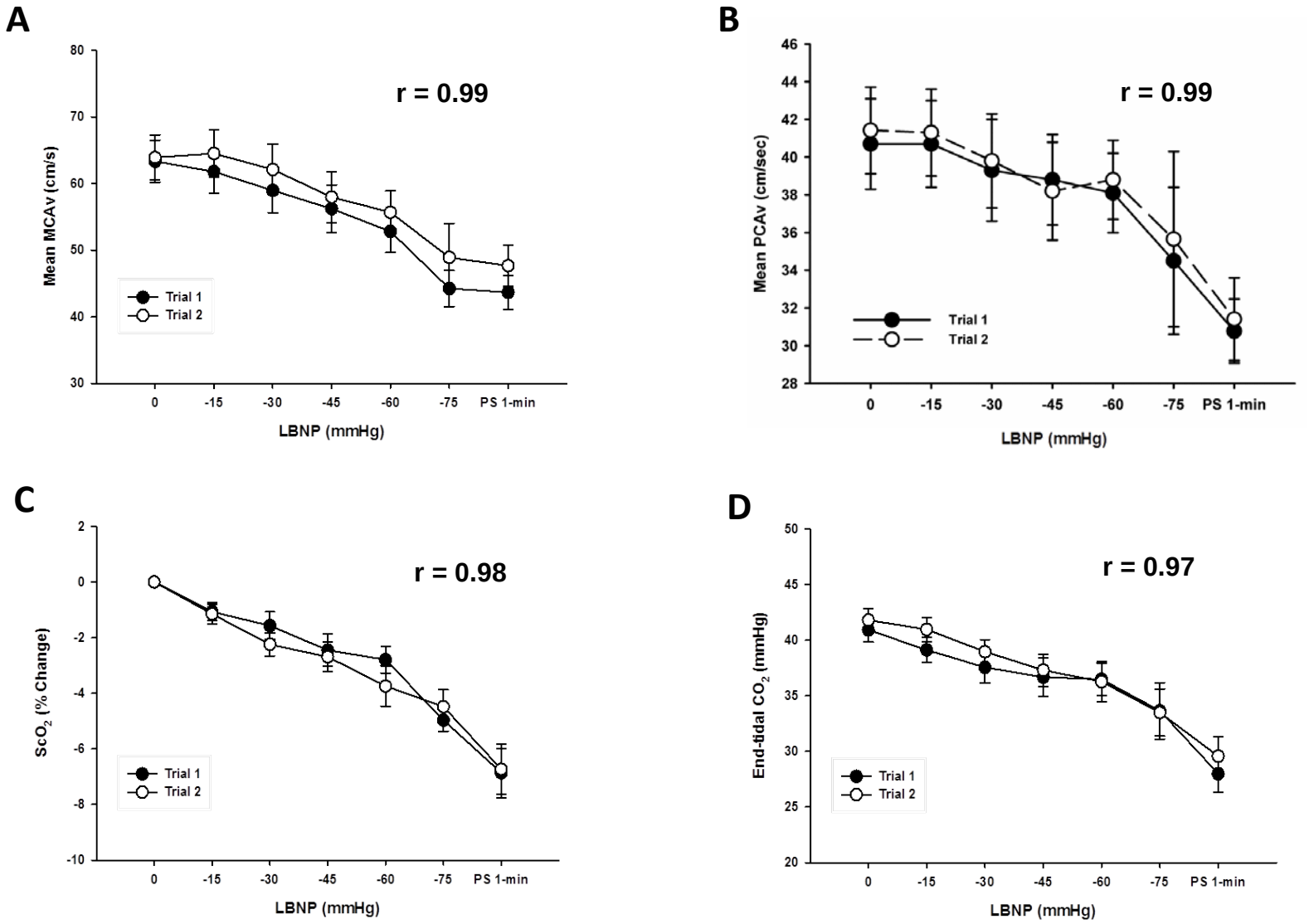

A
HR (beats/min)
120
110
100
90
80
70
60
50
Trial 1
Trial 2
r = 0.99
0
-15
-30
-45
-60
-75
PS 1-min
LBNP (mmHg)
B
MAP (mmHg)
110
100
90
80
70
60
Trial 1
Trial 2
r = 0.99
0
-15
-30
-45
-60
-75
PS 1-min
LBNP (mmHg)

Figure 4


A
Mean MCAv (cm/s)
r = 0.99
Trial 1
Trial 2
LBNP (mmHg)
B
Mean PCAv (cm/sec)
r = 0.99
Trial 1
Trial 2
LBNP (mmHg)
C
ScO2 (% Change)
r = 0.98
Trial 1
Trial 2
LBNP (mmHg)
D
End-tidal CO2 (mmHg)
r = 0.97
Trial 1
Trial 2
LBNP (mmHg)
0
-15
-30
-45
-60
-75
PS 1-min

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CHAPTER III

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

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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 (ScO_2), due in part, to increased low frequency (LF) oscillations in CBF, and prolonged preservation of CBF in the posterior versus anterior cerebral circulation. 20 subjects (8M/12F) completed a presyncopal-limited lower body negative pressure (LBNP) protocol (3 mmHg/min onset rate). ScO_2 (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 ≥70mmHg LBNP were classified as high tolerant (HT; N=9), and low tolerant (LT; N=11) if they completed ≤60 mmHg LBNP. The minimum difference in LBNP tolerance between groups was 214 s. Despite similar reductions in mean MCAv in both groups, ScO_2 decreased in LT subjects at LBNP beyond -15 mmHg ($P \leq 0.04$), but was maintained until -60 mmHg LBNP in HT subjects. Similarly, mean PCAv fell below baseline when LBNP exceeded -30 mmHg in LT subjects ($P=0.013$), but remained unchanged in HT subjects until -60 mmHg LBNP ($P \geq 0.89$).There were no between group differences in LF oscillations for MAP or MCAv at any level of LBNP, nor at presyncope ($P \geq 0.15$). 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.

INTRODUCTION

Hemorrhage accounts for >90% of potentially survivable deaths in the military setting, and is one of the most common injuries in trauma patients in the civilian setting (4, 5, 18, 32, 41). A major factor contributing to death and disability from severe blood loss is poor tissue perfusion and oxygenation of the vital organs (4, 18, 32). Prolonged cerebral hypoperfusion can lead to neuronal cell death, and if the patient survives, long-term cognitive impairment and physical disability (41). Understanding cerebral hemodynamic responses to blood loss is an essential target for improving survival to hemorrhagic injury, and developing effective therapeutic interventions (46). As there is considerable variability in survival time following hemorrhagic injuries (55) as well as tolerance to simulated hemorrhage (11, 21, 34, 36), 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 (9, 14, 60, 66). Two recent studies have validated the use of LBNP as a model for simulated hemorrhage; these studies reported comparable hemodynamic responses to LBNP and blood loss up to 1000 ml in humans (27) and 25% of total blood volume in baboons (23). It is well established that during the initial stages of progressive central hypovolemia (i.e., hemorrhage), reflex cardiovascular responses are initiated (e.g., tachycardia, peripheral vasoconstriction) (10, 11, 21, 25, 34, 53) to protect the vital organs from hypoperfusion. While traditionally, protection of absolute CBF was thought to be essential in determining tolerance to central hypovolemia (34), 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) (26, 37, 38, 47). Most recently, Ogoh et al. (2015) suggested that flow in the posterior cerebral circulation [indexed by flow in the vertebral arteries (VA)] 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 (ICA)]; responses between the two regions with central hypovolemia to presyncope, however, was not evaluated.

Some investigators have also suggested that the pattern of CBF may be more important than the protection of absolute flow (38, 47, 48). Specifically, increased low frequency (LF) oscillations in mean MCAv and mean arterial pressure (MAP) have been associated with increased tolerance to central hypovolemia (38, 47, 48). These oscillations occur spontaneously in the LF range (0.04 - 0.15 Hz) due to sympathetic modulation of arterial pressure via the baroreflex (13, 29, 51), and subsequent transfer to the cerebral circulation. Pulsatile perfusion of the cerebral tissues at other frequencies has also been shown to be protective under conditions of tissue hypoperfusion, such as stroke, cardiac arrest, and severe hemorrhage [see review (49)]. Whole body periodic acceleration in the head-to-foot direction at frequencies of 3-6 Hz increases blood flow to the cerebral tissues due to release of vasodilators from the pulsatile shear stress (1). In a hemorrhage model, periodic acceleration also delayed the onset of irreversible hemorrhagic shock, increased oxygen extraction, and preserved regional blood flow in the brainstem and brain cortex (3). In studies of cardiac bypass surgery, pulsatile perfusion at and above the cardiac frequency (≥1Hz) has been shown to optimize microvascular perfusion (measured in the sublingual mucosa) in humans (33, 42), and decreased neuronal cell damage in a dog model (52). To date, however, there have been no studies examining the potential role of increased CBF

oscillations at lower frequencies (i.e., <1 Hz) on protection of cerebral oxygenation (ScO_2) during central hypovolemia.

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 ScO_2 via assessment of oxy- (HbO_2) 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 (40, 45). As such, following oxygen exchange within the capillaries, a decrease in the HbO_2 concentration and increase in the dHb concentration can be interpreted as an increase in cerebral oxygen extraction from the arterial blood supplying the tissue. Torella et al. reported that HbO_2 decreased, while dHb increased in proportion to mild blood loss in humans (470 ml; approx. 10% total blood volume) (62). 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 (35); 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 (8), the fainters had decreased oxygen extraction in the cerebral tissues (positive oxygenation index, i.e., HbO_2 – 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 (8, 35), 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 ScO_2 and/or increased oxygen extraction plays a role in determining tolerance to this stress; 2) the delivery of oxygen to the cerebral tissues via oscillatory CBF preserves ScO_2; and 3) 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 ScO_2, due in part, to increased LF oscillations in CBF, maintained extraction of oxygen by the cerebral tissues, 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 electrocardiogram (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. All female subjects were tested in the early follicular phase of their menstrual cycle (days 1-4), determined by self-report. 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^{0}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 CO_2 ($etCO_2$) 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). HbO_2, dHb, total hemoglobin concentration (THC; HbO_2 + dHb) and ScO_2 were measured or calculated from the frontal cortex via 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. A catheter was inserted into a vein in the cubital fossa and secured for blood sampling; hematocrit (HCT) was assessed in a subset of subjects to assess hydration status (normal range, women: 35-50%; men: 40-55% (6)).

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 of negative pressure at a decompression rate of 3 mmHg/min until the onset of presyncope, determined by one or more of the following criteria: 1) 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, ScO_2, THC, $etCO_2$) 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 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. The stability of the MAP and mean MCAv signals was assessed using the stationarity function (tendency of the mean and standard deviation to vary with time (44)), where smaller values represent greater stability of the signal.

Oscillatory patterns of MAP, mean MCAv, and ScO_2 were determined with via power spectral analysis. Data was made equidistant by interpolating linearly and resampling at 5 Hz. Data were passed through a low-pass filter with a cutoff frequency of 0.5 Hz. Four-minute data sets were fast Fourier transformed to obtain power spectra, and are expressed as the integrated area within the low frequency (LF, 0.04-0.15 Hz) and very low frequency (VLF, 0.004-0.04 Hz) ranges. Coherence between MAP and mean MCAv in the LF and VLF was calculated by dividing the squared cross-spectral densities of the two signals by the product of the individual autospectra. Transfer function gain between MAP and mean MCAv was calculated only when coherence values were ≥ 0.5.

Statistical Analysis

Since there is variable tolerance to central hypovolemia (21, 34, 36), subjects became presyncopal at different levels of LBNP. As such, subjects were classified as high tolerant (HT) if they withstood -70 mmHg LBNP or greater and low tolerant (LT) if they made it to at least -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 the LT and HT groups. Physiological responses were then 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 higher intensities of LBNP. All time and frequency domain 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), -75 mmHg (Range: -66 to -75 mmHg), -90 mmHg (Range: -81 to -90 mmHg), and -100 mmHg (Range: -96 to -100 mmHg) LBNP. In addition, to compare physiological responses between the HT and LT subjects at presyncope, the final 1-min (PS-1; time domain) and final 4-min (PS-4; frequency domain) immediately prior to presyncope was assessed for each subject. Absolute and percentage changes 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 7 of the 9 HT subjects reached this level of LBNP. Unpaired *t*-tests were used to compare the HT vs. LT group responses at the PS-1 and PS-4 time points or Mann-Whitney tests were run on data that was not normally distributed. Pearson correlations were used to examine the relationships between changes in mean MCAv and ScO_2 from baseline up to -45 mmHg LBNP for the HT and LT groups. A Fisher Exact test was used to

compare the numbers of males and females between the HT and LT groups. All data are presented as mean ± SE (unless otherwise stated), and exact P-values are reported for all comparisons.

RESULTS

LBNP tolerance

Of the 34 subjects who participated in this study, data was analyzed and included from 27 subjects who reached true presyncope, defined as 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 true presyncope, 20 of those subjects (8 male, 12 female; age, 26 ± 3 yrs; height, 171 ± 10 cm; weight, 73 ± 14 kg; means ± SD) were allocated to the HT (n = 9) 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). The remaining 7 subjects reached presyncope between -61 and -69 mmHg LBNP, therefore are not included in this data set. Consequently, the *minimum* difference in LBNP tolerance between the HT and LT groups was 214 s (LT = 1243 ± 185 s vs. HT = 1980 ± 228 s; P<0.001). The LBNP protocol was terminated at or before -45 mmHg LBNP for 4 subjects, between -45 and -60 mmHg LBNP for 7 subjects, between -60 and -75 mmHg LBNP for 7 subjects, between -75 and -90 mmHg LBNP for 7 subjects, and between -90 and -100 mmHg LBNP for 2 subjects. Table 1 compares the baseline and presyncopal characteristics of each group. The only difference between HT and LT groups at baseline, was a 7 mmHg lower MAP in the LT group (P=0.06). The subset of subjects with measures of HCT (n=7) were all euhydrated (HCT: 38 - 46%).

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 ($P<0.001$; Figure 1); the SV reduction in the LT group was greater at -30 and -45 mmHg LBNP ($P\leq0.009$) versus the HT group. However, at the final 1-min prior to presyncope (PS-1), SV decreased by 64 ± 3% in the HT group compared to 42 ± 4% in the LT group ($P<0.001$; Figure 1). Absolute SV was also lower at presyncope in the HT group compared with the LT group (Table 1). 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 ($P<0.001$) maximal HR response at the final 1-min prior to presyncope (PS-1) compared to the LT group (HT: 133 ± 5 beats/min vs. LT: 92 ± 6 beats/min; $P<0.001$; Figure 2A). 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 ($P\leq0.03$). While MAP was lower overall in the LT group throughout LBNP compared to the HT group ($P = 0.08$; Figure 2B), by presyncope it had fallen to similar levels in both groups ($P = 0.81$; Figure 2B).

Mean PCAv decreased below baseline from -30 mmHg LBNP in the LT subjects ($P=0.013$), but remained unchanged in the HT subjects up to -45 mmHg ($P\geq0.86$; Figure 3B), only beginning to fall below baseline at -60 mmHg ($P=0.08$). By presyncope, mean PCAv had decreased by the same magnitude in both HT and LT groups ($P=0.47$; Figure 3B), and to similar absolute values ($P=0.94$; Table 1). 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 ($P\leq0.02$; 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 ($P \leq 0.04$), but no

change in ScO_2 for the HT group at any level of LBNP up to -45 mmHg; ScO_2 only began to fall below baseline from -60 mmHg LBNP in HT subjects (Figure 4A). At presyncope (Figures 3D and 4B), the HT group exhibited greater reductions in both MCAv (HT: -36 ± 3% vs. LT: -23 ± 4%, P=0.02) and ScO_2 (HT: -9 ± 1% vs. -5 ± 1%, P=0.05) than the LT group. This disparate relationship between ScO_2 and CBF for HT and LT subjects is further demonstrated in the data presented in Figure 5. While the coefficients of determination were both high between the changes in mean MCAv and ScO_2 in both the HT (R^2=0.86; P=0.07) and LT subjects (R^2=0.98; P=0.01), in the LT group, for every 3.1% decrease in MCAv, there was 1% decrease in ScO_2, compared with a 8.3% decrease in flow for the HT group to reach the same reduction in ScO_2.

Cerebral HbO_2 and dHb responses are presented in Figure 6. HbO_2 was maintained throughout LBNP up to and including -60 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, HbO_2 had decreased by approximately 10% in both groups (P=0.70). Cerebral dHb progressively increased for both groups, from -15 mmHg LBNP for the LT group (P≤0.01), and from -45 mmHg LBNP for the HT group (P≤0.02). At presyncope, the increase in dHb in the HT was greater than the LT group (P<0.001). 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 in the LT group at every level of LBNP up to -45 mmHg (P≤0.03). $EtCO_2$ progressively decreased from baseline in both groups, with a difference between groups only evident at -45 mmHg LBNP (P=0.03). There were no differences at presyncope between the HT and LT groups for either respiration rate or $etCO_2$.

The absolute LF and VLF oscillations data is presented in Table 3. Stationarity for MAP and mean MCAv did not change from baseline in either the HT group up to -75 mmHg LBNP ($P \geq 0.49$), nor in the LT group up to -45 mmHg LBNP ($P \geq 0.49$), indicating that these signals were relatively stable throughout the protocol (Table 3). MAP LF oscillations increased from baseline in both HT and LT groups at -45 mmHg LBNP, and continued to increase progressively within the HT group up to -75 mmHg LBNP ($P \leq 0.03$). There was no difference between groups in MAP LF oscillations at any level of LBNP ($P \geq 0.25$), nor at presyncope ($P=0.45$); when expressed as a percentage change from baseline, however, MAP LF increased by 292% for HT subjects vs. 121% for LT subjects at presyncope ($P=0.08$). Absolute MAP VLF was only different (reduced) from baseline in the LT group at -15 mmHg LBNP ($P = 0.03$), and there were no differences between groups at any level ($P \geq 0.13$), except at presyncope where MAP VLF power was higher in the LT group ($P=0.08$). In both groups, absolute MCAv LF oscillations did not change from baseline levels at any level of LBNP ($P \geq 0.11$), nor was there a difference between groups at presyncope ($P=0.65$). The HT group did not show increases in MCAv LF even at higher levels of LBNP up to -75 mmHg. Again, in both groups, absolute MCAv VLF oscillations did not change from baseline levels ($P \geq 0.15$), nor were there increases in MCAv VLF in the HT group from baseline up to -75 mmHg LBNP. There was no difference from baseline ($P \geq 0.17$) at any level of LBNP or between groups ($P \geq 0.26$) for ScO_2 LF or VLF oscillations. At presyncope there was no difference between groups for ScO_2 LF. Compared to the HT group, VLF power was higher in the LT subjects at presyncope for MAP, mean MCAv, and ScO_2 ($P \leq 0.08$). MAP-MCAv LF coherence was stable throughout LBNP in both groups ($P \geq 0.15$), with no differences between groups ($P \geq 0.56$). MAP-mean MCAv LF transfer function gain did not change from baseline in the HT group up to -75 mmHg LBNP ($P \geq 0.14$), but

decreased from baseline in the LT group only at -45 mmHg LBNP (P=0.08); there was no difference between groups at any level of LBNP (P≥0.29).

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 and extraction despite early reductions in cerebral blood flow (i.e., delivery); 2) show similar reductions in anterior CBF (indexed by mean MCAv) as LT subjects up to -45 mmHg LBNP, but a greater reduction in anterior CBF at presyncope; 3) have protection of posterior CBF (indexed by mean PCAv) at sub-maximal levels of LBNP; and 4) do not exhibit higher LF oscillations in MAP, mean MCAv, or ScO_2 neither at the final common level of LBNP between groups (i.e., -45 mmHg), at higher levels of central hypovolemia (i.e., up to -75 mmHg), nor at presyncope. Despite there being no difference between groups regarding the oscillatory delivery of cerebral blood flow, our data supports 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 CBF. There is growing evidence, however, that protection of CBF through the MCA is not necessarily associated with tolerance to central hypovolemia (26, 37, 38, 47); 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 LBNP, including the PCA or VAs (17, 43). 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 (61). As such, disruption of posterior cerebral flow may be associated with the symptoms and hemodynamic dysfunction associated with presyncope during central hypovolemia, such as LBNP or hemorrhage (43). 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 (17). Most recently, Ogoh et al. (2015) examined blood flow responses in the VAs feeding the posterior cerebral circulation and ICAs feeding the anterior cerebral circulation up to sub-maximal LBNP of -50 mmHg. 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 -45 mmHg LBNP, but decreased progressively in LT subjects. At presyncope, the reduction in mean PCAv was similar between both groups, 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%) (40, 63). At the last common level of LBNP between groups (i.e., -45 mmHg LBNP), the LT subjects had a lower ScO_2 than the HT group ($P < 0.001$). However, at presyncope, the HT group had greater reductions in ScO_2 than the LT group (HT: -9%; LT: -6%). While previous studies have shown that a 10-15% reduction in ScO_2 is associated with presyncope (8, 22, 39), we have demonstrated that as little as a 6% reduction of ScO_2 can be associated with presyncope in the subjects with low tolerance to central hypovolemia (LT ScO_2 reduction at presyncope ranged from -1 to -7%).

Lewis et al. recently demonstrated that the brain compensates for reductions in cerebral blood flow, elicited by indomethacin or hypocapnia, by increasing oxygen extraction (determined via assessment of cerebral arterial-venous oxygen difference). Reducing the cerebral blood flow reserve prior to exposure to maximal LBNP did not change tolerance, suggesting that increases in oxygen extraction would compensate for the decrease in oxygen delivery; oxygen extraction was not assessed *during* LBNP to directly test this hypothesis (35). While Glaister and Miller reported a decrease in HbO_2 and an increase in dHb during LBNP to presyncope (20), our data are the first, to our knowledge, to demonstrate differential responses of HbO_2 and dHb

within HT and LT subjects. With a decrease in HbO_2 and an increase in dHb on the venous side of the circulation (i.e., NIRS sample volume is 75% venous blood), this can be interpreted as increased 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 HbO_2 and increase in dHb beginning at -15 mmHg LBNP. This increased oxygen extraction was accompanied by a reduction in oxygen delivery (i.e., decreased CBF). In contrast, despite comparable reductions in oxygen delivery as the LT subjects (evidenced by similar decreases in CBF), the HT group exhibited maintenance of HbO_2 until -75 mmHg LBNP, suggesting constant oxygen extraction. Interestingly, despite greater reductions in SV, mean MCAv, and ScO_2 at presyncope in the HT group, both the HT and LT groups experienced the same maximal reduction in HbO_2 (approx. 10%). This finding indicates that reductions in cerebral tissue HbO_2 concentration may be a more accurate indicator of impending presyncope than ScO_2 or CBF through anterior circulation (i.e., mean MCAv).

There are a number of reasons that may account for an increase in oxygen extraction, including reductions in oxygen delivery via decreases in CBF or hypoxia, and/or increased metabolic demand. A reduction in CBF would increase oxygen extraction (35); with less oxygen available to the tissues, extraction would need to increase to compensate for decreased delivery. 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 HbO_2). The LT group started increasing 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 ~22% (at -75 mmHg). 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 experiments were conducted in a normoxic testing environment, we assume hemoglobin concentration would increase in both groups based on similar reductions in central blood volume and subsequent fluid extravasation (7, 59), and central hypovolemia elicited by LBNP stress does not induce any changes in arterial oxygen saturation or PaO_2 (27, 65).

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 22% from baseline, thus contributing to the capacity to tolerate a greater magnitude of central hypovolemia and delay the onset of presyncopal symptoms.

Endogenous cerebral blood flow oscillations

Previous studies have shown that increased oscillations in MAP and MCAv in the LF range are associated with greater tolerance to central hypovolemia (38, 47). The mechanism for this protection may be mediated by increases in shear stress inducing the release of vasodilators

(e.g., nitric oxide, histamine, prostaglandins), thus increasing flow and oxygen delivery (50, 67). In contrast to a previous study by Rickards et al. (2011), our data show increasing MAP LF power in *both* the HT and LT groups, no change in mean MCAv LF power in either group, and no difference in MAP LF or mean MCAv LF between groups at the final common level of LBNP (-45 mmHg), or at presyncope. Additionally, there were no differences in VLF power in MAP, mean MCAv, or ScO_2 between HT and LT subjects, except at presyncope.

Potential reasons for this disparity between studies include the smaller sample size in the current study, compounding the high inter-subject variability for these frequency domain metrics, a difference in the last common level of LBNP (-45 mmHg in the current study vs. -60 mmHg for Rickards et al. (2011)), use of a ramp LBNP protocol which elicits continuous changes in arterial pressure and CBF compared with a stepwise LBNP approach as previously utilized (47), and differences in hemodynamic responses in HT and LT subjects between studies. With a smaller subject pool, and the last common level of comparison between groups at -45 mmHg LBNP, it is unknown if there would have been a group difference in LF oscillations at -60 mmHg LBNP had our LT subjects made it to this level.

Reductions in central blood volume elicit increases in both muscle sympathetic nerve activity (MSNA) and MSNA LF oscillations (13, 19, 30). It has been postulated that MSNA LF oscillations play a role in the variability of MAP and MCAv in the LF range (47), such that sympathetic LF oscillations transfer to pressure, which then transfer to flow. While Convertino et al. (2012) demonstrated that HT subjects have greater increases in MSNA than LT subjects at presyncope, it is unclear if absolute MSNA and/or MSNA LF oscillations differ between groups at submaximal levels of LBNP. Based on similar MAP LF responses to -45 mmHg in our study, we postulate that both MSNA and MSNA LF responses are also similar between HT and LT

subjects until -45 mmHg LBNP; this hypothesis warrants further investigation. In addition to the oscillatory characteristics of MSNA, breathing rate can also impact MAP LF and MCAv LF oscillations if within this range (i.e., 0.04 and 0.15 Hz), equating to breathing frequencies up to 9.0 breaths/min. However, all of the subjects in this study were consistently breathing outside of this range, indicating that breathing frequency should not have impacted LF oscillations.

Finally, application of continuously decreasing LBNP may have influenced subsequent calculations of LF and VLF power spectral density. Traditionally, LBNP is applied in discrete, decreasing steps, with each step lasting anywhere from 3 to 12 minutes (11, 14, 37, 67). However, application of LBNP with this step-wise approach may not accurately mimic actual volume loss (i.e., hemorrhage), as the cardiovascular system is able to compensate and stabilize when the negative pressure is held constant. Therefore, in order to more accurately simulate continuous bleeding, we implemented a ramp LBNP protocol with an onset rate of -3 mmHg/min. We know of only three previous studies that have used this method (2, 12, 28), and none of these studies consistently took their subjects to the point of presyncope for the determination of tolerance. Due to the progressively decreasing pressure during each segment of the analysis, however, the assumption of stationarity of the data for subsequent frequency domain analysis may have been violated. To account for this possibility, we measured stationarity for both MAP and mean MCAv throughout LBNP and found no statistically significant differences when compared with baseline (i.e., the most stable signal since there was no application of LBNP at this time point), so we are confident of the validity of the frequency domain analyses. Together, the frequency domain data suggest that maintenance of ScO_2 and high tolerance to LBNP is occurring via a mechanism other than oscillatory MAP and MCAv in either the LF or VLF ranges.

Clinical implications

While NIRS-derived ScO_2 is often used as a surrogate for CBF (31, 56, 58), our ScO_2 vs. MCAv correlation data suggests caution when using this approach, as ScO_2 does not necessarily decrease in proportion to reductions in MCAv, and the relationships between ScO_2 and mean MCAv are different depending on whether subjects are high or low tolerant. For example, for every 3% decrease in MCAv, there was 1% decrease in ScO_2 in the LT subjects, compared to the HT group where an 8% decrease in flow resulted in the same 1% reduction in ScO_2. 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 utilization extraction and reduce tolerance to hypovolemic stress such as traumatic hemorrhage. Under these conditions, the magnitude of hypovolemia that may induce unconsciousness will depend on individual cerebral metabolic demand; at presyncope, HT subjects had a 64% reduction in SV, compared to a 42% reduction in the LT group. In addition, these data demonstrate the important role of HbO_2 on ScO_2 calculations, the measurement most often reported when using cerebral NIRS monitoring devices. The HT group had stable ScO_2 due to stable HbO_2, whereas the LT group had progressively decreasing HbO_2, which decreased ScO_2. 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.

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 cardiovascular responses elicited by hemorrhage (23, 27).

NIRS is a non-invasive method to obtain ScO_2, HbO_2, and dHb concentrations within the cerebral tissue, but it also may be contaminated by changes in oxygenation of the skin (16, 24, 57, 58). 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 senor placement.

Finally, TCD 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, which may be comparable to our LT group, but not the HT group, as they reached levels of LBNP $\geq$ 70mmHg (54). During greater levels of LBNP,

there is increased sympathetic nerve activity in the periphery (13), 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 than the observed reduction in velocity. If the observed decrease in mean MCAv is an underestimation of CBF, this would only reinforce the dissociation between ScO_2 and CBF, particularly in the HT group; NIRS-related measurements would not be altered as they are not directly dependent on vessel diameter. Recent studies (15, 64) using high resolution magnetic resonance imaging (MRI), suggest that increases in $etCO_2 \geq 9$ mmHg above baseline elicit MCA vasodilation, while decreases in $etCO_2 \geq 13$ mmHg below baseline elicit MCA vasoconstriction. In the current study, $etCO_2$ in both groups only falls ~9 mmHg below baseline, so MCA caliber should be constant. The role of sympathetic activity and variations in arterial CO_2 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 ScO_2 (despite reductions in cerebral flow within the anterior cerebral circulation), and protection of CBF within the posterior cerebral circulation. We postulate that HT subjects maintain relatively constant oxygen metabolism in the brain, thus delaying the onset of presyncope. In addition, our data warrant caution in the clinical use of ScO_2 as a surrogate for flow due to the dissociation between these two variables during central hypovolemia. These findings suggest that the measurements of NIRS-derived HbO_2 and/or posterior CBF may be more sensitive indicators for tracking the onset of presyncope than ScO_2 or measures of anterior cerebral blood flow, and are targets for potential interventions to prolong tolerance to central hypovolemia in the clinical setting.

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

	HT	LT	P-Value
N	9	11	-
LBNP Tolerance Time, s	1980 ± 228	1243 ± 185	< 0.001
Sex, female/male	3/6	5/6	0.17
Age, yrs	27 ± 4	26 ± 3	0.30
Height, cm	170 ± 12	171 ± 9	0.79
Weight, kg	69 ± 12	77 ± 16	0.24
Baseline HR, beats/min	63.6 ± 4.3	60.4 ± 2.3	0.52
Baseline MAP, mmHg	100.3 ± 3.1	93.3 ± 2.2	0.06
Baseline SV, mL	97.0 ± 5.3	99.5 ± 7.1	0.78
Baseline mean MCAv, cm/s	65.1 ± 4.8	63.5 ± 3.8	0.80
Baseline mean PCAv, cm/s	40.7 ± 2.4	38.4 ± 1.2	0.36
Baseline ScO_2, %	67.0 ± 2.6	66.9 ± 1.7	0.98
Presyncopal HR, beats/min	132.8 ± 4.9	92.0 ± 5.5	< 0.001
Presyncopal MAP, mmHg	78.8 ± 2.0	78.0 ± 2.4	0.81
Presyncopal SV, mL	34.5 ± 2.9	58.6 ± 4.7	< 0.001
Presyncopal mean MCAv, cm/s	39.2 ± 2.1	48.7 ± 3.9	0.07
Presyncopal mean PCAv, cm/s	29.4 ± 1.4	29.2 ± 1.6	0.94
Presyncopal ScO_2, %	60.7 ± 2.1	63.1 ± 1.6	0.39

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; SV, stroke volume; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity. Presyncopal time points refer to the 1-min prior to maximal LBNP tolerance.

Table 2: Absolute hemodynamic responses during progressive lower body negative pressure (LBNP) to presyncope in high tolerant (HT) and low tolerant (LT) groups.

	LBNP Level							
	0	**15**	**30**	**45**	**60**	**75**	**PS 1-min**	**P-Value**
SAP, mmHg								
HT	134.7 ± 3.9 †	135.6 ± 4.2 †	130.8 ± 3.8 †	125.6 ± 4.1* †	118.4 ± 3.0 *	112.3 ± 2.1 *	95.1 ± 2.0	P = 0.087
LT	123.7 ± 2.0	124.5 ± 2.6	118.1 ± 1.7 *	113.3 ± 2.7 *	–	–	99.6 ± 1.6	
DAP, mmHg								
HT	77.1 ± 2.6	77.1 ± 2.6	77.0 ± 2.6	76.9 ± 2.7	76.2 ± 2.5	76.6 ± 2.6	69.3 ± 2.2	P = 0.229
LT	73.0 ± 2.0	73.7 ± 1.8	74.2 ± 2.0	72.4 ± 2.8	–	–	65.0 ± 2.5	
MAP, mmHg								
HT	100.3 ± 3.1 †	99.8 ± 3.1	98.1 ± 2.9 †	95.5 ± 2.8 *	92.0 ± 2.4 *	89.6 ± 2.2 *	78.8 ± 2.0	P = 0.810
LT	93.3 ± 2.2	93.8 ± 2.1	91.4 ± 1.9	87.9 ± 3.1 *	–	–	78.0 ± 2.4	
SV, ml								
HT	97.0 ± 5.3	95.7 ± 5.7	86.8 ± 6.1 *	75.1 ± 6.3 *	61.7 ± 5.5 *	52.0 ± 5.4 *	34.5 ± 2.9	P < 0.001
LT	99.5 ± 7.1	97.4 ± 7.6	81.0 ± 6.3 *	70.1 ± 5.9 *	–	–	58.6 ± 4.7	
CO, %Δ								
HT	–	3.1 ± 2.9	-2.8 ± 3.1	-8.9 ± 4.4 *†	-12.0 ± 4.2*	-13.0 ± 4.8 *	-24.6 ± 5.7	P = 0.08
LT	–	0.7 ± 1.7	-4.3 ± 2.6	-0.4 ± 3.7	–	–	-9.7 ± 5.5	
TPR, mmHg/L/min								
HT	16.9 ± 0.8	16.3 ± 0.8	17.1 ± 1.1	18.1 ± 1.6	18.1 ± 1.6	17.8 ± 1.9	18.3 ± 1.6	P = 0.235
LT	16.4 ± 1.2	16.4 ± 1.2	17.1 ± 1.4	15.8 ± 1.6	–	–	15.8 ± 1.4	
PCAv, cm/s								
HT	40.7 ± 2.4	41.0 ± 2.2	40.8 ± 2.4	39.2 ± 2.2	38.5 ± 2.4 *	36.2 ± 2.4 *	29.4 ± 1.4	P = 0.94

LT	38.4 ± 1.2	36.7 ± 1.2	34.9 ± 1.5 * †	32.2 ± 2.0 * †	–	–	29.2 ± 1.6	
MCAv, cm/s								
HT	65.1 ± 4.8	63.7 ± 5.3	61.7 ± 5.0	58.8 ± 4.3 *	52.5 ± 3.9 *	44.2 ± 2.7 *	39.2 ± 2.1	P = 0.071
LT	63.5 ± 3.8	60.7 ± 3.7	56.6 ± 4.0 *	51.9 ± 4.5 *	–	–	48.7 ± 3.9	
ScO_2, %								
HT	67.0 ± 2.6	66.6 ± 2.6	66.4 ± 2.5	66.1 ± 2.2	65.4 ± 2.0 *	63.8 ± 2.6 *	60.7 ± 2.1	P = 0.377
LT	66.9 ± 1.7	65.5 ± 1.6 *	65.9 ± 1.4 *	64.8 ± 1.4 *	–	–	63.1 ±1.6	
HbO_2, µM								
HT	32.6 ± 2.7	31.9 ± 2.6	31.9 ± 2.5	31.8 ± 2.3	31.4 ± 2.2	31.0 ± 2.6 *	28.5 ± 1.8	P = 0.261
LT	35.2 ± 2.2	33.5 ± 1.8	33.6 ± 1.8	33.2 ± 1.8	–	–	31.4 ± 1.7	
dHb, µM								
HT	15.9 ± 1.4	15.9 ± 1.4	16.1 ± 1.4	16.3 ± 1.3 *	16.7 ± 1.3 *	17.7 ± 1.8 *	18.5 ± 1.5	P = 0.913
LT	17.3 ± 1.3	17.6 ± 1.2 *	17.4 ± 1.3 *	18.2 ± 1.5 *	–	–	18.3 ± 1.2	
THC, µM								
HT	48.5 ± 3.1	47.9 ± 3.0	48.0 ± 3.0	48.1 ± 2.9	48.1 ± 2.9	48.7 ± 3.6	47.0 ± 2.7	P = 0.461
LT	52.4 ± 2.9	51.1 ± 2.5 *	51.1 ± 2.8 *	51.4 ± 3.1 *	–	–	49.7 ± 2.4	
Respiratory Rate, breaths/min								
HT	10.0 ± 0.7†	8.5 ± 0.7†	9.0 ± 0.7†	9.4 ± 0.7†	9.5 ± 0.8	10.9 ± 1.1	13.8 ± 1.8	P = 0.904
LT	14.0 ± 1.1	13.1 ± 0.9	12.1 ± 1.0	12.8 ± 1.1	–	–	13.5 ± 0.9	
$etCO_2$, mmHg								
HT	42.5 ± 1.5	41.2 ± 1.5	40.0 ± 1.6	38.6 ± 1.6 *†	36.6 ± 1.7 *	33.6 ± 2.5 *	24.6 ± 2.0	P = 0.171
LT	40.9 ± 1.7	38.6 ± 1.8	36.0 ± 2.4 *	32.9 ± 2.5 *	–	–	28.9 ± 2.2	

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; ScO_2, cerebral oxygen saturation; HbO_2, oxygenated hemoglobin concentration; dHb, deoxygenated hemoglobin concentration; THC, total hemoglobin concentration; $etCO_2$, end tidal carbon dioxide. PS-1 time point refers to the 1-min prior to maximal LBNP tolerance. *P≤0.04 compared to baseline within a group. †P≤0.07 between HT and LT groups.

Table 3: Absolute low frequency (LF, 0.04 – 0.15 Hz) and very low frequency (VLF, 0.004 – 0.04 Hz) oscillatory responses during progressive lower body negative pressure (LBNP) to presyncope in high tolerant (HT) and low tolerant (LT) groups.

	LBNP Level							
	0	**-15**	**-30**	**-45**	**-60**	**-75**	**PS 4-min**	**P- value**
MAP LF, mmHg^2								
HT	9.6 ± 3.0	13.5 ± 4.7	14.8 ± 5.5	19.8 ± 6.2 *	26.8 ± 8.9 *	28.1 ± 8.8 *	33.9 ± 12.8	P = 0.45
LT	8.6 ± 1.7	7.2 ± 1.3	9.7 ± 1.8	16.2 ± 5.6 *	–	–	21.1 ± 6.6	
MAP VLF, mmHg^2								
HT	12.1 ± 3.1	13.2 ± 4.2	11.2 ± 3.5	8.1 ± 2.5	10.0 ± 2.0	7.6 ± 1.4	7.5 ± 1.6	P = 0.08
LT	14.1 ± 3.0	6.7 ± 1.6 *	9.7 ± 3.5	7.7 ± 2.0	–	–	13.7 ± 2.8	
MAP Stationarity								
HT	0.51 ± 0.06	0.58 ± 0.06	0.56 ± 0.07	0.47 ± 0.06	0.53 ± 0.05	0.52 ± 0.04	0.66 ± 0.05	P = 0.78
LT	0.60 ± 0.04	0.66 ± 0.05	0.51 ± 0.04	0.59 ± 0.09	–	–	0.68 ± 0.05	
MCAv LF, (cm/s)^2								
HT	6.7 ± 1.9	6.2 ± 2.3	6.1 ± 2.0	7.9 ± 2.7	9.4 ± 3.7	7.2 ± 2.5	13.3 ± 6.3	P = 0.65
LT	6.6 ± 2.2	4.9 ± 1.4	5.5 ± 1.1	7.0 ± 2.9	–	–	5.6 ± 1.2	
MCAv VLF, (cm/s)^2								
HT	14.3 ± 7.3	6.8 ± 2.7	10.0 ± 5.4	8.0 ± 3.2	7.0 ± 2.4	3.8 ± 1.3	3.4 ± 1.2	P = 0.04
LT	6.5 ± 1.0	8.0 ± 3.6	9.9 ± 6.6	5.3 ± 1.2	–	–	7.7 ± 2.2	
MCAv Stationarity								
HT	0.60 ± 0.04	0.67 ± 0.06	0.69 ± 0.05	0.60 ± 0.05	0.68 ± 0.05	0.62 ± 0.07	0.73 ± 0.05	P = 0.18
LT	0.63 ± 0.06	0.66 ± 0.04	0.59 ± 0.03	0.65 ± 0.07	–	–	0.64 ± 0.04	

ScO_2 LF ($\%^2$)								
HT	0.21 ± 0.1	0.20 ± 0.1	0.19 ± 0.0	0.16 ± 0.0	0.25 ± 0.1	0.29 ± 0.1	0.29 ± 0.1	P = 0.97
LT	0.24 ± 0.1	0.22 ± 0.2	0.21 ± 0.0	0.24 ± 0.0	–	–	0.26 ± 0.0	
ScO_2 VLF ($\%^2$)								
HT	0.25 ± 0.1	0.17 ± 0.0	0.15 ± 0.0	0.12 ± 0.0	0.20 ± 0.1	0.12 ± 0.0	0.16 ± 0.0	P = 0.06
LT	0.25 ± 0.1	0.26 ± 0.1	0.22 ± 0.1	0.31 ± 0.1	–	–	0.44 ± 0.1	
MAP-MCAv LF Coherence								
HT	0.68 ± 0.04	0.70 ± 0.04	0.70 ± 0.04	0.77 ± 0.05	0.78 ± 0.04	0.78 ± 0.06	0.79 ± 0.05	P = 0.10
LT	0.64 ± 0.05	0.59 ± 0.03	0.68 ± 0.05	0.66 ± 0.05	–	–	0.69 ± 0.04	
MAP-mean MCAv LF Gain								
HT	0.71 ± 0.11	0.63 ± 0.09	0.64 ± 0.08	0.60 ± 0.07	0.53 ± 0.04	0.46 ± 0.04	0.53 ± 0.05	P = 0.15
LT	0.90 ± 0.12	0.77 ± 0.09	0.70 ± 0.08	0.67 ± 0.03 $^{\#}$	–	–	0.65 ± 0.05	

Data are presented as means ± SE. LBNP, lower body negative pressure; HT, high tolerance; LT, low tolerance; MAP LF, Mean arterial pressure low frequency oscillations; MAP VLF, Mean arterial pressure very low frequency oscillations; MCAv LF, middle cerebral artery velocity low frequency oscillations; MCAv VLF, middle cerebral artery velocity very low frequency oscillations; ScO_2 LF, cerebral oxygen saturation low frequency oscillations; ScO_2 VLF, cerebral oxygen saturation very low frequency oscillations; PS 4-min refers to 4 mins prior to maximal LBNP tolerance (presyncope). *P≤0.03 compared to baseline within a group. $^{\#}$P=0.08 compared to baseline. No difference between groups at any level of LBNP for any variable.

FIGURE LEGENDS

Figure 1 Stroke volume (SV) % change from baseline in the high tolerant (HT, solid line, closed circles) and low tolerant (LT, dashed line, open circles) groups during lower body negative pressure (LBNP). SV % change from baseline in the HT (filled triangle) and LT groups (open triangle) over the 1-min prior to presyncope ($P<0.001$). *, denotes $P<0.001$ compared with baseline. †, denotes $p<0.001$ between groups.

Figure 2 Heart rate (HR, **panel A**) increases from baseline in the high tolerant (HT, solid line, closed circles) and low tolerant (LT, dashed line, open circles) groups during lower body negative pressure (LBNP). Heart rate comparison between the HT (filled triangle) and LT groups (open triangle) over the 1-min prior to presyncope ($P< 0.001$). Mean arterial pressure (MAP) changes from baseline in the HT and LT groups throughout LBNP (**Panel B**), and presyncopal responses between groups (triangles)**.** *, denotes $P\leq0.03$ compared with baseline. †, denotes $P=0.08$ between groups.

Figure 3 % change from baseline in middle cerebral artery velocity (MCAv, **Panel A**) and posterior cerebral artery velocity (PCAv, **Panel B**) in the high tolerant (HT, solid line, closed circles) and low tolerant (LT, dashed line, open circles) groups during lower body negative pressure (LBNP). PCAv (**Panel B**) and MCAv (**Panel A**) % change from baseline in the HT (filled triangle) and LT (open triangle) groups over the 1-min prior to presyncope. *, denotes $P\leq0.02$ compared with baseline. †, denotes $P\leq0.03$ between groups.

Figure 4 Cerebral oxygen saturation (ScO_2) % change from baseline in the high tolerant (HT, solid line, closed circles) vs low tolerant (LT, dashed line, open circles) group throughout lower body negative pressure (LBNP). % change in ScO_2 during the final 1-min prior to presyncope is shown with filled (HT) and open (LT) triangles. *, denotes $P\leq0.002$ compared with baseline. †, denotes $P\leq 0.01$ between groups. #, denotes $P\leq 0.07$ between groups.

Figure 5 The correlation between % change in middle cerebral artery velocity (MCAv) vs. % change in cerebral oxygen saturation (ScO_2) in the HT group (closed circles) and the LT group (open circles) through LBNP up to -45 mmHg. HT group: $R^2=0.86$, $P=0.07$; LT group: $R^2=0.98$, $P=0.01$.

Figure 6 Oxygenated hemoglobin (Oxy Hb, **Panel A**) and deoxygenated hemoglobin (Deoxy Hb, **Panel B**) % change from baseline in the high tolerant (HT, solid line, closed circles) vs low tolerant (LT, dashed line, open circles) group during lower body negative pressure (LBNP). % change from baseline in Oxy Hb and Deoxy Hb during the final 1-min prior to presyncope is shown with filled (HT) and open (LT) triangles. *, denotes $P\leq0.05$ compared with baseline. †, denotes $P\leq 0.05$ between groups. #, denotes $P\leq 0.07$ between groups.

Figure 1

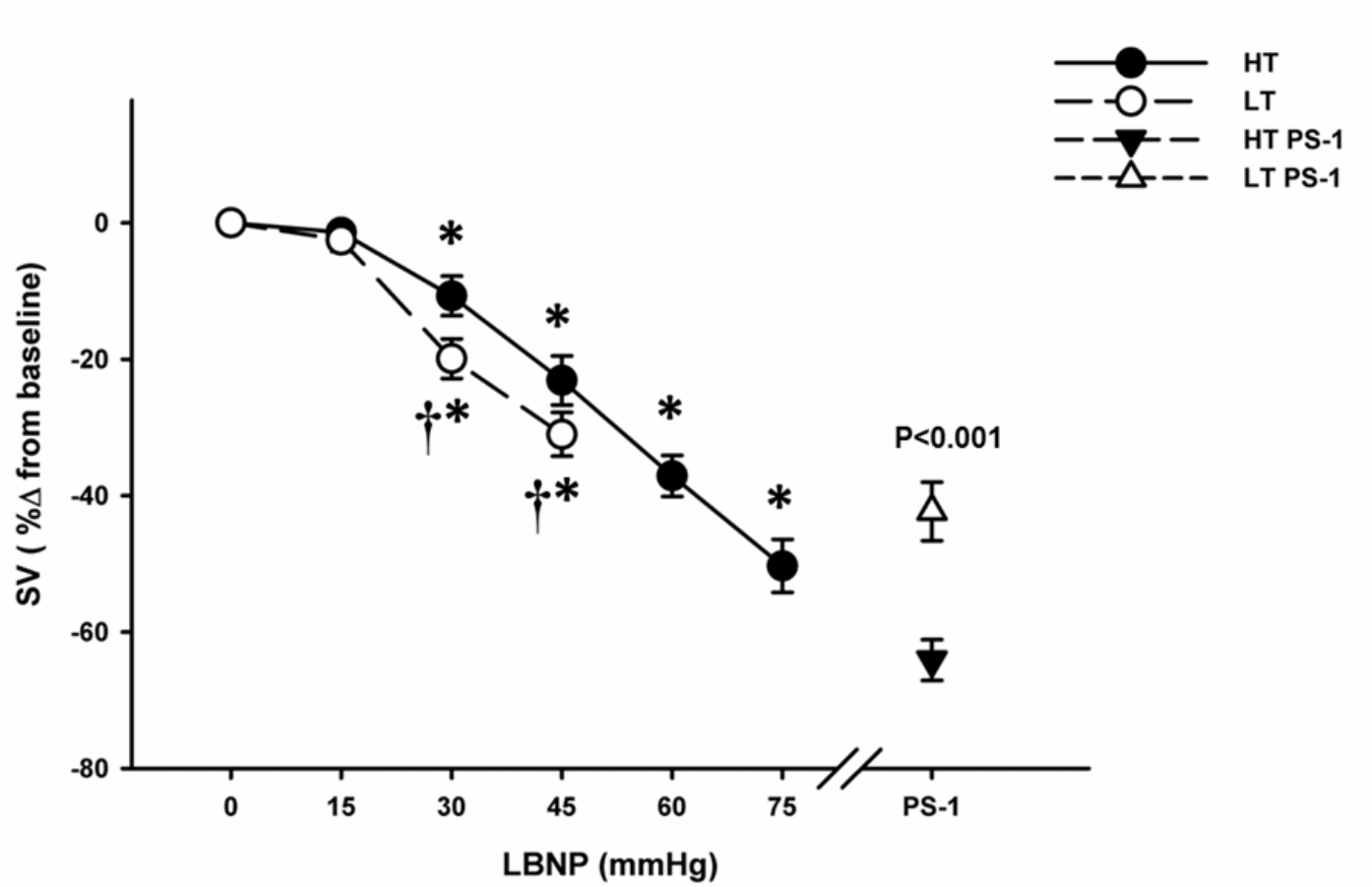

HT
LT
HT PS-1
LT PS-1
SV (%Δ from baseline)
0
-20
-40
-60
-80
0
15
30
45
60
75
PS-1
LBNP (mmHg)
P<0.001

Figure 2

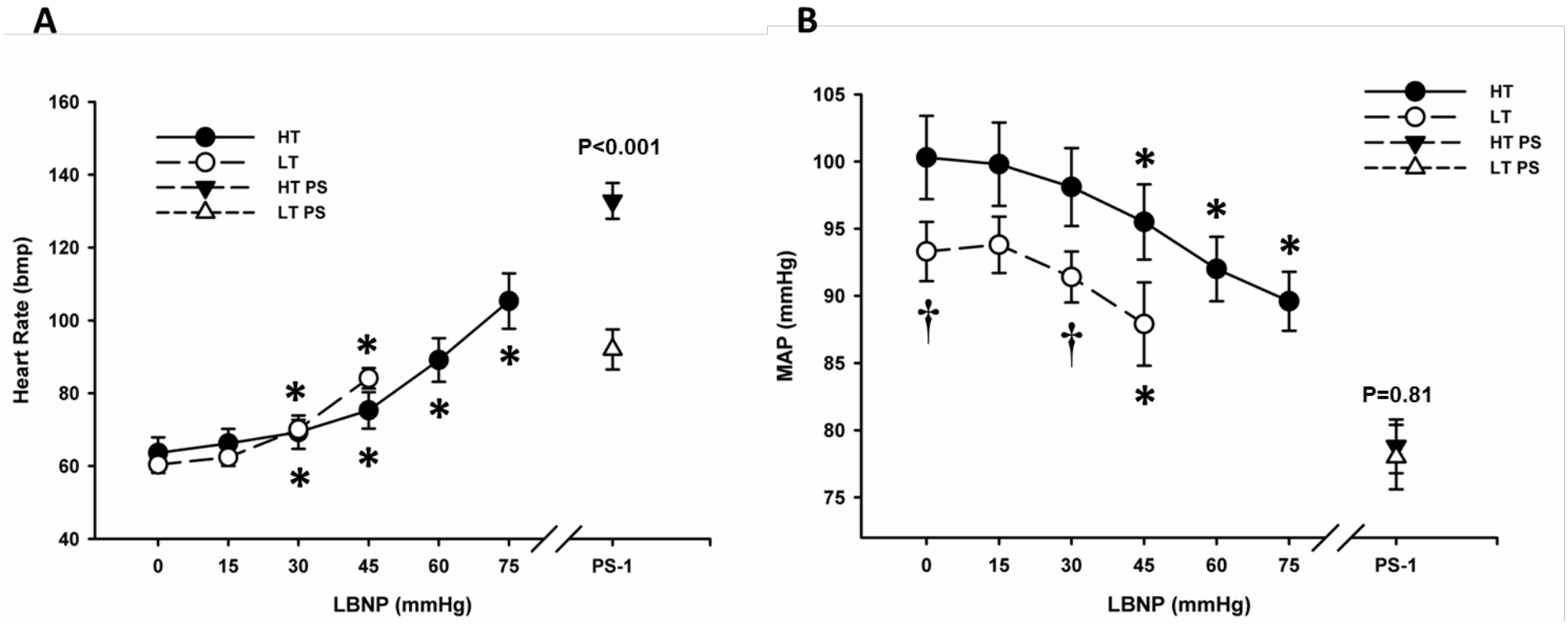

A
160
140
120
100
80
60
40
Heart Rate (bmp)
HT
LT
HT PS
LT PS
P<0.001
0
15
30
45
60
75
PS-1
LBNP (mmHg)
B
105
100
95
90
85
80
75
MAP (mmHg)
HT
LT
HT PS
LT PS
P=0.81
0
15
30
45
60
75
PS-1
LBNP (mmHg)

Figure 3

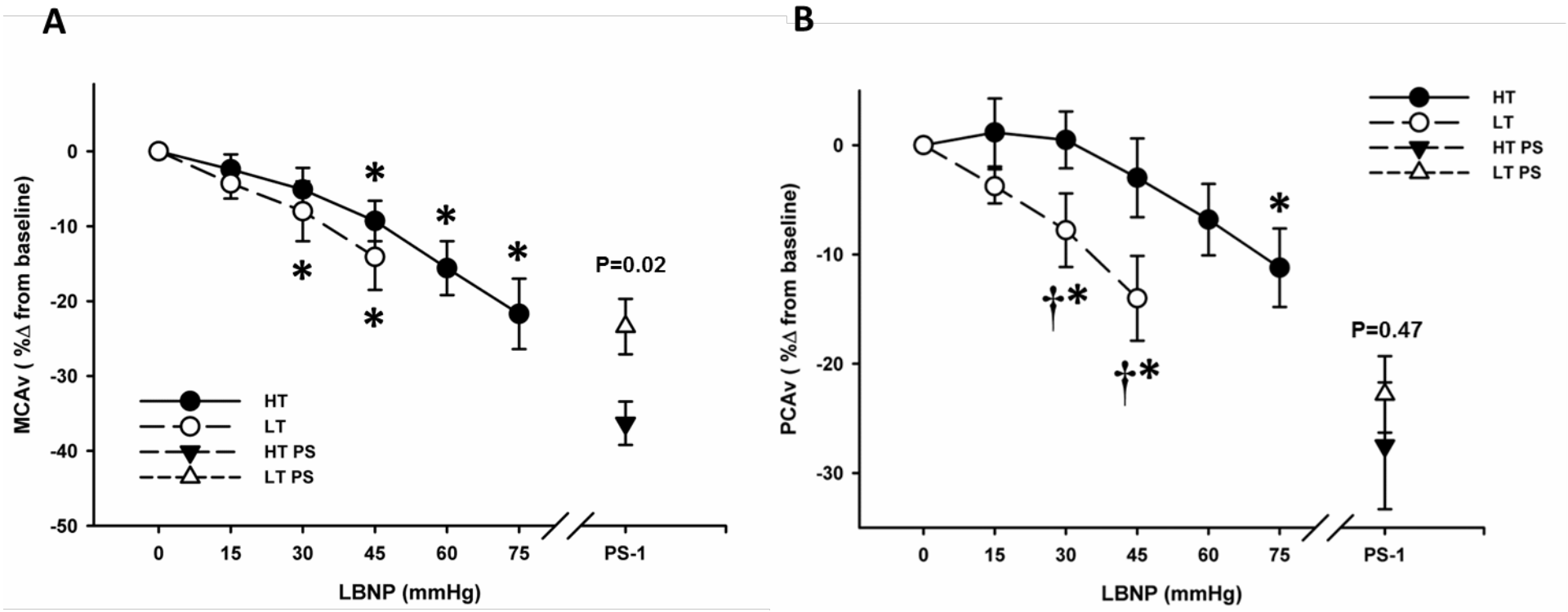

A
MCAv (%Δ from baseline)
0
-10
-20
-30
-40
-50
0
15
30
45
60
75
PS-1
LBNP (mmHg)
HT
LT
HT PS
LT PS
P=0.02
*
B
PCAv (%Δ from baseline)
0
-10
-20
-30
0
15
30
45
60
75
PS-1
LBNP (mmHg)
HT
LT
HT PS
LT PS
P=0.47
*

Figure 4

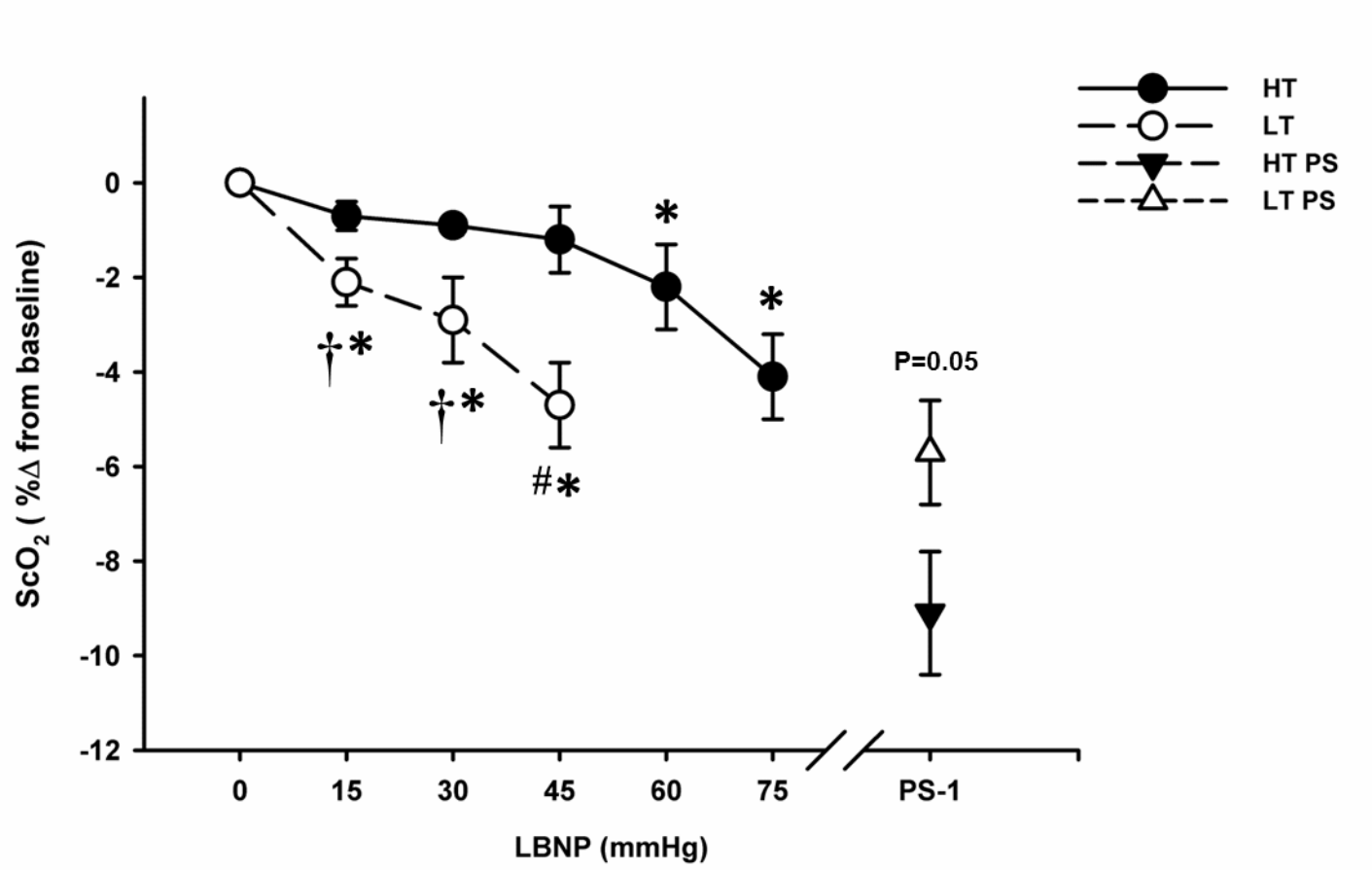

HT
LT
HT PS
LT PS
0
-2
-4
-6
-8
-10
-12
ScO2 (%Δ from baseline)
†*
†*
#*
*
*
P=0.05
0
15
30
45
60
75
PS-1
LBNP (mmHg)

Figure 5

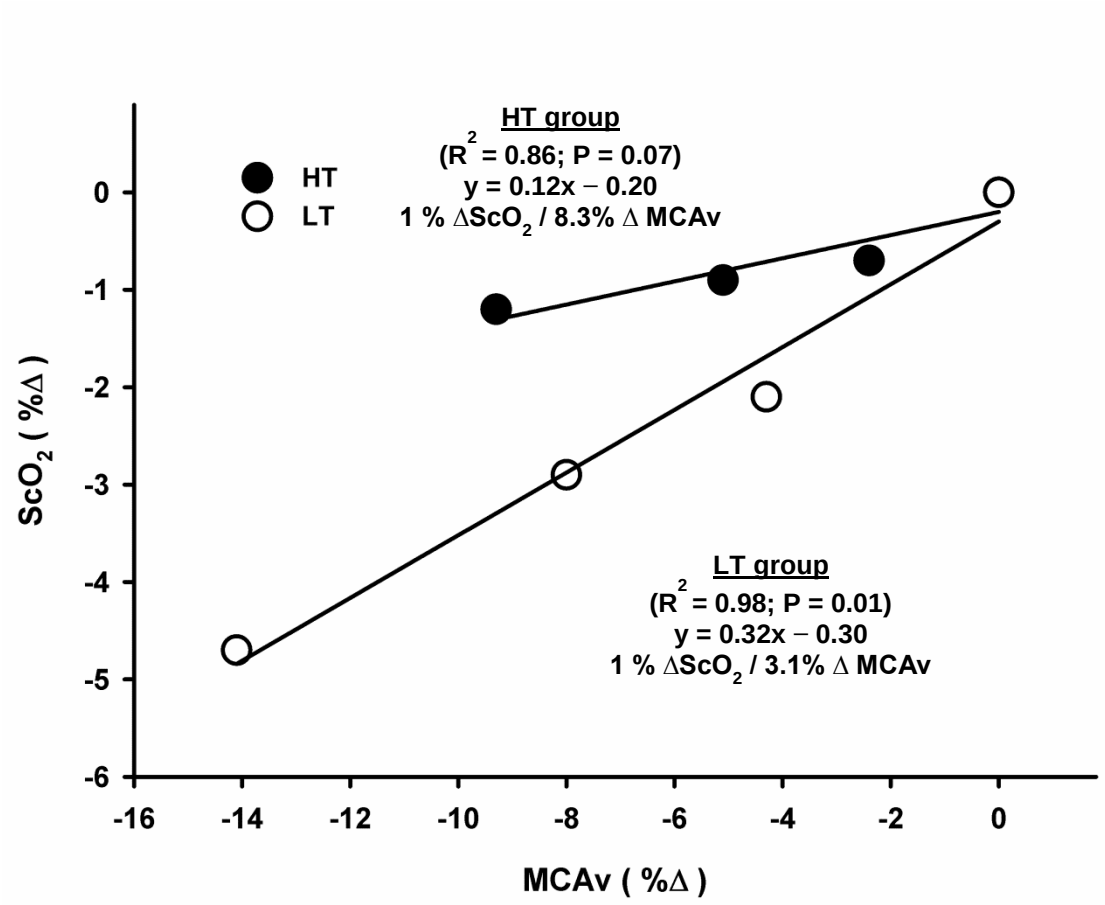
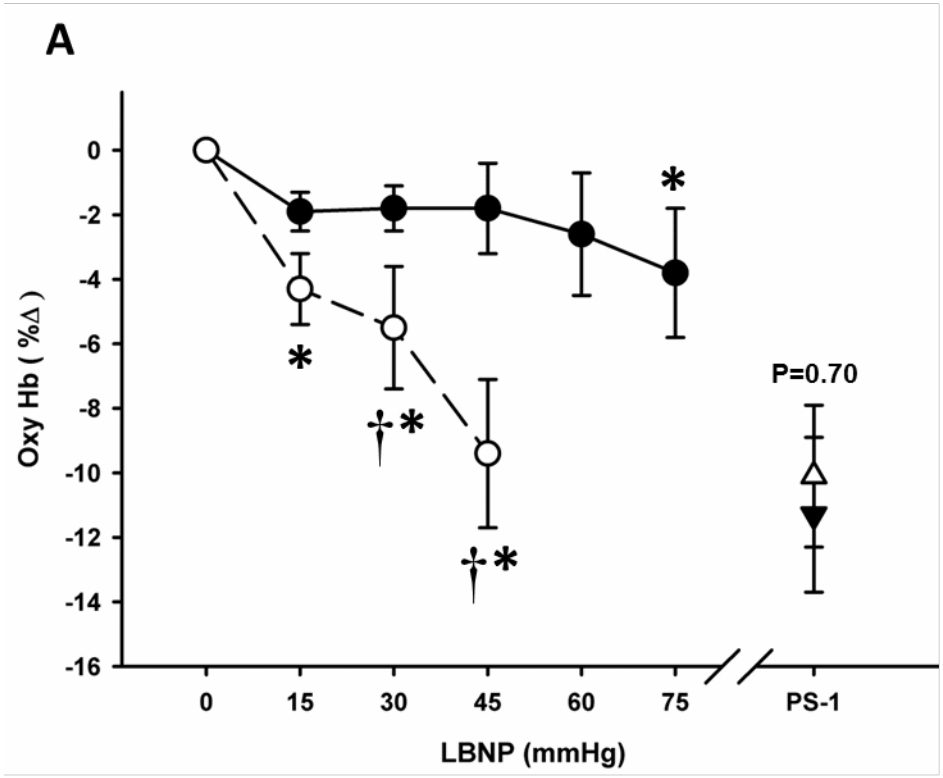
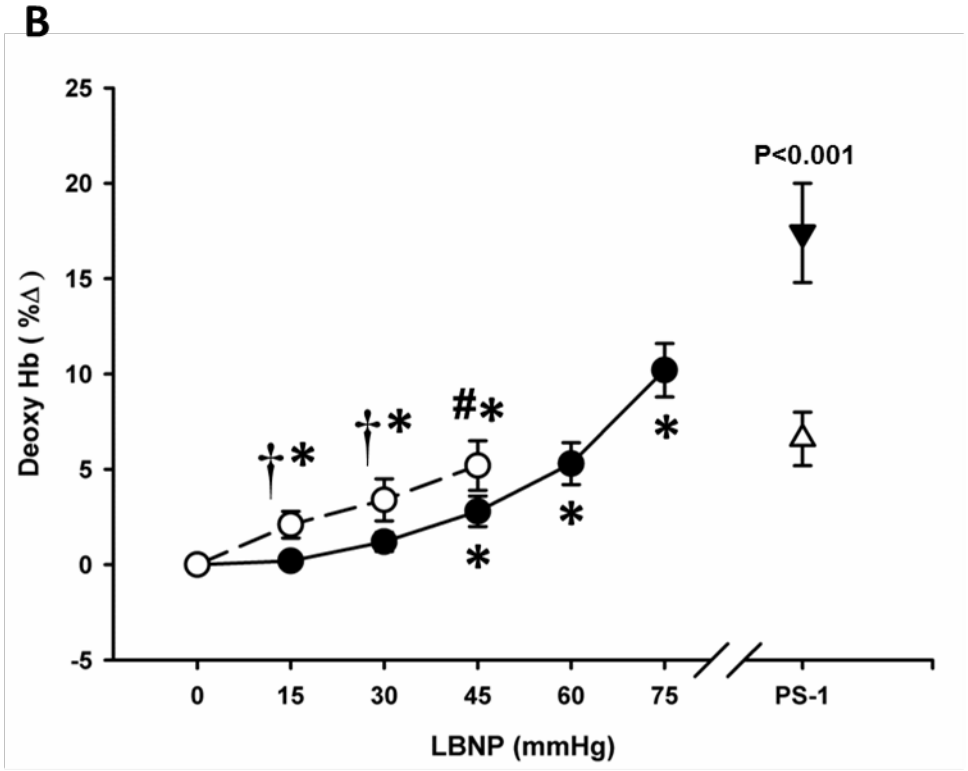

HT group
(R^2 = 0.86; P = 0.07)
y = 0.12x − 0.20
1 % ΔScO2 / 8.3% Δ MCAv
HT
LT
LT group
(R^2 = 0.98; P = 0.01)
y = 0.32x − 0.30
1 % ΔScO2 / 3.1% Δ MCAv
ScO2 (%Δ)
MCAv (%Δ)
0
-1
-2
-3
-4
-5
-6
-16
-14
-12
-10
-8
-6
-4
-2
0

Figure 6


A
Oxy Hb (%Δ)
0
-2
-4
-6
-8
-10
-12
-14
-16
0
15
30
45
60
75
PS-1
LBNP (mmHg)
P=0.70


B
Deoxy Hb (%Δ)
25
20
15
10
5
0
-5
0
15
30
45
60
75
PS-1
LBNP (mmHg)
P<0.001

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CHAPTER IV

Cerebral Oxygenation and Regional Cerebral Perfusion Responses with Resistance Breathing and Central Hypovolemia

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ABSTRACT

Resistance breathing has been shown to improve tolerance to central hypovolemia induced by lower body negative pressure (LBNP), but this was not related to protection of anterior cerebral blood flow (indexed by mean middle cerebral artery velocity, MCAv). We hypothesized that inspiratory resistance breathing improves tolerance to central hypovolemia by maintaining cerebral oxygenation (ScO_2), and protecting cerebral blood flow in the posterior cerebral circulation (indexed by posterior cerebral artery velocity, PCAv). Eight subjects (4 M, 4 F) completed two experimental sessions of a presyncopal-limited LBNP protocol (3 mmHg/min onset rate) with and without (Control) resistance breathing via an impedance threshold device (ITD). ScO_2 (via near-infrared spectroscopy), MCAv and PCAv (both via transcranial Doppler ultrasound), and arterial pressure (via finger photoplethysmography) were measured continuously. Hemodynamic responses were analyzed between the Control and ITD condition at baseline (T1) and 10 seconds prior to presyncope in the Control condition (T2). Breathing on the ITD increased tolerance from the control experiment from 1506 ± 75 s to 1704 ± 88 s (P=0.003). Both mean MCAv and mean PCAv were similar between conditions at T2 ($P \geq 0.46$), and decreased by the same magnitude with and without ITD breathing ($P \geq 0.53$). ScO_2 decreased by approximately 9% with or without ITD breathing at T2 (P = 0.972), and there were also no differences in deoxygenated (dHb) or oxygenated hemoglobin (HbO_2) between conditions ($P \geq 0.43$) at T2. There was no evidence that protection of regional cerebral blood flow (i.e., anterior or posterior cerebral circulation), nor cerebral oxygen extraction played a key role in the determination of tolerance to central hypovolemia with resistance breathing.

INTRODUCTION

Prolonging the therapeutic window for treatment is imperative for survival from a multitude of life-threatening events such as hemorrhage, cardiac arrest, and stroke. Hemorrhage accounts for 30-40% of traumatic deaths (20), most often occurring within 6 hours of injury (2, 20); up to 80% of battlefield deaths from hemorrhage are considered potentially survivable (14). A major factor contributing to death and disability from severe blood loss is prolonged cerebral hypoperfusion and reduced tissue oxygenation (28). As such, investigating methods for improving perfusion and oxygenation of the cerebral tissues is essential for improving survival and reducing long-term complications from these injuries.

Inspiratory resistance breathing has been extensively investigated under various conditions of reduced vital organ perfusion such as cardiac arrest (24, 31, 42) and hemorrhage (25, 37, 40, 41). Resistance breathing works to enhance the physiological processes that occur during normal inspiration, that is, a decrease in intrathoracic pressure (ITP), which results in increased venous return, cardiac output (CO), and mean arterial pressure (MAP) (7, 25). A commonly investigated resistance breathing device, the impedance threshold device (ITD), consists of a rubber valve and spring mechanism that requires additional inspiratory effort (-7 cm H_2O; 5mmHg) before inflow of air can be achieved (8). Upon inspiration through the ITD, ITP is further reduced, which then augments circulation back to the heart and further increases arterial pressure (8). In a number of animal studies, resistance breathing has been shown to reduce intracranial pressure (ICP) and, subsequently, increase cerebral perfusion pressure (CPP) (40, 41).

Use of inspiratory resistance increases survival time from severe hemorrhage and cardiac arrest in swine (40-42), and increases tolerance to central hypovolemia (via application of lower body negative pressure, LBNP) in healthy conscious humans (6, 8, 27, 33). This protective effect in humans under conditions of acute central hypovolemia has been associated with the maintenance of stroke volume (SV), CO, and arterial blood pressure (4, 26), but not the protection of cerebral blood flow (indexed by mean middle cerebral artery velocity, MCAv), despite maintenance of CPP (via maintained MAP, and presumably, reduced ICP) (33). Instead, it was postulated that the observed increase in low frequency (LF; 0.04-0.15 Hz) oscillations in MCAv may be associated with the delayed reporting of presyncopal symptoms, possibly related to the release of local vasodilators that could enhance tissue perfusion and oxygenation (32, 33). Cerebral tissue oxygenation (ScO_2) could also be enhanced via compensatory increases in oxygen extraction with decreased cerebral blood flow, as recently proposed by Lewis et al. (21). This response may explain, in part, the growing body of evidence that cerebral blood flow per se, is not a determinant of tolerance to central hypovolemia (17, 22, 33). The role of resistance breathing on ScO_2 has not been assessed under conditions of cerebral hypoperfusion with central hypovolemia.

An alternative explanation for the observed increased LBNP tolerance is that ITD breathing may also preferentially protect the posterior cerebral circulation feeding the brain stem, the location of autonomic and respiratory control centers. Recently, Ogoh et al. (29) proposed that blood flow to the cerebral tissues may differ between the internal carotid arteries (ICA) which feed the anterior cerebral circulation, and the vertebral arteries (VA) that feed the posterior cerebral circulation under conditions of hypovolemia. They reported that blood flow in the ICA is weakly correlated ($r=0.29;P=0.03$) with reductions in central blood volume induced

by graded LBNP up to -50 mmHg, whereas flow through the VA was unchanged (r=0.17; P=0.22) (29). Although Ogoh et al. did not specifically investigate tolerance to central hypovolemia, they speculated that posterior cerebral blood flow would only decrease with severe orthostatic stress, and suggested that differences in regional cerebral blood flow may play an important role in tolerance to reduced blood volume.

The effect of resistance breathing on regional cerebral perfusion and cerebral oxygenation has not been investigated under any condition. We hypothesized that inspiratory resistance breathing would maintain ScO_2 to compensate for reductions in anterior cerebral blood flow (indexed by mean MCAv), and would also protect cerebral perfusion in the posterior cerebral circulation (indexed by posterior cerebral artery velocity, PCAv), thus improving tolerance to central hypovolemia.

METHODS

Subjects

Twenty-seven 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 electrocardiogram (ECG) and blood pressure measurements. Females underwent a urine pregnancy test and were excluded if pregnant; the pregnancy test was repeated immediately prior to each experiment. All female subjects were tested in the early follicular phase of their menstrual cycle (days 1-4), determined by self-report. 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 the day prior to each experiment. 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^{0}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 SV via infrared finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). Respiration rate and end tidal CO_2 ($etCO_2$) were measured on a breath-by-breath basis through a sealed facemask (7940 Series, Hans Rudolph Inc., Shawnee, KS) via capnography (ML206 Gas

Analyzer, AD Instruments, Bella Vista, NSW, Australia). Inspiratory and expiratory pressures were directly recorded from the facemask using a pressure transducer (Digimano 1000, Netech Corporation, Farmingdale, NY). 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). Oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (dHb,) total hemoglobin concentration (THC; HbO_2 + dHb), and ScO_2 [(HbO_2/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. During the ITD experimental day, subjects breathed through an impedance threshold device (ITD, ResQGARD7®, Advanced Circulatory Systems Inc., Roseville, MN) that was attached to a custom adapter (Hans Rudolph Inc., Shawnee, KS) on the facemask.

Each subject underwent 3 experimental sessions separated by at least one month. The first experimental session ("Baseline") was used to assess the subject's baseline tolerance to a presyncopal-limited LBNP protocol. The protocol consisted of a 5-min rest period followed by continuous application of negative pressure at a decompression rate of 3 mmHg/min until the onset of presyncope, determined by one or more of the following criteria: 1) 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. Only the time to presyncope data from the "Baseline" experiments is reported for the current study; all hemodynamic data is included in a separate manuscript (currently in review; Chapter 2).

When subjects returned to the laboratory for their second LBNP exposure, they participated in either the "Control" or "ITD" LBNP protocols (randomized, cross-over design to account for the possible order effect of the ITD intervention protocol always following the control protocol):

1) **"Control" LBNP Protocol**: The "Control" protocol was identical to the "Baseline" protocol described previously. Subjects were exposed to progressively decreasing LBNP at a rate of 3 mmHg/min until the onset of presyncopal symptoms.

2) **"ITD" LBNP Protocol**: Subjects were exposed to progressively decreasing LBNP at a rate of 3 mmHg/min, but 5-min prior to the pre-determined time of presyncope (determined from the "Baseline" protocol on day 1), *or* with a 30% reduction in SV from baseline (whichever came first), the ITD was placed on the facemask, and subjects were instructed to breathe spontaneously at a rate and depth most comfortable to them. Application of LBNP continued at the same rate (3 mmHg/min) until the onset of presyncopal symptoms.

Data analysis

All continuous waveform data (ECG, arterial pressure, SV, MCAv, PCAv, ScO_2, THC, $etCO_2$) were collected at 1000 Hz (PowerLab and 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 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. Cerebrovascular resistance (CVR) was calculated as MAP divided by MCAv, and MAP divided by PCAv. Total peripheral resistance (TPR) was calculated as MAP divided by CO.

Oscillatory patterns of MAP, mean MCAv, mean PCAv, and ScO_2 were determined via power spectral analysis. Data was made equidistant by interpolating linearly and resampling at 5 Hz. Data were passed through a low-pass filter with a cutoff frequency of 0.5 Hz. Four-minute data sets were fast Fourier transformed to obtain power spectra, and are expressed as the integrated area within the low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz) ranges.

All time domain and frequency domain variables were analyzed from the final 4 minutes of the rest period prior to initiation of LBNP during both the “Control” and “ITD” experiments, and were designated as time “T1”. To examine the hemodynamic effects of resistance breathing via the ITD during LBNP, the time point of presyncope during the “Control” trial was first identified for each subject (“T2”). Data were then analyzed for both the “Control” and “ITD” experiments at this T2 reference time point. All time domain variables were calculated from the 10 seconds prior to T2 in order to capture the dynamic responses of cardiovascular collapse and to examine the potential protective effects of resistance breathing at this time. Respiration rate was calculated for the 60 seconds prior to T2, as 10 seconds is not sufficient to accurately assess

this variable. The frequency domain variables were analyzed over the 4 minutes prior to T2 during the "Control" and "ITD" experiments. Data were also compared for the last 60 seconds prior to presyncope (PS-1) between the two experiments.

Statistical Analysis

A one-way repeated measures analysis of variance (ANOVA) was used to compare LBNP tolerance times between the Baseline, Control, and ITD experiments, followed by Tukey post-hoc tests. Two-way (time, T1 vs. T2; experiment, Control vs. ITD) repeated measures ANOVAs, followed by Tukey post-hoc tests were used for comparison of all hemodynamic variables. Paired t-tests were used to compare the PS-1 data from the ITD vs. Control experiments. All data are presented as mean ± SE (unless otherwise stated), and exact P-values are reported for all comparisons.

RESULTS

Subject Selection

Of the 27 subjects who participated in this study, data was analyzed and included from 8 subjects (4 male, 4 female; age 26 ± 3 yrs; height, 170 ± 11 cm; weight, 74 ± 11 kg; means ± SD). Subjects were only included in the final analysis if they 1) reached true presyncope during both experimental conditions (i.e., Control and ITD); 2) reached adequate "cracking" pressures during ITD breathing (i.e., at least -7 cm.H_2O); and 3) exhibited a difference in tolerance between the two experiments of at least 60-s. First, true presyncope was defined as average SAP < 100 mmHg for the 1-min prior to presyncope and/or minimum SAP ≤ 90 mmHg within the 1-min prior to presyncope; subjects who reported subjective symptoms only without reaching

this objective arterial pressure threshold for both experiments were not included (n=4). Second, as we were investigating the role of resistance breathing on cerebral blood flow and oxygen regulation, we had to ensure that subjects breathing on the ITD consistently reached cracking pressures of at least -7 cm.H_2O; 4 subjects did not reach this criterion, likely reflecting a leak in the mask set-up. Finally, as the ITD was being investigated as an intervention for improving tolerance to central hypovolemia, subjects were only included in the final analysis if tolerance was different between experiments by at least 60 s, as less than 60 s was not considered clinically significant; 9 subjects were excluded based on this criterion. Additionally, 2 subjects exhibited reduced tolerance to LBNP when using the ITD (-95 s and -164 s), and were excluded from analysis; this small sample size does not facilitate statistical comparison of these two subjects with the 8 subjects who exhibited improved tolerance with ITD breathing. Reliable PCAv measurements were only obtained in 4 of the 8 subjects under both conditions at all time-points of interest.

LBNP Tolerance

There was no difference in time to presyncope between the Baseline and Control experiments (P=0.89). Breathing on the ITD increased LBNP tolerance from the control experiment (without ITD) from 1506 ± 75 s to 1704 ± 88 s (P=0.003), an average of 3-min and 18-s. Tolerance was also increased from the baseline experiment from 1480 ± 119 s to 1704 ± 88 s (P=0.004) when breathing on the ITD.

Hemodynamic Responses to Resistance Breathing During LBNP (T2 Comparisons)

All of the time domain variables at rest (T1) were similar between the Control and ITD experiments ($P \geq 0.29$), except $etCO_2$ was higher for the ITD condition ($P=0.02$; Table 1). During the Control experiment, SV, CO, and arterial pressure (SAP, DAP, MAP) all decreased during LBNP until the point of presyncope (T2); these decreases were attenuated with resistance breathing (Figures 1-2 & Table 1). Given that HR and SV are both contributing factors to CO, the protection of CO during resistance breathing is primarily due to protection of SV, as HR increased to the same degree in both experiments at T2 (Control: 96 ± 11 beats/min vs. ITD: 102 ± 7 beats/min; $P=0.29$) (Figure 2). TPR did not increase with LBNP under either condition ($P \geq 0.28$), and there was no difference in TPR between conditions at T2 ($P=0.11$; Table 1).

Both absolute mean MCAv and mean PCAv were similar between conditions at T2 ($P \geq 0.46$; Figure 3), and decreased by the same magnitude with and without ITD breathing ($P \geq 0.53$) (Table 1). Resistance breathing increased MCAv CVR from baseline to T2 (1.5 ± 0.1 vs. 2.0 ± 0.3 mmHg/cm/s; $P=0.003$), but MCAv CVR did not change from baseline in the control condition (1.5 ± 0.1 mmHg/cm/s vs. 1.5 ± 0.1 mmHg/cm/s; $P=0.53$); at T2 MCAv CVR was higher in the ITD condition (2.0 ± 0.3 mmHg/cm/s) compared to the Control condition (1.5 ± 0.1 mmHg/cm/s; $P=0.003$). PCAv CVR did not differ between conditions at T2 (Control: 2.7 ± 0.5 mmHg/cm/s vs. ITD: 3.2 ± 0.1 mmHg/cm/s; $P=0.23$), but did increase from baseline with resistance breathing (T1: 2.5 ± 0.3 mmHg/cm/s vs. T2: 3.2 ± 0.1 mmHg/cm/s; $P=0.07$). ScO_2, HbO_2, and THC all decreased, and dHb increased for both experiments ($P \leq 0.013$; Figure 4 and Table 1), but were also similar between the control and ITD conditions at T2 ($P \geq 0.35$). ScO_2 decreased by approximately 9% with or without ITD breathing at T2 ($P=0.97$). HbO_2 decreased by 13 ± 3% in the control condition at T2, and by 14 ± 4% in the ITD condition ($P=0.69$), while

dHb increased by 12 ± 2% in the control condition, and by 9 ± 3% in the ITD condition (P=0.16). While there was a reduction in respiration rate at T2 with ITD breathing compared with the control condition (Control: 15.0 ± 3.0 breaths/min vs. ITD: 11.0 ± 1.7 breaths/min; P=0.002), the fall in $etCO_2$ was not protected with resistance breathing (P=0.37; Δ from baseline, Table 1).

Responses to Resistance Breathing at Presyncope:

When comparing the 60-s prior to presyncope for both the ITD and Control conditions, MAP, PCAv, and $etCO_2$ all fell to similar levels between groups (P≥0.36) (Table 2). However, with resistance breathing, there were greater increases in HR (P=0.01), and greater reductions in SV (P=0.04), ScO_2 (P=0.01), HbO_2 (P=0.09), and MCAv (P=0.03) compared to the control condition.

Oscillatory Responses to Resistance Breathing During LBNP:

Absolute LF oscillatory power for MAP, mean MCAv, mean PCAv, and ScO_2 all increased numerically from T1 to T2 but were not statistically distinguishable (P≥0.11), nor were they different between conditions at T2 (P≥0.13; Table 3). The percentage change from baseline for both MAP LF and MCAv LF increased with ITD breathing (P=0.05 for both), but not under the control condition (P≥0.46); only MCAv LF (% change) was different between conditions at T2 (P=0.06). However, with resistance breathing, HF power for MAP, mean MCAv, mean PCAv, and ScO_2 all increased at T2 compared to T1 (P≤0.02; Figure 5), and were all higher compared with the control condition at T2 (P≤0.02), except for ScO_2 (P=0.18). Respiration rate for the 4-min prior to T2 was compared between the Control and ITD conditions (this timeframe

is coincident with the frequency domain analysis), and was lower with resistance breathing (P=0.02; Table 3), but was still within the HF range (0.15-0.4 Hz; 9-24 cycles/min).

DISCUSSION:

In this study we examined the role of cerebral oxygenation and regional cerebral blood flow on tolerance to central hypovolemia with inspiratory resistance breathing. The key findings of this study demonstrate that increased tolerance to central hypovolemia via resistance breathing is associated with 1) an attenuated reduction in SV and MAP; and 2) increased HF oscillatory power of MAP, MCAv, PCAv, and ScO_2. There was no evidence that protection of regional cerebral blood flow (i.e., anterior or posterior cerebral circulation), nor cerebral oxygen saturation or extraction played key roles in the determination of tolerance to central hypovolemia with resistance breathing.

Both hemorrhage and LBNP result in central hypovolemia due to decreased venous return which leads to reduced SV, CO, and a subsequent reduction in arterial pressure (10, 15, 18). The ITD was specifically designed to augment the physiological responses of inspiration, by further decreasing negative ITP thereby causing an increase in venous return, SV, and CO (7, 25). Resistance breathing following severe hemorrhage in pigs has been shown to decrease ICP and right atrial pressure, resulting in increased CPP, coronary perfusion pressure, and MAP (37, 40, 41). With increased perfusion to vital organs such as the heart and brain, both acute and 24-hour survival was increased following severe hemorrhage in pigs (37). In studies of resistance breathing in healthy, conscious humans, ITD breathing protected SV, CO, and MAP during progressive LBNP, resulting in delayed presyncopal symptoms, and increased tolerance (33, 34).

Convertino et al. also recently demonstrated increased systolic and diastolic blood pressures with inspiratory resistance in patients with hypotension secondary to blood loss or trauma (5).

This study is the first to report cerebral blood velocity responses within both the anterior and posterior cerebral circulations, and ScO_2 responses with inspiratory resistance breathing during central hypovolemia to presyncope. Consistent with previous studies, we found that resistance breathing during progressive LBNP protects SV, CO, and MAP thus delaying presyncope, while having no effect on HR, or cerebral blood velocity in the anterior circulation (indexed by mean MCAv) (33, 34). Moreover, resistance breathing did not prevent the fall in ScO_2 in the frontal cortex, nor did it protect the reduction in cerebral blood velocity in the posterior circulation (indexed by mean PCAv). A previous study from our laboratory comparing high versus low tolerant subjects during presyncopal-limited LBNP [Kay & Rickards, In review; Chapter 2], demonstrated that high tolerant subjects exhibit greater reductions in SV, MCAv, and ScO_2 at presyncope; the findings of the current study are consistent with this prior investigation as subjects reached a greater magnitude of central hypovolemia while breathing on the ITD.

While cerebral blood velocities within both the MCA and PCA were not protected, a number of findings from this study provide indirect evidence that cerebral vessel diameter may be increasing with resistance breathing, challenging the assumption of constant vessel caliber, and subsequent equivalence of cerebral blood velocity to cerebral blood flow. Resistance breathing ameliorated the reduction in MAP in the current study, and has also been shown to decrease ICP in a number of animal studies (37, 40, 41). Together, protection of MAP and a reduction in ICP should result in protection of CPP. If CPP is maintained, but cerebral blood flow *velocity* is not protected in either the MCA or PCA, this indirectly suggests that the vessel diameter may be increasing, resulting in increased flow, but, subsequently, decreased velocity.

Alternatively, disparity between responses of cerebral perfusion pressure and cerebral blood flow could indicate increased cerebrovascular resistance; while this would result in a decrease in flow, it would increase velocity, which did not occur. The observed increase in cerebrovascular resistance with ITD breathing (calculated from MAP and MCAv or PCAv) should be interpreted with caution as it is possible that the reduction in velocity is not an accurate representation of CBF dynamics.

Rickards et al. previously demonstrated that the ITD breathing-induced increase in tolerance to central hypovolemia is not associated with protection of absolute MCAv (33), a finding replicated in the current study. Instead, it was suggested that the increase in amplitude of LF MCAv oscillations may elicit a shear-stress mediated vasodilation, with a subsequent increase in flow and oxygen delivery. In the current study, we similarly report a relative increase in LF power of MCAv with resistance breathing (% change from baseline), in addition to the novel finding of markedly enhanced HF oscillatory power of arterial pressure, cerebral blood velocity (MCAv and PCAv) and ScO_2, associated with higher tolerance to decreased central blood volume. LF oscillations are predominantly due to sympathetic modulation of arterial pressure via the baroreflex (9, 19, 35), with subsequent transfer to the cerebral circulation. HF oscillations in the range assessed in the current study (0.15-0.4 Hz) are predominantly influenced by respiration (1, 3, 13), with greater tidal volumes eliciting increases in the amplitude of HF hemodynamic oscillations (i.e., increased power) (30). Respiration can also impact LF oscillations if the breathing rate is within this frequency range (0.04-0.15 Hz), equating to 2.4 to 9.0 breaths/min; in the current study, however, most subjects were breathing in the HF range (i.e., ≥9 breaths/min) under both Control and ITD conditions. ITD breathing was associated with a lower (P=0.001) respiration rate at T2 (11.0 ± 1.7 breaths/min) compared with the control

condition (15.3 ± 3.0 breaths/min), and a higher amplitude of HF oscillations in MAP, MCAv, PCAv, and ScO_2. Together, these findings suggest that resistance breathing may induce an increase in the depth of breathing (i.e., tidal volume), a proposal supported by a previous study demonstrating an increase in tidal volume with ITD breathing at rest (0.86 vs. 0.67 liters) (7). In addition, Lucas et al. compared the effect of controlled breathing (6 breaths/min; 0.1 Hz) vs. spontaneous breathing (16-20 breaths/min) on tolerance to central hypovolemia (combined head-up tilt and LBNP). Subjects breathing at 0.1 Hz had increased tolerance to central hypovolemia, and also exhibited much larger tidal volumes compared with the spontaneous breathing condition (2.2 vs. 1.1 liters) (23). The greater tidal volumes likely represent greater reductions in ITP and ICP which may further augment the decreases in ITP and ICP elicited by ITD breathing, culminating in further increases in CPP, which could play a major role in the observed increase in tolerance to central hypovolemia. As previously speculated (33, 43), it is also possible that the greater amplitude of HF oscillations elicits a shear stress effect on the cerebrovasculature causing release of local vasodilators that increase vessel diameter, and subsequently, flow. It is possible that increasing the depth of breathing has the same physiological effect as increasing the resistance to breathing. Studies assessing tolerance to central hypovolemia with controlled variations in both the depth and frequency of breathing, in addition to direct measures of CBF, and measurement of vasoactive mediators (e.g., nitric oxide, prostaglandins) could further elucidate this mechanism.

In the current study, we also measured ScO_2 in the frontal cortex using NIRS, a measurement that is not dependent on the assumption of constant vessel caliber. Under both ITD and control conditions, however, ScO_2, THC, HbO_2, and dHb all decreased from baseline to T2,

and there were no differences in these responses between the two conditions. By presyncope with ITD breathing, however, HbO_2 and ScO_2 were both less than in the control condition, suggesting an increase in oxygen extraction due to greater decreases in CBF, likely reflecting the greater magnitude of central hypovolemia (Table 2). These data suggest that ITD breathing did not modify oxygen extraction within the cerebral tissues at T2, reflecting similar metabolic demand. Direct measurement of cerebral blood flow (i.e., oxygen delivery) and cerebral oxygen extraction (via cerebral arterial to venous oxygen difference), however, is necessary to confirm this speculation.

Methodological Considerations:

Although LBNP does not simulate all the responses observed in traumatic hemorrhage such as pain, tissue trauma and whole red blood cell loss, this model of inducing central hypovolemia has recently been validated against actual hemorrhage (15, 18). These studies demonstrated that LBNP elicits similar reflex cardiovascular responses to compensate for reductions in central blood volume as actual hemorrhage. However, hemorrhage elicits reductions in hematocrit and hemoglobin, due to loss of whole red blood cells (15, 18), while hematocrit and hemoglobin increase during LBNP due to the shift in plasma from the intravascular space to the extravascular space (15). This experimental model of central hypovolemia in healthy, conscious humans allows for the isolation of hemodynamic and cerebrovascular responses without the many confounding factors associated with traumatic hemorrhage.

For the measurements of ScO_2, HbO_2, and dHb concentrations within the cerebral tissue, we used the non-invasive NIRS technique. It has been suggested that this method of

measurement could be contaminated by flow and oxygenation within the skin (12, 16, 38). To reduce the likelihood of skin contamination in the current study, we used a spatially resolved NIRS sensor with 4 light emitters (2.0, 2.5, 3.0, and 3.5 cm from the detector). Since the use of multiple emitter distances enables the measurement of HbO_2 and dHb from different depths, mathematical corrections can be applied to remove extracranial sample volume contamination (i.e., skin, muscle, and fat). We also speculate that posterior cerebral oxygenation could play a key role in tolerance to central hypovolemia, but we are limited by the capabilities of our NIRS device to only measure frontal lobe oxygenation.

We are also limited by the fact that TCD is used to measure cerebral blood velocity only, and not actual flow. While Serrador et al. has demonstrated with magnetic resonance imaging (MRI) that MCA diameter remains unchanged with application down to -40 mmHg LBNP (36), it is unknown if cerebral vasoconstriction occurs at higher intensities of LBNP to the limit of tolerance. Other recent studies utilizing higher resolution MRI have indicated that hypercapnia $\geq$ 9 mmHg above baseline elicits MCA vasodilation, while hypocapnia to $\geq$ 13 mmHg below baseline elicits MCA vasoconstriction (11, 39). In our current study, during both conditions, $etCO_2$ decreased by $\geq$14 mmHg, suggesting that cerebral vasoconstriction may be taking place, and measurements of cerebral blood velocity via TCD may be overestimating actual cerebral blood flow. The magnitude change in $etCO_2$ was similar between ITD and Control conditions, however, so this potential confounding factor affected both conditions equally.

Finally, the small number of subjects with PCAv data may limit interpretation of these findings. However, the homogeneity of these responses within this sub-set of subjects (4 out of the 8 total subjects), and the robust statistical comparison provide confidence in these responses, despite the small sample size.

***Conclusions**:*

Although inspiratory resistance breathing improved tolerance to central hypovolemia, these findings suggest that it does not do so by protecting ScO_2 to compensate for reductions in anterior cerebral blood flow (indexed by mean MCAv), nor does resistance breathing protect cerebral blood flow in posterior cerebral circulation (indexed by PCAv). However, we speculate that improved tolerance to central hypovolemia via resistance breathing may be due, in part, to ITD breathing causing a decrease in respiration rate and an increase in depth of breathing, subsequently decreasing ICP, and increasing CPP, thus delaying the onset of presyncope.

Table 1: Hemodynamic responses during progressive lower body negative pressure (LBNP) to presyncope with (ITD) and without inspiratory resistance breathing (Control).

Time Domain Parameter	Control			ITD			T2 vs. T2 P-value
	T1	T2	P-Value	T1	T2	P-Value	
SAP, mmHg	128.5 ± 3.2	85.6 ± 3.5	< 0.001	131.5 ± 4.7	108.3 ± 3.1	0.001	< 0.001
DAP, mmHg	74.3 ± 2.5	55.6 ±4.1	< 0.001	74.0 ± 1.8	72.2 ± 2.3	0.64	< 0.001
TPR, mmHg/l/min	17.5 ± 1.6	16.6 ± 1.7	0.47	17.0 ± 1.1	18.5 ± 2.0	0.28	0.11
Resp. Rate, breaths/min	12.9 ± 1.4	15.0 ± 3.0	0.31	12.5 ± 1.2	11.0 ± 1.7	0.47	0.002
$etCO_2$, mmHg	40.1 ± 2.5	25.7 ± 1.5	0.002	46.2 ± 1.0*	30.0 ± 3.6	< 0.001	0.09
$etCO_2$, Δ from T1	-	-14.4 ± 3.1	0.002	-	-16.2 ± 3.5	< 0.001	0.37
Mean MCAv, % Δ from T1	-	-34.2 ± 5.0	< 0.001	-	-30.5 ± 4.4	< 0.001	0.53
Mean PCAv, % Δ from T1**	-	-32.3 ± 4.6	0.001	-	-31.6 ± 4.8	0.002	0.84
Total Hb, µM	52.4 ± 5.8	49.7 ± 5.5	0.01	50.6 ± 5.5	48.0 ± 6.1	0.01	0.48

Data are presented as means ± SE. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; TPR, total peripheral resistance, etCO2, end tidal carbon dioxide; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; Total Hb, total hemoglobin. *, P=0.02 for T1 vs. T1 time points; **, N=4 for PCA.

Table 2: Hemodynamic responses at the point of presyncope during lower body negative pressure (LBNP) in the Control vs. ITD condition.

	Control	ITD	P-value
HR, beats/min	99.0 ± 8.1	121.6 ± 7.6	0.01
SV (% Δ from T1)	-46.1 ± 5.2	-56.4 ± 3.7	0.04
MAP, mmHg	77.1 ± 2.7	79.4 ± 2.2	0.40
TPR, mmHg/l/min	16.8 ± 1.3	17.3 ± 1.7	0.64
ScO_2,%	61.6 ± 2.6	57.2 ± 3.0	0.01
HbO_2, µM	31.1 ± 3.9	27.3 ± 4.3	0.09
dHb, µM	19.2 ± 2.3	20.0 ± 2.7	0.27
Mean MCAv, cm/sec	48.9 ± 3.5	41.2 ± 4.4	0.03
Mean MCAv (% Δ from T1)	-27.8 ± 4.2	-38.40 ± 3.0	0.08
Mean PCAv, cm/sec**	27.4 ± 3.5	24.3 ± 0.9	0.36
Mean PCAv (% Δ from T1)**	-27.7 ± 6.5	-39.3 ± 3.3	0.14
Resp. Rate, breaths/min	15.0 ± 3.0	13.5 ± 2.0	0.37
$etCO_2$, mmHg	27.7 ± 2.1	25.8 ± 3.3	0.51

Data are presented as means ± SE. HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; TPR, total peripheral resistance MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; ScO_2, cerebral oxygen saturation; HbO_2, oxygenated hemoglobin concentration; dHb, deoxygenated hemoglobin concentration; THC, total hemoglobin concentration; $etCO_2$, end tidal carbon dioxide. **, N=4 for PCA.

Table 3: Low frequency (LF) oscillatory responses during progressive lower body negative pressure (LBNP) to presyncope.

Frequency Domain Parameter	Control			ITD			T2 vs. T2 P-value
	T1	T2	P	T1	T2	P	
MAP LF, $mmHg^2$	6.0 ± 1.3	19.1 ± 6.9	0.43	11.6 ± 5.4	39.4 ± 25.2	0.11	0.17
MAP LF, (%Δ)	-	235.8 ± 99.1	0.46	-	667.2 ± 426.4	0.05	0.19
MCAv LF, $(cm/s)^2$	7.0 ± 1.8	8.2 ± 3.6	0.79	6.4 ± 2.8	14.1 ± 7.5	0.12	0.13
MCAv LF, (%Δ)	-	13.3 ± 23.9	0.91	-	238.7 ± 153.4	0.05	0.06
PCAv LF, $(cm/s)^2$ **	1.6 ± 0.4	2.4 ± 1.3	0.68	2.2 ± 0.7	4.4 ± 1.9	0.22	0.17
ScO_2 LF, $\%^2$	0.19 ± 0.08	0.26 ± 0.08	0.22	0.20 ± 0.07	0.25 ± 0.05	0.43	0.75
Resp. Rate, breaths/min	12.9 ± 1.3	14.0 ± 2.7	0.90	12.5 ± 1.2	11.2 ± 1.5	0.30	0.02

Data are presented as means ± SE. LF, low frequency; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity; ScO_2, cerebral oxygen saturation. **, N=4 for PCA.

FIGURE LEGENDS:

Figure 1 Percent change from baseline for stroke volume (SV; Panel A) and cardiac output (CO; Panel B) in the Control condition (black bar) vs. ITD condition (white bar) at T2 (the time point of presyncope in the control condition).

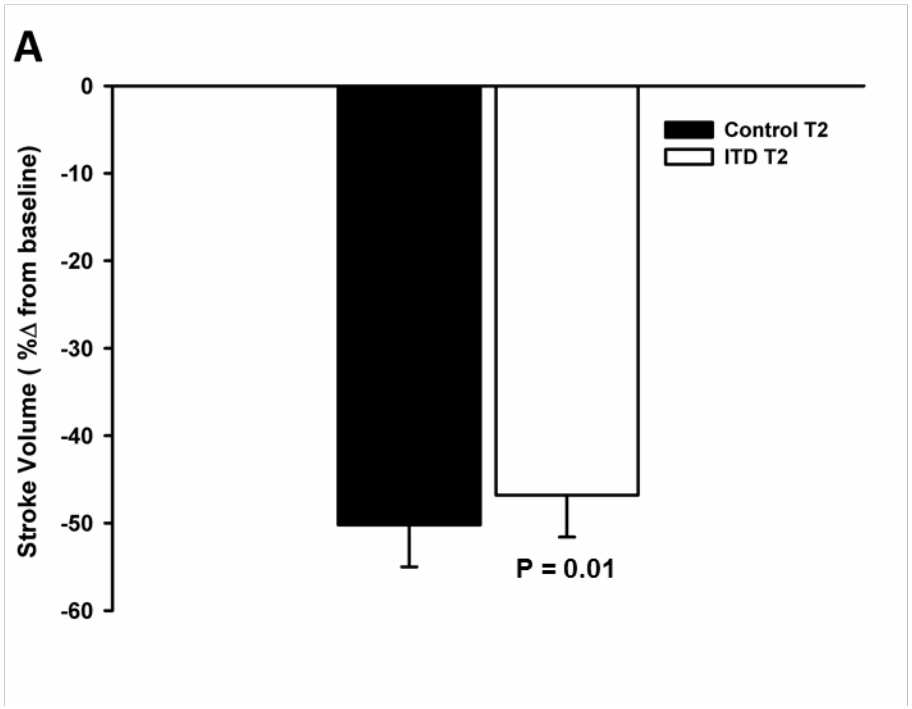
Figure 2 **Panel A:** Mean arterial pressure (MAP) decreased from baseline (T1, black bars) to T2 (time point of presyncope in the control condition, white bars) in both the Control condition (left) and ITD condition (right). **Panel B:** Heart rate (HR) increased from baseline to T2 in the Control condition (left) and ITD condition (right). *, denotes $P \leq 0.02$ compared with baseline within conditions; †, denotes P=0.001 between conditions (T2 vs. T2).

Figure 3 Absolute reductions in middle cerebral artery velocity (MCAv, **Panel A**) and posterior cerebral artery velocity (PCAv, **Panel B**) from baseline (T1, black bars) to T2 (time point of presyncope in the control condition, white bars). *, denotes $P \leq 0.001$ compared with baseline within conditions.

Figure 4 Cerebral oxygen saturation (ScO_2, **Panel A**), and oxygenated hemoglobin (HbO_2, **Panel B**) decreased from baseline (T1, black bars) to T2 (time point of presyncope in the control condition, white bars). Deoxygenated hemoglobin (dHb, **Panel C**) increased from T1 to T2 within each condition. *, denotes $P \leq 0.002$ compared with baseline within groups.

Figure 5 High frequency (HF) oscillatory power for mean arterial pressure (MAP, **Panel A**), cerebral oxygenation (ScO_2, **Panel B**), middle cerebral artery velocity (MCAv, **Panel C**), and posterior cerebral artery velocity (PCAv, **Panel D**). Resistance breathing via the ITD increased HF oscillations in MAP, ScO_2, MCAv, and PCAv. *, denotes $P \leq 0.02$ compared with baseline within conditions; †, denotes P=0.02 between conditions.

Figure 1


A
Stroke Volume (%Δ from baseline)
0
-10
-20
-30
-40
-50
-60
Control T2
ITD T2
P = 0.01

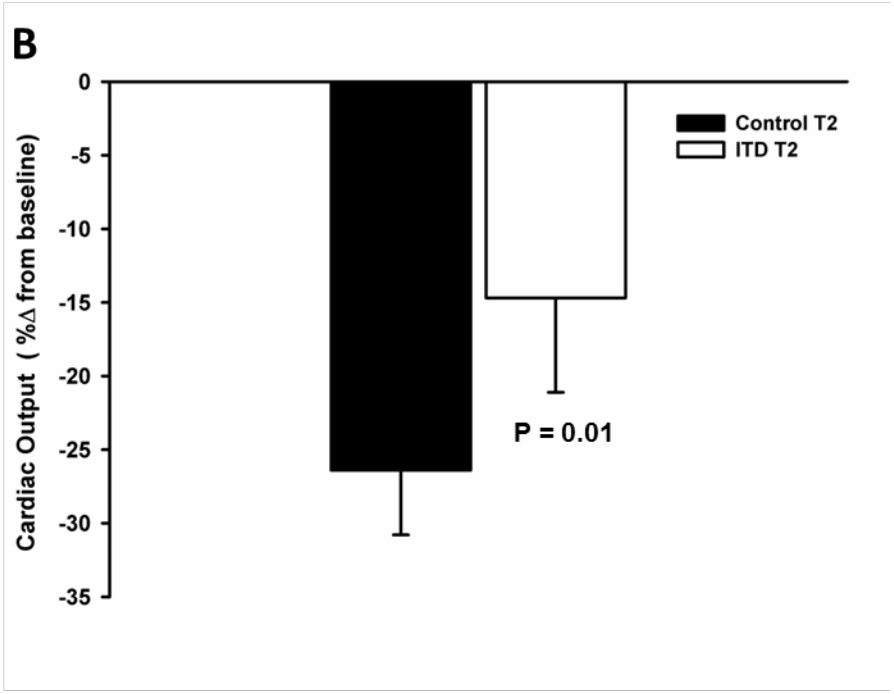

B
Cardiac Output (%Δ from baseline)
0
-5
-10
-15
-20
-25
-30
-35
Control T2
ITD T2
P = 0.01

Figure 2

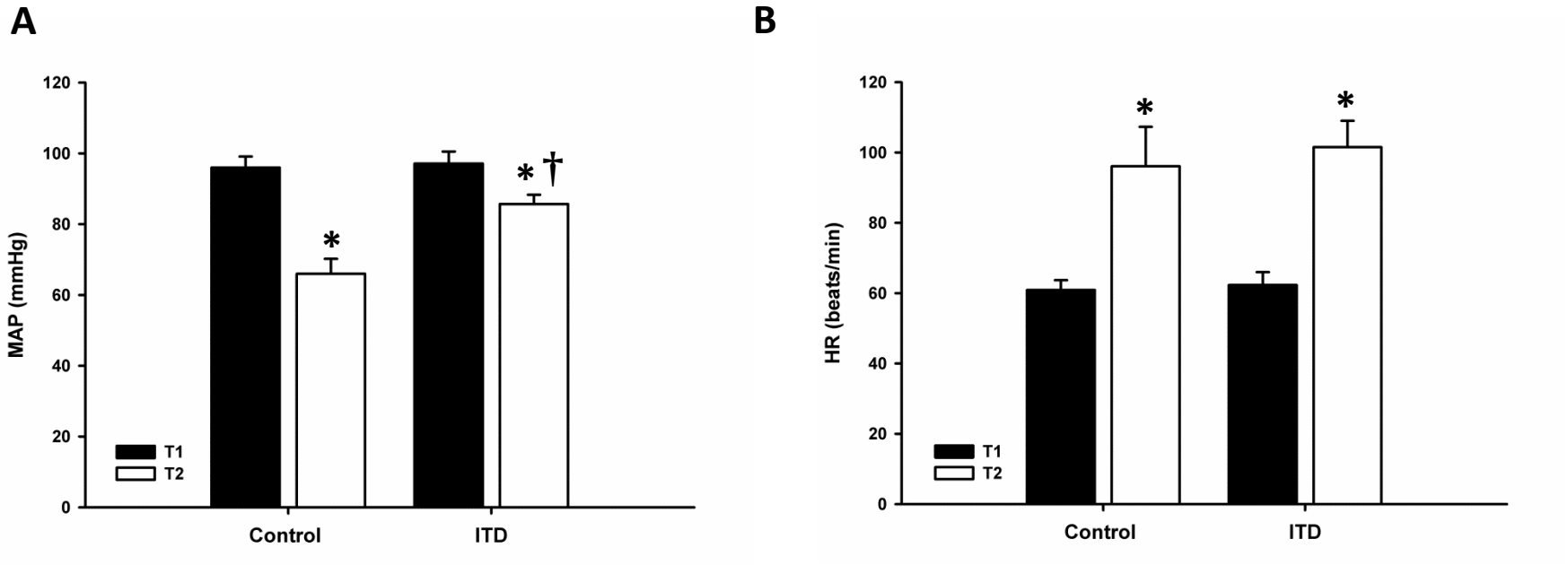

A
MAP (mmHg)
120
100
80
60
40
20
0
T1
T2
*
* †
Control
ITD
B
HR (beats/min)
120
100
80
60
40
20
0
T1
T2
*
*
Control
ITD

Figure 3

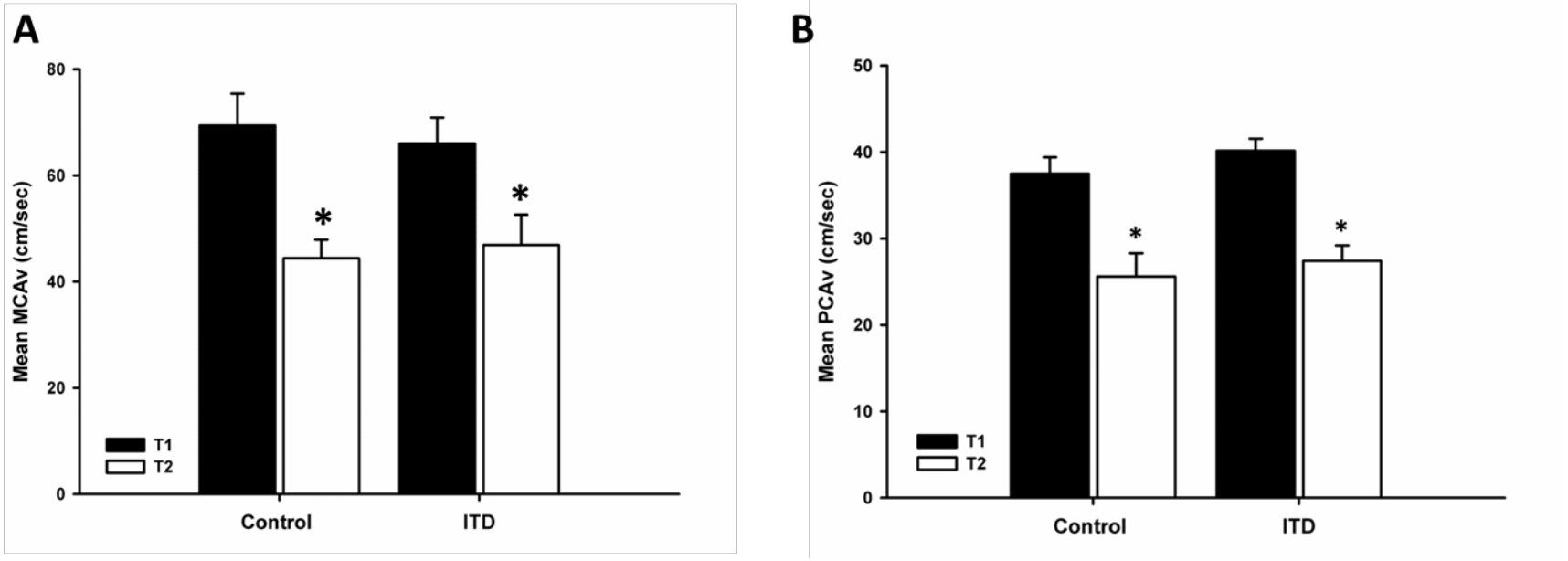
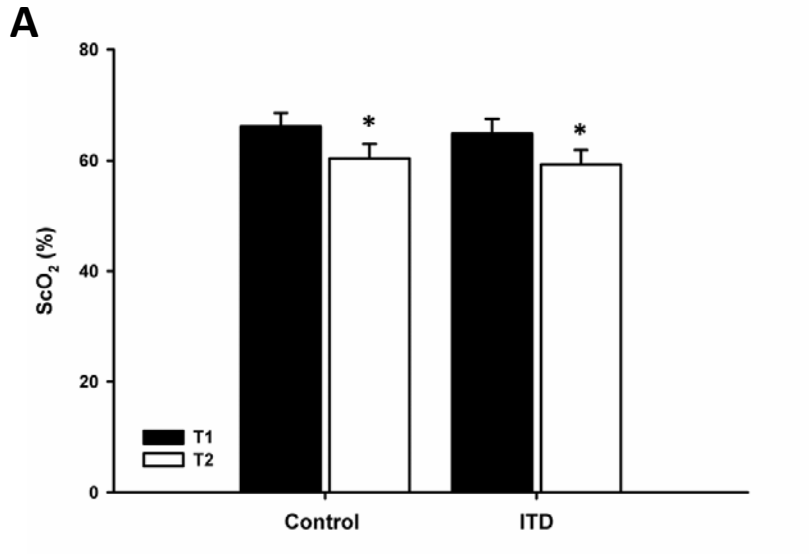
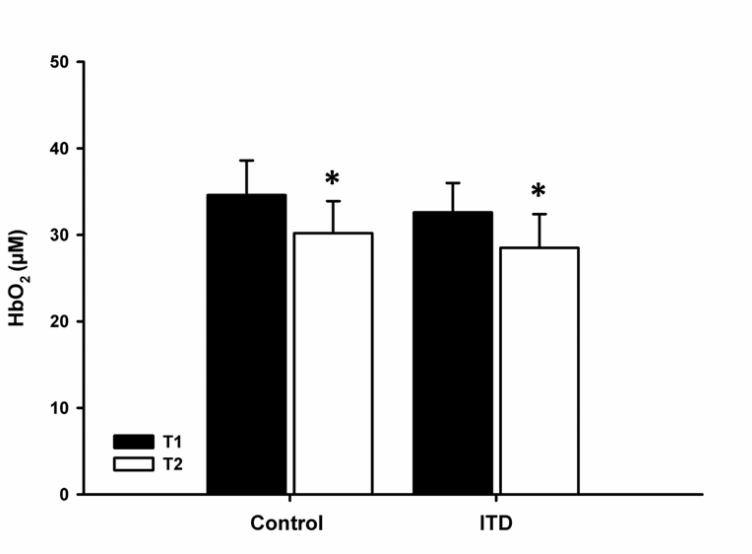

A
Mean MCAv (cm/sec)
80
60
40
20
0
T1
T2
*
*
Control
ITD
B
Mean PCAv (cm/sec)
50
40
30
20
10
0
T1
T2
*
*
Control
ITD

Figure 4


A
80
60
40
20
0
ScO2 (%)
T1
T2
*
*
Control
ITD


B
50
40
30
20
10
0
HbO2 (µM)
T1
T2
*
*
Control
ITD

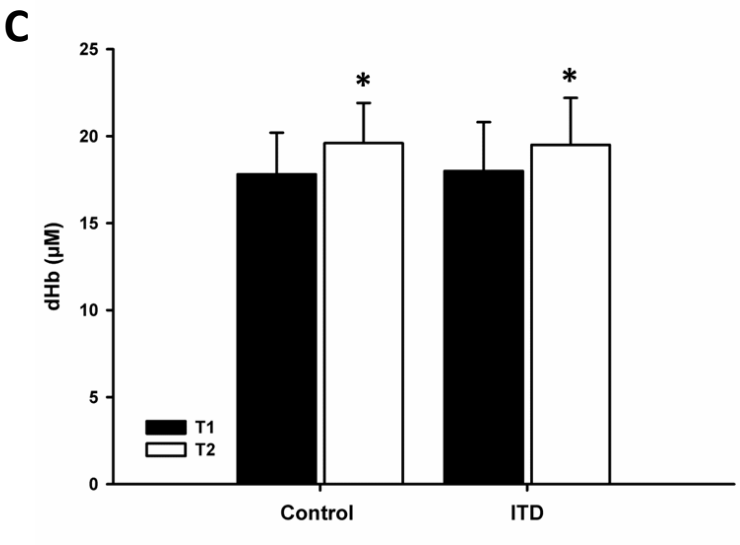
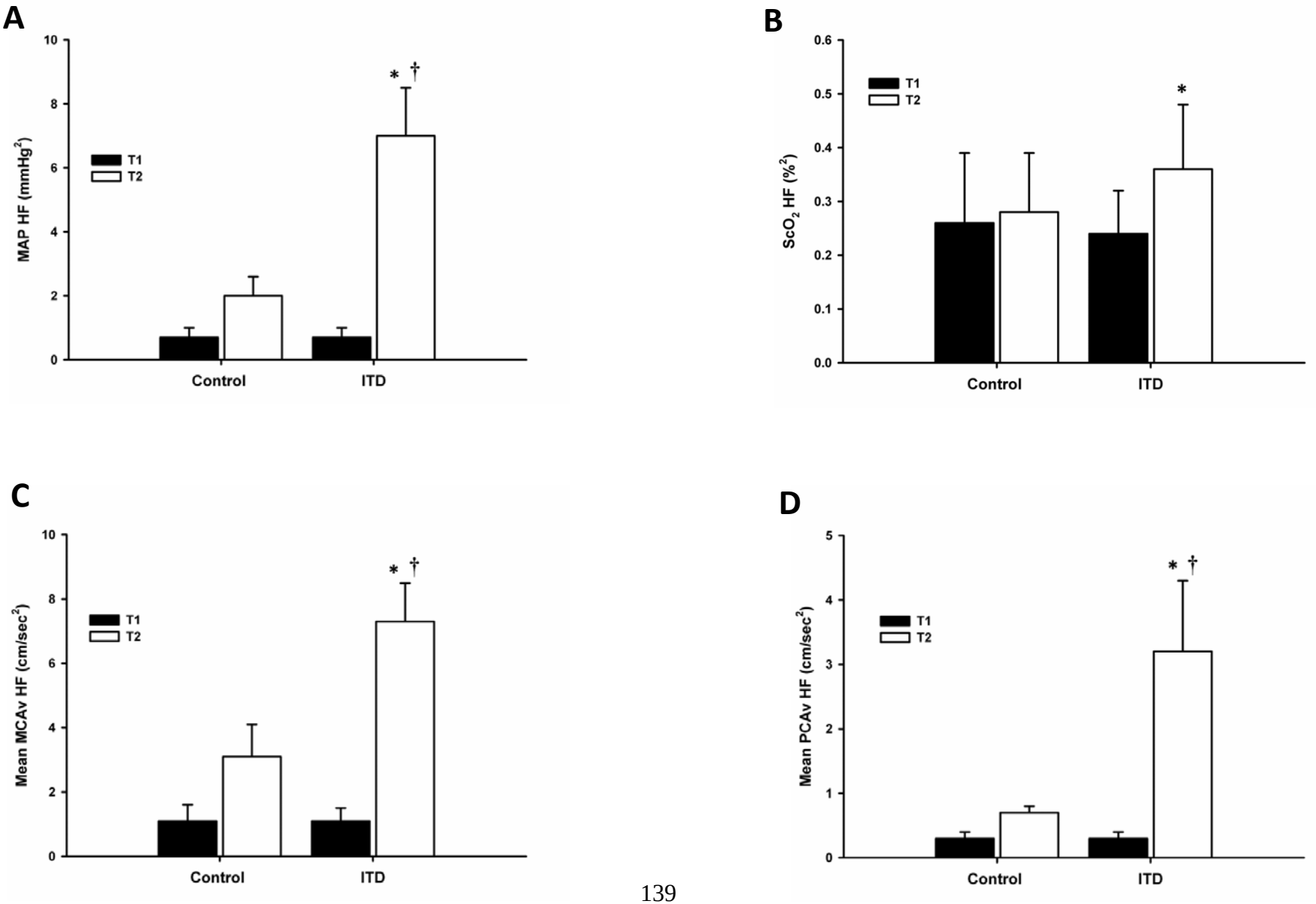

C
25
20
15
10
5
0
dHb (µM)
T1
T2
*
*
Control
ITD

Figure 5


A
MAP HF (mmHg²)
T1
T2
Control
ITD
* †
B
ScO₂ HF (%²)
T1
T2
Control
ITD
*
C
Mean MCAv HF (cm/sec²)
T1
T2
Control
ITD
* †
D
Mean PCAv HF (cm/sec²)
T1
T2
Control
ITD
* †

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CHAPTER V

CONCLUSIONS & FUTURE DIRECTIONS

The overall goal of this research was to understand the physiological mechanisms underlying tolerance to central hypovolemia, with a focus on cerebral perfusion and oxygenation. The studies presented in this dissertation examined three main topics related to this overall goal in cohorts of healthy human subjects:

1) Reproducibility of a novel ramp-LBNP profile for simulating hemorrhage;
2) The role of cerebral oxygenation and regional cerebral perfusion on tolerance to central hypovolemia
3) The effect of an inspiratory resistance breathing intervention on cerebral oxygenation and regional cerebral perfusion, and subsequent tolerance to central hypovolemia.

The first investigation (Chapter II) demonstrated that a ramp-LBNP profile with a decompression rate of 3 mmHg/min is reproducible in regards to tolerance time, hemodynamic, and cerebrovascular responses. This continuous pressure profile may more accurately simulate hemorrhage compared with a traditional step-wise pressure profile, as it simulates a continuous bleed that would occur in the clinical setting prior to intervention and hemostasis. Future studies are necessary to compare hemodynamic and cerebral blood flow responses between ramp-LBNP and actual blood loss to confirm this speculation. Additionally, as ramp LBNP may provide an

alternative methodological approach to the traditional step-wise LBNP protocol, quantification of differences and/or similarities in hemodynamic and cerebral circulation responses would be a meaningful future study.

The second investigation (Chapter III) explored mechanisms that play a vital role in determining tolerance to central hypovolemia, and delaying the onset of presyncope. Although it has been well established that there are differences in tolerance to central hypovolemia, the role of regional cerebral blood flow and oxygen regulation had not been fully elucidated in subjects taken to presyncope. The findings in this investigation demonstrated that subjects with increased tolerance to central hypovolemia had protection of posterior cerebral circulation and cerebral oxygenation (ScO_2), despite reductions in anterior cerebral circulation. Based on the maintenance of oxygenated hemoglobin responses, we also suggest that high tolerant subjects maintain constant cerebral metabolism until more severe levels of central hypovolemia are reached compared to their low tolerant counterparts. Future studies are required to directly assess oxygen extraction within the cerebral tissues via a combination of measuring actual cerebral blood flow, and arterial-venous differences to more accurately assess cerebral oxygen metabolism. In addition, measurement of glucose concentration and metabolism could provide insight into the role of substrate utilization in subjects with high vs. low tolerance to central hypovolemia. The data presented in this investigation also warrants caution for the use of ScO_2 as a surrogate for cerebral blood flow in the clinical setting for a number of important reasons, including; 1) ScO_2 and cerebral blood flow show only weak linear associations during central hypovolemia representing a reduction in SV of 30%; 2) tolerance dependent differences in the linear associations between reductions in cerebral blood flow and ScO_2; and 3) at the point of presyncope, there are greater maximal reductions in cerebral blood flow (~35%) than ScO_2 (~12

%). This study indicated that NIRS-derived oxygenated hemoglobin and/or posterior cerebral blood flow may be more sensitive indicators for tracking the onset of presyncope than the measurement of anterior cerebral blood flow and/or ScO_2.

The aim of the third investigation (Chapter IV) was to determine if protection of ScO_2 and/or posterior cerebral circulation were potential mechanisms underlying the observed improvement in tolerance to central hypovolemia with inspiratory resistance breathing. In contrast to the findings of study 2 (Chapter III), where high tolerant subjects exhibited protection of posterior cerebral circulation and ScO_2, we demonstrated that resistance breathing improved tolerance to central hypovolemia, but this was not associated with protection of ScO_2, maintained cerebral oxygen metabolism, or protection of posterior cerebral circulation. Although resistance breathing did not protect ScO_2 or posterior cerebral blood flow, subjects progressed to higher levels of LBNP compared to the control condition, and exhibited greater reductions in cerebral blood flow and ScO_2 at presyncope. We also observed that resistance breathing caused a decrease in respiration rate, potentially increasing the depth of breathing, which subsequently, could further decrease intracranial pressure and increase cerebral perfusion pressure, potentially underlying the observed delay in the onset of presyncope. In future studies, tidal volume and respiration rate could be measured during resistance breathing and LBNP to determine the influence of these factors on tolerance. Additionally, modifying tidal volume and respiration rate during LBNP could further facilitate examination of the role of breathing characteristics on tolerance to central hypovolemia. Potential clinical applications of resistance breathing could also be explored, including other stressors that are known to decrease central and cerebral blood flow and oxygenation, such as stroke, traumatic brain injury, orthostasis, and dehydration.

Due to the design of these investigations, we were able to make a unique comparison of endogenous (Chapter III) vs. induced (Chapter IV) oscillations in cerebral blood flow and subsequent cerebral blood flow and oxygenation responses to presyncopal limited central hypovolemia. Although there was no difference in endogenous LF oscillations between high vs. low tolerant subjects, resistance breathing-induced increases in LF oscillations in cerebral blood flow may be a contributing factor to improved tolerance with this intervention. A novel finding in this study was the markedly enhanced HF oscillations in arterial pressure, cerebral blood flow, and ScO_2, and a reduction in respiration rate with resistance breathing. As increases in the amplitude of HF oscillations are associated with greater tidal volumes, resistance breathing may alter the characteristics of breathing to further decrease intracranial pressure and subsequently increase cerebral perfusion pressure, thus delaying the onset of presyncope. A potential clinical application of this finding includes the treatment of hemorrhaging trauma patients with "pulsatile perfusion therapy" that could essentially enhance the delivery of oxygen to the cerebral tissues, therefore decreasing neuronal damage and cognitive impairment. Continued investigation into the role of oscillatory cerebral perfusion on cerebral blood flow and oxygenation is warranted.

In conclusion, the findings of these investigations enhance our understanding of the role of cerebral oxygenation and regional cerebral blood flow on tolerance to central hypovolemia, and provide important future directions for assessing the role of breathing characteristics on tolerance to conditions that compromise cerebral perfusion. It is anticipated that these findings could eventually be applied to clinical conditions such as hemorrhage, stroke, traumatic brain injury, orthostasis, and dehydration.