Anderson, Garen K. Hemodynamic Oscillations: Physiological Consequences and Therapeutic Potential. Doctor of Philosophy (Biomedical Sciences), March 29th, 2022, 175 pp., 11 Tables, 17 figures, bibliography.

Hemorrhage, or massive blood loss, continues to be a leading cause of preventable death. Therapeutic approaches that protect vital organ function are needed to improve outcomes from hemorrhage. In this dissertation, I explored the use of hemodynamic oscillations below the respiratory frequency (i.e., oscillations in arterial pressure and cerebral blood flow) as a novel technique for protecting tissue oxygenation during hemorrhage. In the first study of this dissertation, I hypothesized that hemodynamic oscillations would contribute to improved tolerance to central hypovolemia simulating hemorrhage. In further assessing the role of arterial blood gases on the physiological responses to forcing hemodynamic oscillations during a simulated hemorrhage, I hypothesized that forcing hemodynamic oscillations during simulated hemorrhage would protect tissue oxygenation during conditions of hypoxia and isocapnia, and improve cerebral blood flow. I also hypothesized that this protection would occur equally for both females and males. To address these hypotheses, I conducted five independent studies using lower body negative pressure as a method of simulating hemorrhage in healthy, conscious humans: in one study I utilized a maximal step-wise LBNP protocol to assess endogenous hemodynamic oscillations and tolerance to simulated hemorrhage, and in the remaining 4 studies, I utilized oscillatory and non-oscillatory LBNP to assess the potential therapeutic utility of forcing hemodynamic oscillations during simulated hemorrhage.

The major findings from these investigations were: 1) greater amplitude of low frequency oscillations in arterial pressure are associated with greater LBNP tolerance, but the relative time to peak oscillatory power was not dependent on tolerance; 2) forced hemodynamic oscillations protect cerebral tissue oxygenation without protecting cerebral blood flow during the combined stress of simulated hemorrhage and hypobaric hypoxia; 3) isocapnia with simulated hemorrhage prevents the reduction in cerebral blood flow and tissue oxygenation, and forced hemodynamic oscillations during this stress protects stroke volume and arterial pressure; 4) females exhibit protected muscle tissue oxygenation to simulated hemorrhage, and the reduction in muscle tissue oxygenation in males can be attenuated with forced hemodynamic oscillations; and 5) forced hemodynamic oscillations at high altitude are greater in amplitude and result in similar protection of cerebral tissue oxygenation as low altitude conditions.

These findings contribute to the growing body of literature highlighting the potential utility of oscillatory hemodynamics for therapeutic application.

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HEMODYNAMIC OSCILLATIONS: PHYSIOLOGICAL CONSEQUENCES AND THERAPEUTIC POTENTIAL

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HEMODYNAMIC OSCILLATIONS: PHYSIOLOGICAL CONSEQUENCES AND THERAPEUTIC POTENTIAL

DISSERTATION

Presented to the Education Council of the

School of Biomedical Sciences

University of North Texas

Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

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March 29, 2022

AKNOWLEDGMENTS

I thank my mentor, Dr. Caroline Rickards, for her guidance and teaching. Her encouragement and patience during unprecedented times has been invaluable in the completion of this dissertation. She has helped me grow in my scientific knowledge and ability to approach scientific questions. Most of all, she has taught me invaluable lessons of mentorship and what it means to support others in their academic and life pursuits.

I thank the members of my committee, Drs. Stella Goulopoulou, Steven Romero, Tom Cunningham and Nicole Phillips, for their valuable guidance in the development of my dissertation and mentorship in becoming a better scientist. I thank those who have worked alongside me in the laboratory over the last few years: Dr. Justin Sprick, Flora Park, Dr. Alexander Rosenberg, My-Loan Luu, Haley Barnes, Ryan Rusy, Austin Davis, and Nasrul Bhuiyan. Each of them played an important role in my development and progression through this program. I thank the members of the high-altitude expedition team for adding expertise and an opportunity to address scientific questions in a unique setting. I thank Dr. Glen Foster for taking time to train me to use end-tidal forcing. I thank Dr. Stephen Payne for his training on using the continuous wavelet transform.

Finally, I thank my family. I thank my parents for their never-ending love and their support for my scientific interests. I thank my wife, Elizabeth, for her love and unfailing support through all the ups and downs of a graduate education. I thank my children, London, Eloise and Bennett, for always brightening my day and keeping me grounded through the twists and turns of this experience.

Funding for these projects was provided by a National Institutes of Health-supported Neurobiology of Aging Training Grant (T32 AG020494, Principal Investigator: N Sumien), an

American Heart Association Predoctoral Fellowship (20PRE35210249; GK Anderson), an American Heart Association Grant-in-Aid (GRNT 17GRNT33671110; CA Rickards), multiple seed grants from the Department of Physiology & Anatomy, University of North Texas Health Science Center (CA Rickards), a Natural Sciences and Engineering Research Council of Canada Discovery Grant (RGPIN-2016-04915; TA Day, Chapter III), a University of Calgary Research Grant Committee (RJA Wilson, Chapter III), and a NSERC Discovery grant (RJA Wilson, Chapter III).

PEER REVIEWED PUBLICATIONS

Anderson GK, Rosenberg AJ, Barnes HJ, Bird J, Pentz B, Byman BRM, Jendzjowsky N, Wilson RJ, Day TD, Rickards CA. Peaks and Valleys: Oscillatory cerebral blood flow at high altitude protects cerebral tissue oxygenation. *Physiol Meas.* 2021 Jun 29;42(6).

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PRESENTATIONS AND ABSTRACTS

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Anderson GK, Rosenberg AJ, Barnes HJ, Kay VL, Sprick JD, Rickards CA. Low frequency hemodynamic oscillations during simulated hemorrhage: the influence of sex. Women's Cardiovascular & Brain Health Symposium, University of North Texas Health Science Center, January 2020.

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LIST OF ABBREVIATIONS

BOLD	Blood oxygen-level dependent
CaO_2	Arterial oxygen content
CSF	Cerebrospinal fluid
CVCi	Cerebrovascular conductance index
dHb	Deoxygenated hemoglobin
DO_2	Delivery of oxygen
$etCO_2$	End-tidal CO_2
etO_2	End-tidal O_2
FFT	Fast Fourier transform
Hb	Hemoglobin
HbO_2	Oxygenated hemoglobin
HT	High tolerant
ICA	Internal carotid artery
IPAD	Intramural periarterial drainage
LBNP	Lower body negative pressure
LF	Low frequency
LT	Low tolerant
MAP	Mean arterial pressure
MCAv	Middle cerebral artery velocity

MRI	Magnetic resonance imaging
MSNA	Muscle sympathetic nerve activity
NIRS	Near infrared spectroscopy
NREM	Non-rapid eye movement
OLBNP	Oscillatory lower body negative pressure
PaO_2	Arterial partial pressure of oxygen
PCAv	Posterior cerebral artery velocity
PCO_2	Partial pressure of carbon dioxide
$PetCO_2$	Partial pressure of end-tidal CO_2
$PetO_2$	Partial pressure of end-tidal O_2
PO_2	Partial pressure of oxygen
ScO_2	Cerebral tissue oxygen saturation
SmO_2	Muscle tissue oxygen saturation
SpO_2	Arterial oxygen saturation
SVR	Systemic vascular resistance
THC	Total hemoglobin concentration
VLF	Very low frequency

CHAPTER I

LITERATURE REVIEW

The cardiovascular system is tightly regulated to maintain the delivery of oxygen and nutrients to metabolically active tissues, and the removal of waste products. Both intrinsic and extrinsic mechanisms regulate the cardiovascular system to facilitate the matching of blood flow to metabolic demand, including neurogenic, myogenic, and humoral control. Given the complexity and interactive nature of these multiple physiological inputs, there is inherent variability in the measurable indices of the cardiovascular system (such as heart rate, arterial pressure, and blood flow), hence the term "hemodynamic". This variability can occur across multiple time scales, and is associated with a variety of underlying physiological mechanisms. Aside from the term "variability", there are numerous other terms used for describing hemodynamic variability in the literature, such as pulsatility, fluctuations, and oscillations. For this review, a definition of each term being used will be provided to enhance clarity.

While now common knowledge, in 1733 Stephen Hales was the first to directly measure arterial pressure (in the horse), and recognize both its magnitude and pulsatile nature that was coincident with the heart beat (1). Fluctuations in blood vessel diameter occurring at a rate slower than the heart beat were recognized as early 1853, and were concomitant with changes in blood flow at the same rate (2). Since these early fundamental observations, research examining

cardiovascular variability has expanded across many parameters such as heart rate and R-R intervals, arterial pressure, systemic and microvascular blood flow, and even tissue oxygenation, and the range and complexity of analytical approaches has also grown. When examined, hemodynamic variability can provide important information beyond that of a single or series of average measurements, and can improve our understanding of regulatory mechanisms of the cardiovascular system (3, 4).

It is important to note that when hemodynamic variability is assessed, a wide array of timescales can be examined. These time frames range from very fast beat-to-beat measures, down to very slow visit-to-visit variability, which can be measured week-to-week, month-to-month, over 6 month intervals, or even over years (5). Intermediate time scales can also be explored including variability due to respiration, sympathetic activity, vasomotion, hormonal cycles, and circadian rhythms (6). For a more comprehensive assessment of measures of blood pressure variability over slower timescales (e.g., visit-to-visit), the reader is referred to reviews focused on this topic (4, 7). There has also been extensive investigation into the physiological mechanisms (8-10), and the potential clinical application, of heart rate variability for diagnostic and treatment purposes (11-15), but this is also beyond the scope of the current report. The focus of this review is on short term variability of arterial pressure and blood flow, measured over minutes, and within frequency ranges typically centered around 0.1 Hz (low frequency) and 0.05 Hz (very low frequency), which equate to 10-s and 20-s cycles. The physiological mechanisms underlying variability within these time scales will be explored, including how these measurements can be used for clinical diagnostic and treatment purposes.

Methods for Quantifying Hemodynamic Oscillations

Various methods have been developed for quantifying the oscillatory characteristics of cardiovascular parameters using techniques in both the time domain and frequency domain. Within the time domain, measurements such as standard deviation (10), coefficient of variation, root mean squared standard deviation, Poincare plots (16, 17), complex demodulation (18), and pulsatility indices (19, 20) (such as Gosling's pulsatility index for cerebral blood velocity (21)) are commonly used. While time domain metrics are relatively easy to calculate, and helpful as a measure of general cardiovascular variability, they are limited in that the time scale of the variation within a signal is absent, and subsequently, the physiological underpinning of the variability is not always apparent.

More complex methods have been developed for distinguishing variability at different time scales, which can then be related to physiological mechanisms (e.g., via pharmacological blockade). Most popular among these methods is the Fourier transformation (9, 22-26), but other methods such as autoregressive techniques and wavelet analysis have also been employed (7, 27, 28). Using these advanced analytical techniques, the oscillations occurring at various frequencies within a time series signal can be extracted (both frequency and amplitude characteristics; see figure 1). These techniques can be categorized as "frequency domain" or "spectral analysis" approaches, and have a number of strengths and weaknesses that should be considered (29).

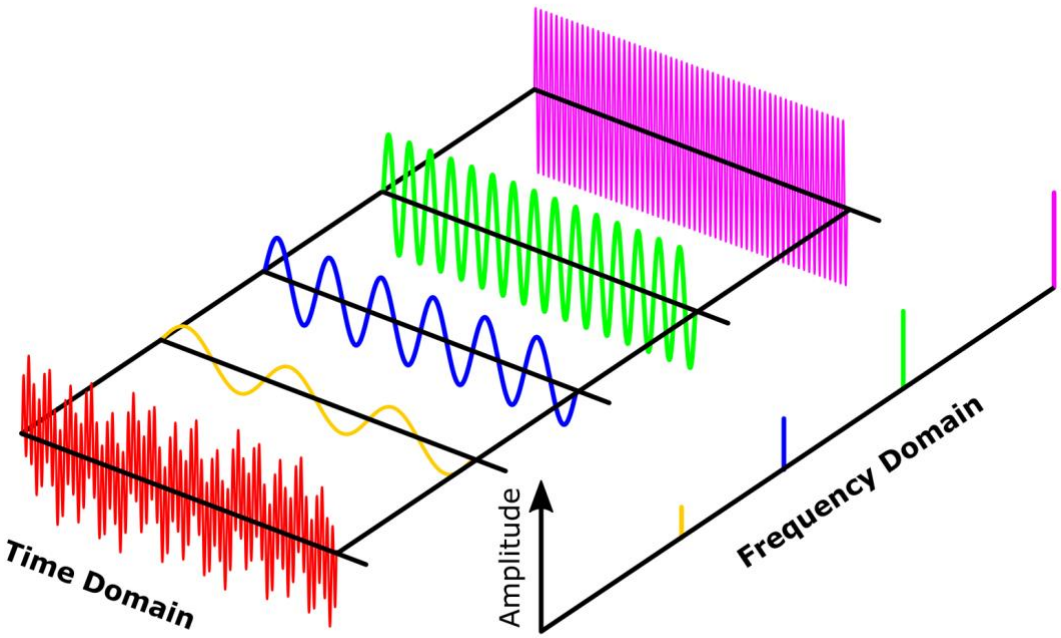

Figure 1. Representation of a cardiovascular time domain signal and the various oscillatory components that can be extracted from this signal at different frequencies of interest.

If the red signal was arterial pressure, the pink tracing would be related to heart rate and would occur at around ~1 Hz (for a heart rate of 60 beats/min); the green signal would be related to respiratory effects on arterial pressure, occurring between 0.15-0.5 Hz (9-30 breaths/min); the blue signal would be related to the effects of sympathetic activity on arterial pressure, occurring at ~0.1 Hz (6 cycles/min, or 10-s cycle); and the yellow tracing would be related to the myogenic/neurohumoral effect on arterial pressure, occurring at ~0.05 Hz (3 cycles/min, or 20-s cycle).

The fast Fourier transform can be used to generate frequency spectrums over a given time interval (dictated by the user), but without any localization of when a particular frequency of oscillation is occurring in time (aside from the specific time frame that was selected for analysis).

This approach can be used to capture the total variability in a time series directly, both at a specific frequency of interest, or across a range of frequencies (i.e., broadband) (22). A further limitation to this approach is that the oscillatory frequencies and amplitudes of cardiovascular signals can change over time (non-stationarity), which can either dilute or broaden estimates of amplitude (or power) around a frequency range of interest (27).

Autoregressive techniques create a statistical model of the time series by regressing a measurement at one time point by another measurement from a previous time point, and subsequently estimating the frequency spectrum (30). An advantage to this approach is finer resolution within the frequency domain compared to the Fourier transform, allowing for improved identification of peaks within the frequency spectrum (31). A critique to using this method, however, is that the frequency domain measures are not generated directly from the measured time series but are estimated from the time series, and the order of the model (how many samples from the past are included in the prediction) must be explicitly chosen, both of which could introduce error (29, 32).

Wavelet analysis of time series data provides both measures of magnitude at different frequencies, and localization of these frequencies in time (28, 29). This could, for example, allow for the identification of when in time arterial pressure has the largest amplitude of oscillation within a given frequency range during a physiological stress, and is better suited for non-stationary data (33, 34). Use of the wavelet transform, however, requires greater familiarity with the technique for accurate implementation (for example, choosing the correct wavelet for analysis). Of these advanced approaches, the Fourier transformation is the most well-known and widely used throughout the signal processing, physiology, and clinical literature. Regardless of the method, however, spectral analysis of hemodynamic time series data provides important

information that is otherwise lost when simply calculating the averages of these time series (3, 4, 35).

Mechanisms of Oscillations in Arterial Pressure and Blood Flow

The waveform oscillations shown as an example in figure 1 are due to different underlying physiological mechanisms. In brief, oscillations around 1 Hz are due to the cardiac cycle (for a heart rate of 60 beats/min), around 0.2 Hz are due to respiration (for a respiratory rate of 12 breaths/min), around 0.1 Hz are due to sympathetic activity (36), and around 0.05 Hz are likely due to a number of circulating and local vasoactive substances, or may be the result of the intrinsic constriction and relaxation of smooth muscle around the arterioles (i.e., myogenic control) (37).

Oscillations in arterial pressure have been widely assessed in the literature for potential use as a non-invasive biomarker of physiological function, such as sympathetic activity (36, 38-41), or as an index of condition severity such as in stroke (42, 43). Much of the literature surrounding oscillations or variability in arterial pressure focuses on negative clinical outcomes in a myriad of conditions, including transient ischemic attack, stroke, and hypertension (7, 43-45). However, this focus on the negative consequences of arterial pressure variability may be an oversimplification, as variability occurs across different time scales, with different underlying mechanisms and consequences. Day-to-day or visit-to-visit variability, often focused on in the clinical literature, may indeed represent hemodynamic instability and impaired physiological function (4). Studies assessing beat-to-beat metrics of variability focused around the cardiac frequency (~1 Hz), also known as pulsatility, have also shown associations with negative clinical outcomes, such as stroke (46-48). However, while associations between blood pressure

variability and poor clinical outcomes are prevalent in the literature, experimental evidence demonstrating a causative role between high blood pressure variability (including around the 0.1 Hz frequency) and organ damage is lacking. Indeed, this variability may occur secondary to the initial insult, so may be an indicator of overall damage rather than the being the cause of the damage. We contend that it is also possible that oscillations around 0.1 Hz are present with disease and/or compromised physiological states as a compensatory mechanism, rather than the cause of the damage (see "Blood Flow Oscillations and the Protection of Tissue Oxygenation" section for details). More research is needed to elucidate this potential "cause and effect" relationship.

Early observations of oscillations in arterial pressure below the respiratory frequency were made in experimental conditions of reduced tissue perfusion (38, 49). Notably, Arthur Guyton and colleagues conducted a series of studies in dogs, seeking to understand the source of these oscillations (38). After removing 25% of total blood volume via hemorrhage, Guyton and Harris observed consistent "waves" in blood pressure occurring at ~0.04 Hz (~25-s cycles), which then disappeared when the animals were heavily sedated with sodium pentobarbital or with denervation of the baroreceptors, both of which block direct neural regulation of the vasculature (38); hence this was postulated as an underlying mechanism for the oscillations. Auer & Gallhofer conducted a series of studies in cats to observe the oscillatory characteristics of the blood vessels in the brain during different physiological stimuli (e.g. hemorrhage, hypercapnia, hyperoxia, sympathetic blockade/stimulation), and noted consistent oscillations at various frequencies including 5-8 cycles/min (~0.1 Hz) (50). More recently, oscillations around 0.1 Hz (6 cycles/min) were experimentally observed in the cerebral microcirculation at rest and during hemorrhage in rats (51). Hudetz et al. demonstrated that ~0.1 Hz oscillatory amplitudes of

cerebral blood flow (assessed via microcirculatory flux with laser Doppler flow) consistently increased with progressively decreasing mean arterial pressure. Much of the research following these experiments has sought to further investigate the physiological mechanisms responsible for the arterial pressure and blood flow oscillations.

Mechanistically, short-term oscillations in arterial pressure below the rate of respiration, and the resulting oscillations in blood flow, may be linked to different physiological systems depending on the species under investigation (36, 52, 53). Rhythmic oscillations in blood vessel diameter or vascular tone (often referred to as "vasomotion") are an important contributor to the oscillations observed in arterial pressure and blood flow. Vasomotion can occur intrinsically, or via extrinsic stimuli, so serves as a "control site" where other physiological systems can then modulate the frequency and amplitude of hemodynamic oscillations. Observations of vasomotion at frequencies below the rate of respiration, date as far back as 1853 when T. Wharton Jones documented oscillations in the diameter of bat wing venules at a rate of 10 cycles per min (~0.17 Hz) at rest (2). The range of vasomotion frequencies varies based on location of the vessels within the arterial tree, and the animal species. In a study of hamster skinfold preparations, larger arterioles (ranging from 50-100 μm) dilate and constrict 2-3 times per minute (0.03-0.05 Hz), while terminal arterioles dilate and constrict anywhere from 10 up to 25 times per minute (0.17-0.42 Hz) (54). While similar systematic studies have not yet been conducted in humans, in studies using laser Doppler flux in human skin at rest, oscillations have been demonstrated within the microvasculature at various frequencies within the range of 0.005-2.0 Hz (55). These oscillations appear to be intrinsic to the vascular wall (56), and are altered by physiological inputs, such as neural and hormonal factors, including local calcium currents (assessed via calcium imaging), sympathetic stimulation, and release of vasoactive factors (54, 57, 58).

Stauss and Kregel (59) explored how stimulation of the sympathetic nerves at frequencies between 0.05 and 2 Hz in conscious rats would affect arterial pressure oscillations and mesenteric blood flow. Splanchnic nerve stimulation in these rats generated blood pressure and blood flow oscillations between 0.2-0.5 Hz (59). In comparison, the frequency of sympathetically mediated oscillations in arterial pressure and blood flow in humans is around 0.1 Hz (36, 53). The relationship between sympathetic activity and oscillations in arterial pressure has been explored in humans during increasing steps of central hypovolemia and hypotension induced by lower body negative pressure (LBNP) (60). In this study, Cooke et al. demonstrated an increase in muscle sympathetic nerve activity (measured directly by microneurography) when diastolic arterial pressure decreased. Importantly, this hypovolemic stimulus resulted in increased amplitude of oscillations in the 0.04-0.15 Hz range for both diastolic arterial pressure and muscle sympathetic nerve activity (presumably eliciting vasomotion at the same frequencies), as well as increased coherence between diastolic arterial pressure and muscle sympathetic nerve activity within this frequency range. Furthermore, Cevese et al. performed alpha$_1$-adrenoreceptor blockade in supine resting humans using Urapidil, and showed a decrease in arterial pressure oscillations at around 0.1 Hz, adding further evidence for the role of the sympathetic nervous system in generating these oscillations (61).

In the skin vasculature of rats, Stauss et al. determined that sympathetic oscillations occurred between 0.05-0.075 Hz (62). In comparison, when stimulating the median nerve skin sympathetic fibers in humans across frequencies from 0.01-0.5 Hz, Stauss et al. showed that skin blood flow (indexed via measurement of skin laser Doppler flux) oscillated between 0.075-0.1 Hz (63), suggesting that sympathetic activity was also responsible for the generation of these oscillations. However, there is some contention about the sympathetically-induced oscillatory

range of skin vasomotion in humans, as vasomotion in the 0.02-0.05 Hz range was also reduced in human denervated skin flaps compared to an innervated control skin site (assessed via laser Doppler flux) (64). Interestingly, Salvi et al. observed an increase in skin vasomotion at 0.1 Hz (also assessed by laser Doppler flux) in humans during high altitude ascent to 5050 m, a stimulus known to increase sympathetic activity (65). Collectively, these studies demonstrate two important characteristics of hemodynamic oscillations in arterial pressure and blood flow: 1) they occur at different frequencies between various vascular beds, and; 2) they occur at different frequencies for different species.

For oscillations in arterial pressure and blood flow occurring in the very low frequency range (i.e., around 0.05 Hz in humans), the exact mechanism/s of generation are more complex. In isolated blood vessels, rhythmic, spontaneous oscillations in tone have been observed, demonstrating that the machinery necessary to produce oscillations is intrinsic to the vessels, leading to an oscillatory myogenic response in the arterioles (56, 66). For example, when using an L-type Ca^{2+} channel blocker (nifedipine) to prevent the myogenic response, very low frequency (0.02-0.2 Hz for rats) oscillations in blood pressure are reduced in conscious rats at rest (37). Similarly, when oscillations in arterial pressure are induced via periodic occlusion of the abdominal aorta in rats, Ca^{2+} channel blockade (via nifedipine) suppresses the amplitude of oscillations between 0.008-0.05 Hz (67). In humans, Ca^{2+} channel blockade (via nimodipine) reduced resting oscillations in arterial pressure and cerebral blood flow between 0.02-0.07 Hz (68), but did not affect the amplitude of forced oscillations at 0.05 Hz (induced via application of oscillatory LBNP) (69). Interestingly, in a similar study, also in humans, Ca^{2+} channel blockade via nicardipine augmented forced arterial pressure oscillations across the 0.03-0.08 Hz range (again via oscillatory LBNP), but had no effect on cerebral blood flow oscillations (70). Blood

pressure and blood flow oscillations within this very low frequency range are mechanistically complex, however, because they are influenced by a number of factors other than just variations in myogenic tone (71). Pharmacological blockade studies in animals and humans have also suggested an influence of the renin-angiotensin system (72), endothelial nitric oxide (73), and even circulating catecholamines (74) on the generation of very low frequency oscillations in arterial pressure and blood flow.

While there is a paucity of experimental evidence about the physiological role of oscillatory arterial pressure and blood flow, the evidence that does exist revolves around the possible protection of tissue oxygenation, and improved clearance of interstitial fluid. These topics will be the focus of the next sections of this review.

Blood Flow Oscillations and the Protection of Tissue Oxygenation

While past research efforts have focused on the mechanisms contributing to hemodynamic oscillations at ~0.1 Hz, there is accumulating evidence pointing to a possible role of these oscillations in the protection of tissue oxygenation. Recent evidence has alluded to a potential benefit of 0.1 Hz oscillations in arterial pressure and blood flow in humans in a model of reduced tissue perfusion, via application of LBNP. Rickards et al. compared the arterial pressure and cerebral blood velocity responses of human participants who were classified as "high tolerant" (N=93) or "low tolerant" (N=42) to a maximal LBNP protocol, which was terminated with the onset of presyncopal signs and symptoms (26). When comparing the final common LBNP stage between these two groups, high tolerant participants were found to have higher low frequency (0.04-0.15 Hz) power (i.e., amplitude) in mean arterial pressure and middle cerebral artery velocity (MCAv), an index of cerebral blood flow, when compared with low

tolerant participants. *One limitation in this approach is the inability to measure the amplitude of these oscillations continuously over time (see section above on methods for quantifying hemodynamic oscillations). To address this limitation, I measured the amplitudes of hemodynamic oscillations over time in high and low tolerant participants using the wavelet transform approach (Specific Aim 1; Chapter II).* Another limitation of this study was the observational nature of the experimental design, where spontaneous hemodynamic oscillations were retrospectively assessed, rather than induced or blocked to assess the "cause and effect" of oscillations on tolerance to central hypovolemia.

To address this limitation, Lucas et al. developed a protocol to induce hemodynamic oscillations at 0.1 Hz by coaching participants to breath at a frequency of 6 cycles per min (75). Participants then underwent a test to presyncope (via head-up tilt plus LBNP) with paced breathing at 0.1 Hz, or spontaneous breathing (average respiration rate was 16-20 breaths/min (0.26-0.30 Hz) across the protocol). Importantly, the amplitude of 0.1 Hz oscillations increased during the paced breathing protocol for both mean arterial pressure and MCAv compared to the spontaneous breathing condition, demonstrating that robust low frequency hemodynamic oscillations can be induced experimentally via physiological maneuvers. As a result, tolerance time to head-up tilt plus LBNP was increased during the paced breathing protocol by ~4.5 min. There was also an attenuated rate of decline in mean arterial pressure and MCAv during the paced breathing protocol, hinting at a protection of cerebral blood flow.

When considering the findings of these two studies (26, 75), it should be noted that the impact of the increased amplitude of oscillations in arterial pressure and cerebral blood flow on cerebral tissue oxygenation was not measured. Accordingly, potential mechanisms contributing to improved tolerance to central hypovolemia were speculative. To address this limitation, we

have assessed the responses of both cerebral blood velocity and cerebral tissue oxygenation while inducing hemodynamic oscillations at specific frequencies (76). Unlike Lucas et al. (75), who utilized the physiological maneuver of breathing to induce hemodynamic oscillations, we used the physical maneuver of oscillating LBNP chamber pressure at two frequencies of interest: 0.1 Hz (which is related to the effects of sympathetic activity on blood pressure) and 0.05 Hz (which is somewhat related to the effects of myogenic activity on blood pressure; see discussion in section above). Importantly, we first induced a state of central hypovolemia by initially decreasing chamber pressure to -60 mmHg (simulating a blood loss of ~15 ml/kg (77)), then applied the oscillations for ~10-min. Accordingly, participants completed three experimental conditions: 1) a static profile where LBNP chamber pressure was lowered to -60 mmHg and held constant (0 Hz); 2) a 0.1 Hz oscillatory condition where chamber pressure was lowered to -60 mmHg and then oscillated between -30 and -90 mmHg every 5-s (10-s cycle), and; 3) a 0.05 Hz profile, similar to the 0.1 Hz profile except with 10-s at -30 and -90 mmHg chamber pressures (20-s cycle). This experimental design ensured that participants were exposed to the same average LBNP of -60 mmHg for each profile. We observed protection of cerebral tissue oxygenation of about 2-3% during oscillatory LBNP at both the 0.1 Hz and 0.05 Hz frequency compared with the control profile, which was also coincident with improved tolerance to the LBNP stimuli (i.e., prolonged time without experiencing presyncopal signs or symptoms). Surprisingly, this protection of cerebral tissue oxygenation occurred without the simultaneous protection of cerebral blood flow indexed by MCAv, suggesting no difference in delivery of oxygen through the major intracranial arteries. *A limitation in this design is the index of cerebral blood flow through the MCA. Transcranial Doppler ultrasound does not allow for the measurement of blood vessel diameter, and is therefore limited to only velocity measures. To*

address this limitation, I measured the internal carotid artery (ICA) flow utilizing duplex Doppler ultrasound (Specific Aim 2, Chapters III and IV), which enables the measurement of both velocity and diameter in the ICA. In this way, blood flow can be calculated to assess the effects of hemodynamic oscillations on blood flow to the brain.

These observations in humans are corroborated with evidence from both mathematical models and empirically via animal experiments, examining the effect of low frequency oscillations in microvascular diameter and blood flow on tissue oxygenation. Using mathematical models, Tsai and Intaglietta were among the first to propose a role for vasomotion in protecting tissue oxygenation (78). Using a model of Krogh cylinder geometry, which is a simplified model of tissue around a capillary, coupled with oscillating red blood cell flux through this cylinder, they determined that vasomotion could create a pump-like effect in the microvasculature, where brief periods of high red blood cell velocity and high hematocrit could extend perfusion of oxygenated blood further into tissues compared to conditions of no vasomotion. This phenomenon was further evaluated by Goldman and Popel using another series of computational models based on the capillary network of a hamster cheek pouch retractor muscle (79). When accounting for the varying amplitudes and frequencies of vasomotion, the presence or absence of myoglobin, and tissue metabolic rate, the authors confirmed that vasomotion could produce similar effects as those stated by Tsai and Intaglietta (79). The most pronounced improvement in oxygenation from their models occurred with vasomotion between 1.5-3 cycles per minute (0.025-0.05 Hz), in tissues that are relatively hypoxic (modeled by a computational doubling of oxygen consumption), and do not contain myoglobin (with important implications for the brain, which also does not contain myoglobin). In both computational studies, increasing amplitude of vasomotion increased the tissue oxygenation. One of the only

studies to experimentally assess the effects of vasomotion on tissue perfusion was by Rücker et al., using a rat model with stepwise reductions in femoral artery blood flow (80). In response to reduced femoral artery blood flow, vasomotion at about 2 cycles per minute, or 0.03 Hz, spontaneously occurred in the skeletal muscle vasculature. When vasomotion was blocked via administration of a Ca^{2+} channel blocker (felodipine), perfusion in the skeletal muscle was maintained, but surrounding tissues (skin, subcutis, and periosteum), which had previously been protected by vasomotion, experienced a reduction in tissue perfusion as measured by functional capillary density. This protection may be due to cyclical increases in blood velocity and hematocrit being perfused into the capillaries as a result of vasomotion, which may allow for improved oxygen distribution throughout the tissue (54, 81).

Figure 2 shows the potential mechanisms of protection with increased amplitude of arterial pressure and blood flow oscillations (both endogenous and forced). At rest, intrinsic vasomotion creates localized and cyclical increases in red blood cell velocity and hematocrit within the microcirculation (81). During conditions of reduced tissue oxygenation such as hemorrhage, this intrinsic vasomotion may be augmented and synchronized across a tissue with the compensatory increase in sympathetic nerve activity. The subsequent increased amplitude of blood flow oscillations could then create even greater waves of blood with increased hematocrit and red blood cell velocity within the capillaries and facilitate a protection in tissue perfusion and oxygenation (81). These waves of increased hematocrit and red blood cell velocity could also account for improved functional capillary density during reduced perfusion. When hemodynamic oscillations are forced, such as with LBNP, oscillatory amplitudes in arterial pressure and blood flow are increased even further, which could then amplify the sympathetic response and the subsequent oscillatory vasomotion effect within the microcirculation. While the

evidence is currently limited, this potential mechanism for the protection of tissue perfusion with oscillatory blood flow could be utilized in treatment of clinical conditions of tissue hypoperfusion.

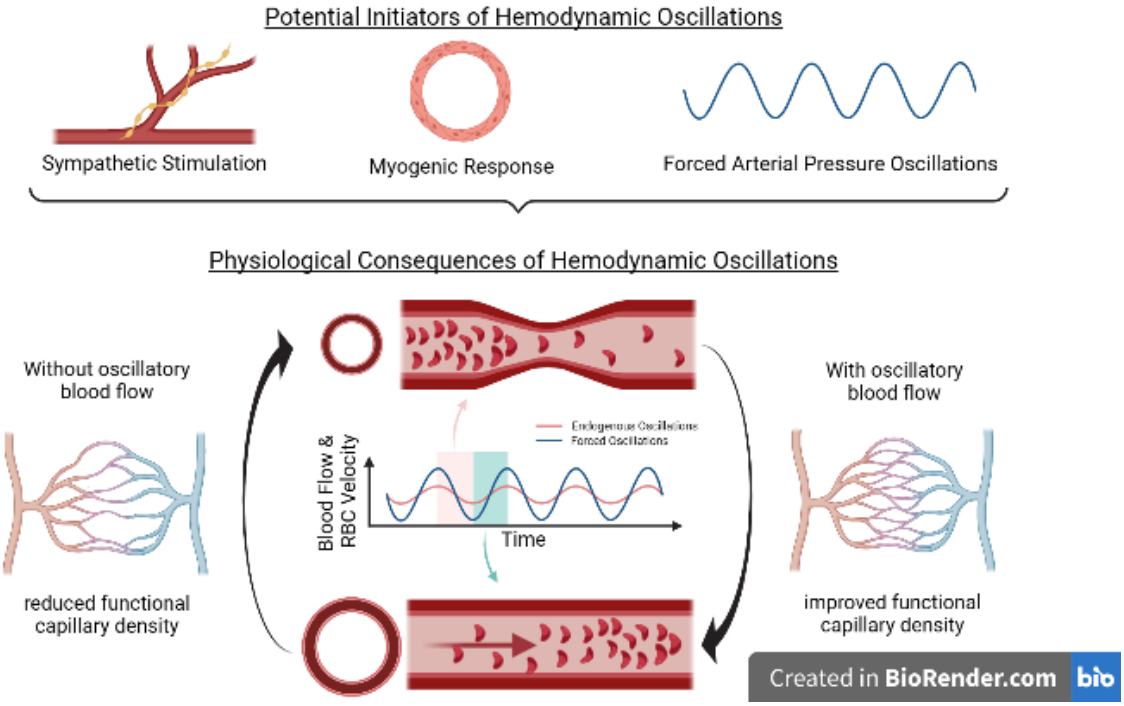

Figure 2. Proposed mechanism of improved perfusion and tissue oxygenation with oscillatory blood flow.

Cyclical vasoconstriction (light red square) and vasodilation (light green square) creates brief increases in red blood cell velocity and hematocrit (81). Several physiological/mechanical mechanisms can initiate/enhance this response such as increased sympathetic activity, intrinsic myogenic responses or myogenic responses to changes in arterial pressure and blood flow, and forced arterial pressure and blood flow oscillations. In conditions of reduced perfusion (e.g., hemorrhage), oscillatory blood flow preserves functional capillary density (80). This effect is enhanced when oscillatory arterial pressure and blood flow are forced systemically. Forcing

oscillations in arterial pressure and blood flow would subsequently increase sympathetic activity and increase hemodynamic oscillatory amplitude.

As a primary focus of this dissertation (Specific Aim 2), I will be assessing the utility of forcing hemodynamic oscillations during simulated hemorrhage, as an example of a clinical condition of tissue hypoperfusion. I will also be assessing the role of arterial blood gas concentrations and biological sex on the utility of forced hemodynamic oscillations for protection of tissue oxygenation during simulated hemorrhage. The first two studies in this aim are focused on assessing the role of arterial blood gases during forced hemodynamic oscillations. The last two studies in this aim will seek to further inform potential clinical applications of forced hemodynamic oscillations by assessing the role of biological sex, and potential differences between high and low altitude conditions.

Summary

Evidence regarding the role of hemodynamic oscillations as a mechanism to protect tissue oxygenation is accumulating. To better characterize the timing of endogenous generation of hemodynamic oscillations, and assess their role in tolerance to simulated hemorrhage, I have compared the responses of high and low tolerant individuals with continuous measures of oscillatory amplitude via the wavelet transform during LBNP to presyncope. To explore the utility of these oscillations as a potential therapeutic, I have assessed the physiological effects of inducing hemodynamic oscillations during simulated hemorrhage. In particular, I have explored the use of these oscillations with consideration of arterial blood gases and biological sex. These

studies provide important information for the future development of therapeutic applications utilizing hemodynamic oscillations.

SPECIFIC AIMS

Specific Aim 1: Determine whether the characteristics of endogenous low frequency hemodynamic oscillations influences tolerance to simulated hemorrhage.

Hypothesis 1: We hypothesized that 1) the amplitude of low frequency (LF) oscillations in arterial pressure and cerebral blood flow would be higher in participants with greater tolerance to a presyncopal LBNP stress, and; 2) the time of maximum magnitude of LF oscillations would be further from baseline for high tolerant vs. low tolerant participants.

Approach 1: In a group of participants subjected to presyncopal LBNP, the continuous wavelet transform was used to quantify the magnitude of low frequency oscillations in arterial pressure and middle cerebral artery velocity over time, and the relationship to LBNP tolerance was explored. These participants were then separated into high and low tolerance groups and their responses were compared in regard to maximum magnitude of LF oscillations in arterial pressure and cerebral blood flow. This aim is addressed in Chapter II of this dissertation.

Specific Aim 2: Assess the effect of forced hemodynamic oscillations during simulated hemorrhage on cerebral blood flow and tissue oxygenation, with consideration of arterial blood gases and sex.

Hypothesis 2: Forcing hemodynamic oscillations during simulated hemorrhage will protect cerebral and muscle tissue oxygenation, and cerebral blood flow. This protection of tissue oxygenation will remain during conditions of altered blood gases (i.e., hypoxia and isocapnia), and between the sexes.

Approach 2: Participants underwent simulated hemorrhage with and without forced hemodynamic oscillations during both hypoxic (hypobaric hypoxia; Chapters III and VI) and

isocapnic (clamped arterial PCO_2; Chapter IV) conditions, to assess the effect of changing blood gases on cardiovascular responses to forced oscillations. A comparison was also conducted to assess the role of biological sex on cardiovascular responses to forced hemodynamic oscillations (Chapter V).

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CHAPTER II

Time-Frequency Analysis of Hemodynamic Oscillations during Presyncopal Lower Body Negative Pressure (LBNP) in Humans

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ABSTRACT

Greater amplitude of low frequency (LF) oscillations (~0.1 Hz) in arterial pressure and cerebral blood flow, quantified by fast Fourier transform (FFT) analysis, have been associated with higher tolerance to central hypovolemia. The FFT is reliable for analysis of stationary data, which is an uncommon characteristic of hemodynamic data in conscious humans. The continuous wavelet transform can also be used to quantify amplitudes of LF hemodynamic oscillations, but allows for time localization, and is better suited for non-stationary data. We

hypothesized that 1) the magnitude of LF oscillations in mean arterial pressure (MAP) and cerebral blood flow (indexed by middle cerebral artery velocity, MCAv) would be higher in participants with greater tolerance to central hypovolemia induced via step-wise lower body negative pressure (LBNP), and; 2) the time of maximum magnitude of LF oscillations would be further from baseline for high tolerant participants vs. low tolerant participants. Healthy human participants (N=22; 11 male, 11 female) underwent step-wise LBNP to presyncope. MAP was measured via finger-photoplethysmography, and MCAv was measured using transcranial Doppler ultrasound. The continuous wavelet transform was used to quantify the maximum magnitude of oscillations in MAP and MCAv in the LF range (0.07-0.15 Hz). Presyncopal time was positively correlated with increases in both MAP LF ($r = 0.64$, $p = 0.001$) and MCAv LF ($r = 0.45$, $p = 0.06$) oscillations. Relative time to maximum magnitude of LF oscillations (where 1.0 is presyncope) was no different between high and low tolerant individuals for MAP (HT: 0.82 ± 0.13, LT: 0.66 ± 0.25; $p = 0.11$) or MCAv (HT: 0.70 ± 0.27, LT: 0.72 ± 0.25; $p = 0.91$). Overall, the increase in oscillatory amplitudes in the LF range for arterial pressure and cerebral blood flow are positively associated with tolerance to central hypovolemia, despite no difference in the relative timing of the maximum amplitude of these oscillations.

INTRODUCTION

Evidence accumulated over the past 30 years has alluded to a protective role of oscillatory blood flow during conditions of reduced blood flow and/or hypoxia. Early work using computational approaches provides evidence for a protection of tissue oxygen with oscillatory blood flow when blood flow to the tissue is reduced (1, 2). Empirical evidence from Rücker et al. furthered understanding of these oscillations using an animal model of reduced tissue perfusion.

Tourniquets were used on the hindlimbs of rats to reduce blood flow through the Femoral artery with or without calcium channel blockade (via felodipine). This model effectively induced tissue ischemia with or without vasomotion (one mechanism for producing oscillatory blood flow). Compared to reduced blood flow without vasomotion, vasomotion was shown to protect perfusion of otherwise vulnerable tissue, as measured by functional capillary density (3). Recent evidence from human studies has shown a protection of cerebral tissue oxygen saturation when low frequency (LF; ~0.1 Hz) oscillations in arterial pressure and cerebral blood flow are induced during a condition of cerebral hypoperfusion (via application of lower body negative pressure, LBNP) (4, 5). Spontaneously occurring LF oscillations in arterial pressure and cerebral blood flow have also been associated with improved tolerance to the cerebral hypoperfusion induced by LBNP (6, 7).

A common method for quantifying oscillations or variability within the cardiovascular system is the fast Fourier transform (FFT) (8-12). This technique allows for the quantification of amplitude or power of oscillations at specific frequencies of interest. While a powerful tool, the FFT has several limitations within the context of physiological systems. First, when used to measure amplitude or power, FFT is most reliable for time-series signals that are stationary; meaning the signal has a regular, predictable oscillatory pattern, without irregularities or deviations from this pattern. However, stationarity is not a typical characteristic for many cardiovascular variables under all conditions, such as blood pressure or blood flow, where multiple intrinsic and extrinsic factors can alter oscillatory patterns (e.g., deep sighs, coughing, occasional sympathetic activity bursts, hypoxia, etc.). Another important consideration when using the FFT is that this method does not allow for the localization of when oscillations are occurring in time. Instead, researchers are limited to assessing amplitude or power of a signal

across the entire time frame for which the FFT was performed. For example, in the LBNP studies referred to earlier, quantification of oscillatory amplitudes was limited to each 5-min step of LBNP, which may not accurately pinpoint the precise time when amplitudes were highest. Because this approach averages the data over a specific time frame set by the user, periods of low oscillatory amplitude within this time frame may reduce the average oscillatory amplitude. Other techniques have been developed to quantify the frequency components of a time-series signal, including the wavelet transform. Importantly, the wavelet transform facilitates assessment of both the magnitude and timing of oscillations. For a more detailed understanding of the theory of the wavelet transform see the following reviews (13, 14).

In this study, we retrospectively analyzed data collected from multiple LBNP studies using the wavelet transform approach, to leverage the advantage of isolating the timing of peak oscillatory amplitude of arterial pressure and cerebral blood flow. We hypothesized that 1) the amplitude of LF oscillations in arterial pressure and cerebral blood flow would be higher in participants with greater tolerance to a presyncopal LBNP stress, and; 2) the time of maximum magnitude of LF oscillations would be further from baseline for high tolerant vs. low tolerant participants.

METHODS

Participants

Participants for this analysis were selected from a database of three experimental protocols which were reviewed and approved by the North Texas Institutional Review Board (Protocol Numbers: 2012-163, 2014-127, 2018-120). Data from these studies and participants have been reported in prior publications which focused on independent research questions (15-

19). Participants were free from cardiovascular, respiratory, metabolic, or inflammatory diseases. All participants were familiarized with the study protocol and equipment prior to providing written informed consent. Participants abstained from exercise, alcohol, caffeine, and medications 24-h prior to testing. Female participants were tested on days 1-4 of their menstrual cycle (self-reported), or during the blank or no pill days if taking oral contraceptives. All experiments were performed in the morning in a temperature-controlled laboratory (22-24°C).

Instrumentation

Upon arrival to the laboratory, participants laid supine in a LBNP chamber (VUV Analytics Inc., Austin, TX) with their iliac crest in line with the opening of the chamber. Participants were subsequently sealed into the chamber at the waist using a plastic sleeve and neoprene waistband. Heart rate was monitored using a standard lead II configuration ECG (shielded leads, cable and amplifier, AD Instruments, Bella Vista, NSW, Australia). Continuous measurements of arterial pressure (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) and stroke volume (ModelFlow®) were taken via finger photoplethysmography. Transcranial Doppler ultrasound was used to obtain measures of middle cerebral artery velocity (MCAv) by placing a 2-MHz probe (ST3, Spencer Technologies, Seattle, WA) on the right temporal window, which was held in place by adjustable headgear. Cerebral tissue oxygenation (ScO_2) was measured via near-infrared spectroscopy over the temporal lobe (OxiplexTS; ISS, Champaign-Urbana, IL, USA). End tidal gases (etO_2 and $etCO_2$) were measured using an oral-nasal cannula connected to a gas analyzer (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW, Australia). Respiration rate was detected from the continuous CO_2 waveform.

Experimental Protocol

Participants completed a LBNP tolerance protocol to presyncope. Following a 5-min baseline, LBNP chamber pressure was lowered every 5-min to -15, -30, -45, -60, -70, -80, and -90 mmHg, or until the onset of presyncope. The protocol was terminated when systolic arterial pressure fell below 80 mmHg, or upon subject termination due to subjective symptomology (i.e., dizziness, light-headedness, nausea, sweating, grey-out). At protocol termination, chamber pressure was immediately released, and participants were monitored for a 10-min recovery period.

Data Analysis

Continuous data were collected at 1000 Hz (LabChart 8, AD Instruments, Bella Vista, NSW, Australia) and saved for offline analysis using specialized software (WinCPRS, Absolute Aliens, Turku, Finland). R-waves from the ECG were automatically detected and used to gate the remaining cardiovascular waveform data. Beat-to-beat MAP and mean MCAv were calculated as area under the curve from the continuous arterial pressure and MCAv waveforms. After calculating respiratory rate from the CO_2 waveform, participants who were breathing below 10 breaths per minute were removed from the analysis to ensure that respiration was not influencing the generation of hemodynamic oscillations in the LF range (i.e., 0.07-0.15 Hz or 4.2-9 breaths/min). Participants were also removed from the analysis if a large single breath was taken during the protocol due to the resultant large swings in arterial pressure or cerebral blood velocity, leading to noise in calculating oscillatory amplitudes. Given the observational nature of this study, removing participants from analysis based on these criteria facilitates a clearer

understanding of the specific outcome variables of interest, rather than trying to interpret data that is affected by multiple simultaneous and uncontrolled physiological inputs.

Beat-to-beat MAP and mean MCAv data from the start of baseline to presyncope were then imported into MATLAB (The MathWorks, inc., Natick, MA). MAP and mean MCAv were resampled at 10 Hz using cubic spline interpolation. Both signals were high pass filtered with a fifth-order Butterworth filter and cut-off frequency of 0.005 Hz, and then low pass filtered with fifth-order Butterworth filter with a cut-off frequency of 0.8 Hz. This filtering process removes noise from baseline shift and variability due to the cardiac cycle (20). The resulting signals were 10 Hz continuous MAP and mean MCAv from the start of baseline to presyncope for each subject.

The continuous wavelet transform was then calculated for MAP and mean MCAv using the cwt() function with the Morlet wavelet in the wavelet toolbox of MATLAB. The absolute value of the wavelet coefficients was used to calculate the magnitude of oscillations for each frequency. The magnitude of oscillations within the low frequency range (0.07-0.15 Hz) was then averaged at each sampling point to provide the magnitude of LF oscillations over time for each subject. To identify periods of sustained oscillations, a 1-min moving average was calculated from these signals, and the maximum of MAP and mean MCAv was identified.

The magnitude of LF oscillations at rest for MAP and mean MCAv was calculated by averaging the magnitude of the wavelet coefficients for the last 3-min of baseline. The change in LF oscillations was subsequently calculated as the difference between baseline magnitude and the maximum magnitude divided by baseline magnitude and multiplied by 100. The relative timing of maximum oscillatory magnitude was calculated by dividing the time at maximum magnitude of oscillations by presyncopal time.

For each participant, a 3-min average of ScO_2 was taken at the end of baseline and a 10-sec average was taken at the time of maximum magnitude in oscillations for both MAP and MCAv to understand the effect of pressure and blood flow oscillations on this variable. Lastly, participants were classified as high tolerant (HT) if they completed the -60 mmHg stage of LBNP (i.e., 1500-s) and low tolerant (LT) if they reached presyncope before this time.

Statistical Analysis

All statistical analysis was performed in R (R Core Team, 2021). Baseline demographics and cardiovascular parameters were compared between tolerance groups using unpaired t-tests. Welch's t-tests were also used to assess differences between high and low tolerant groups when there was non-homogeneity of variance in the data (i.e., relative increases in MAP and MCAv magnitudes). Correlation coefficients between the magnitude of LF oscillations and LBNP tolerance time were calculated using Pearson's coefficients. Two-way linear mixed models with subject as a random effect were used to analyze values over time between high and low tolerant participants using the lme4 package (21). Post-hoc testing was performed with the estimated marginal means from the linear mixed models using the *emmeans* package (22). Specific pairwise comparisons between tolerance groups at each time point and within tolerance across time were performed and corrected using Holm's method.

RESULTS

Of the original 52 participants who completed the LBNP experiments, 21 were removed for consistent respirations rates below 10 breaths per min during LBNP. Of the remaining 31 participants, 9 recordings of MAP and 12 recordings of MCAv were removed for sudden large

breaths that affected the frequency domain analysis, as previously indicated. Subsequently, 22 recordings of MAP and 19 recordings of mean MCAv were included in the final analysis. No differences were observed in the baseline demographics or resting MAP or mean MCAv values between tolerance groups (Table 1).

A representative tracing of the magnitude of MAP oscillations over time is shown in figure 1. When assessing the relationship between presyncopal time and the maximum magnitude of oscillations for MAP, a weak correlation was observed ($r=0.37$; $p=0.09$) (Figure 2, panel A). However, when adjusting for the change from baseline in LF MAP oscillations, the relationship between presyncopal time and the maximum magnitude of oscillations strengthened ($r=0.64$; $p=0.001$) (Figure 2, panel B). There was no relationship between presyncopal time and maximum magnitude of LF MCAv oscillations ($r=-0.25$; $p=0.31$) (Figure 2, panel C), but this relationship also improved when assessed with the relative change from baseline ($r=0.45$; $p=0.06$) (Figure 2, panel D).

After separating participants into high and low tolerance groups, the time from the start of baseline to the maximum magnitude of LF oscillations for MAP and MCAv was calculated. As shown in figure 3, the absolute time from baseline at which maximum LF oscillations occurred for both MAP and MCAv was longer for high tolerant participants compared to low tolerant participants (Figure 3, panels A and C). As this response is likely due to higher tolerant participants simply lasting longer through the LBNP protocol, a relative time to maximum LF oscillations was calculated as described in the methods section. When using this approach, maximum oscillations in MAP and MCAv occurred at a relatively similar time for both high and low tolerant participants, although the between subject variability was much higher in the low tolerant group (Figure 3, panels B and D).

Finally, the magnitude of maximum oscillations (LF max) was compared between high and low tolerant individuals. Both MAP and mean MCAv LF oscillations increased compared to baseline for both tolerance groups (Figure 4, panels A & C), but there was no difference between groups at baseline or at LF max. When calculating the change in magnitude of LF oscillations from baseline, a greater increase was observed for high tolerant individuals for MAP ($p=0.02$) but not for MCAv ($p=0.15$) (Figure 4, panels B & D). The reduction in ScO_2 was minimal and no different between the high and low tolerant groups at the time points of either the maximum MAP oscillations or maximum MCAv oscillations (Figure 4, panels F & H).

DISCUSSION

The aim of this study was to examine the relationship between LBNP tolerance time and the magnitude of LF hemodynamic oscillations using the wavelet transform approach. The key findings of this study include: 1) presyncopal time is positively associated with the increase in magnitude of LF oscillations in arterial pressure and cerebral blood flow, 2) this increase in oscillatory magnitude occurs at a relatively similar time, regardless of tolerance to the LBNP stress, and 3) high tolerant individuals exhibited a greater relative increase in arterial pressure LF oscillations but not cerebral blood flow LF oscillations.

Rickards et al. was the first to associate increases in hemodynamic oscillations to tolerance to the central hypovolemic challenge of LBNP (6). In response to the same step-wise LBNP protocol as used in the current study, LF oscillations in MAP and mean MCAv were quantified for each LBNP step using FFT. High tolerant individuals exhibited greater amplitudes of MAP and MCAv LF oscillations compared with individuals with low tolerance at the final common stage of LBNP (-60 mmHg), and during the final 3-min before onset of presyncope.

Similarly, we observed an association between LBNP tolerance of an individual and the maximum magnitude of MAP and MCAv LF oscillations. However, in this study the time point of interest was not at any pre-determined step of LBNP; rather, the continuous wavelet transform was used to identify the precise time where LF oscillations in MAP and MCAv were at their greatest amplitude. Based on this analysis, we demonstrate that tolerance to LBNP is most strongly related to the relative increase of LF oscillations in arterial pressure, and to a lesser extent, cerebral blood flow (Figures 2 and 4). While Rickards et al. observed increases in both MAP and MCAv oscillations for the high tolerant group compared to the low tolerant group, we observed a greater increase in oscillatory amplitude only for MAP in the high tolerant group. Both the high and low tolerant groups experience similar decreases in ScO_2 in our study, which may be related to the similar amplitude of oscillations in MCAv. These data suggest that oscillations in arterial pressure may be having other systemic effects outside of protecting tissue oxygenation (such as augmenting sympathetic activity) that could contribute to LBNP tolerance.

In both the current study and the Rickards et al. study (6), high tolerant individuals had greater amplitudes of oscillation during LBNP, but both high and low tolerant individuals exhibited increases in arterial pressure oscillations from baseline. Endogenous generation of arterial pressure oscillations around 0.1 Hz have been linked to the effects of sympathetic neural activity on the cardiovascular system (23, 24). Convertino et al. measured the muscle sympathetic nerve activity (MSNA) between high and low tolerant individuals to a similar step-wise LBNP protocol (25). While both groups exhibited an increase in MSNA, the high tolerant group exhibited a greater increase in MSNA at presyncope compared to the low tolerant group. As MSNA increases in absolute terms with LBNP, oscillations in MSNA within the low frequency range also increase, as does coherence between MSNA and arterial pressure

oscillations (26). This may help to explain the results in our current study, where arterial pressure oscillations displayed a similar response between tolerance groups albeit for a different absolute time point during LBNP, where sympathetic activity was likely higher.

Two computational studies have linked the amplitude of hemodynamic oscillations within the microvasculature with greater protection of tissue oxygenation (1, 2). Tsai and Intaglietta (1) first developed mathematical models of vasomotion and its effect on tissue oxygenation. In their model, amplitude of vasomotion (and the resulting flow through the microvasculature) was modelled at ±50% and ±90% of the average flow. In their scenarios, the larger amplitude of oscillations (modelled at ±90% of the average flow) always produced a greater protection of tissue oxygenation (1). Goldman and Popel expanded on these earlier models to include hypoxic tissue without myoglobin, and compared the effect of varying amplitudes of hemodynamic oscillations. Similar to Tsai and Intaglietta, greater amplitudes of oscillations resulted in greater protection of tissue oxygenation (2). Experimental evidence of this phenomenon is limited, however. While tissue oxygenation was not measured, early studies have recognized a consistent increase in hemodynamic oscillations around 0.1 Hz during hemorrhage. For example, Auer & Gallhofer described these oscillations in cerebral pial arteries during hemorrhage in cats (27). Hudetz et al. took these observations further and quantified the response across step-wise decreases in blood pressure via hemorrhage in rats (28). They observed linear increases of oscillatory amplitude at around 0.1 Hz in microvascular flux (measured via laser Doppler flux) with each decrease in arterial pressure.

Two of our studies have also assessed the effect of forcing hemodynamic oscillations in arterial pressure and blood flow at 0.1 Hz in humans during a simulated hemorrhage. Our first study utilized LBNP with or without superimposed oscillations at both 0.1 Hz and 0.05 Hz in

chamber pressure in order to drive hemodynamic oscillations during a simulated hemorrhage (4). This approach protected against reductions in cerebral and muscle tissue oxygenation compared to a control, non-oscillatory condition. Subsequently, we repeated this study under the added stress of hypoxia and found a similar protection in both cerebral and muscle tissue oxygenation when arterial pressure and cerebral blood flow are oscillated at 0.1 Hz during simulated hemorrhage (see Chapter III) (5). Hockin et al. also conducted a study in humans using intermittent inflation of calf cuffs at ~0.07 Hz during the central hypovolemic challenge of combined head-up tilt and LBNP (29). While arterial pressure, stroke volume, and cerebral blood flow were protected, and tolerance to central hypovolemia was improved with cyclical leg cuff inflations, the amplitude of subsequent oscillations in arterial pressure and cerebral blood flow were not reported. It is known, however, that leg cuffs oscillating within the LF range can produce robust oscillations in MAP and MCAv at rest (30). Additional experimental evidence is needed to compare the effects of varying amplitudes of hemodynamic oscillations on tissue oxygenation under hypoperfused conditions.

In this study, the absolute time at which maximal LF oscillations occurred in MAP or MCAv was further from baseline in the high tolerant vs. low tolerant participants. However, when calculated as a relative time from baseline, there was no difference between tolerance groups, and most participants exhibited the largest amplitudes of oscillations around 80% of the way through their individual protocol (Figure 3). This finding suggests a similar relative time profile for the maximal generation of endogenous LF oscillations regardless of tolerance. Rickards et al. (6) measured LF oscillatory amplitudes in MAP and MCAv at each step of LBNP, and showed that amplitudes of oscillations at the -60 mmHg stage of LBNP were higher in the higher tolerant individuals compared to low tolerant individuals (6). The time course for these

oscillations was not measured past -60 mmHg LBNP for high tolerant individuals, but our results indicate that the amplitude of LF oscillations may continue to increase in high tolerant individuals, at least in MAP. It may be that the general pattern of responses in LF oscillations is similar between high and low tolerant individuals, except high tolerant individuals are able to continue increasing LF oscillatory amplitude where low tolerant individuals cannot.

Methodological Considerations

Respiration rate and depth were not controlled in this study. To account for the effects of respiration on hemodynamic oscillations within the LF range, participants were removed from the analysis if they had a respiration rate below 10 breaths per min (i.e., 0.07-0.15 Hz or 4.2-9 breaths/min). This approach ensures that oscillatory amplitudes were not augmented by the respiratory pump, focusing instead on sympathetically generated LF oscillations (23). Participants were also removed from the analysis if a single large breath interfered with identifying oscillations due to sympathetic activity. Previous studies in a canine model of hemorrhage by Guyton and Harris, highlighted the effect of small perturbations in pressure (via reinfusion of blood) during their hemorrhage protocol (31). Reinfusion of blood in these animals triggered small chains of oscillations in arterial pressure that lasted between 15-30 seconds. Similarly, when inspecting the individual recordings in our study, large breaths augmented arterial pressure (via the respiratory pump) and triggered a short chain of oscillations in arterial pressure and cerebral blood flow. This introduced noise into the analysis, as these oscillations were sometimes of greater amplitude than the sympathetically induced oscillations occurring independent of respiration. Application of these necessary exclusion criteria resulted in a reduced number of participants in this study, but allowed for a more focused analysis, without the

confounding effect of respiration on the outcomes of interest. Future studies should consider explicitly examining the role of respiratory rate and/or depth on the generation of hemodynamic oscillations, and the subsequent effect on tissue perfusion and oxygenation.

As this was a retrospective observational study, the amplitudes of the LF oscillations in MAP and MCAv were not controlled. While we, and others, have demonstrated that increasing the amplitude of hemodynamic oscillations in the LF range can improve tolerance to LBNP (4, 7), less is known about the effect of varying amplitudes of oscillations on hemodynamic responses. Hamner et al. explored the relationship between forcing various oscillatory amplitudes and frequencies in arterial pressure and cerebral blood flow via varied amplitudes and frequencies of oscillatory LBNP (32). Participants in this study were exposed to oscillatory LBNP at 3 frequencies (0.03, 0.05, and 0.1 Hz) and 2 amplitudes (0-20 mmHg and 0-40 mmHg). Generally, the greater the amplitude of forced oscillations in LBNP, the greater the amplitude of oscillations in arterial pressure and cerebral blood flow. However, these investigators did not address the effects of oscillations on tolerance to LBNP or tissue oxygenation. Only Hockin et al. has explored varying the amplitudes of forced oscillations via leg cuffs during a hypovolemic stress, which demonstrated that greater amplitudes of oscillations in cuff pressures led to increased tolerance to central hypovolemia (29). They did not, however, quantify the resulting amplitudes of oscillations in arterial pressure or cerebral blood flow, or the effect on tissue oxygenation. Future experimental work is needed in this area to understand the role of oscillatory amplitude on physiological outcomes.

The data from this study demonstrates that 1) the greater the increase in amplitude of LF oscillations in arterial pressure, the greater the tolerance to the central hypovolemia induced by LBNP, but; 2) the relative time profiles for when the maximum amplitude of oscillations occurs

is independent of LBNP tolerance. This indicates that therapies aimed at increasing low frequency oscillations in arterial pressure and blood flow could provide therapeutic benefit in extending tolerance to hypovolemic conditions, such as blood loss.

Table 1. Demographics and baseline values for all participants (N=22), and participants separated by tolerance group.

	All Participants	High Tolerant	Low Tolerant	P-value
N	22 (11 M/11 F)	14 (9 M/5 F)	8 (2 M/6 F)	
Age (y)	25 ± 3	25 ± 3	25 ± 3	0.93
Height (cm)	166 ± 10	168 ± 10	163 ± 10	0.23
Weight (kg)	68 ± 14	69 ± 14	68 ± 13	0.83
MAP (mmHg)	94.8 ± 10	94.3 ± 10.9	95.7 ± 8.8	0.74
MCAv (cm/s)	65.6 ± 13.0	62.0 ± 11.4	72.0 ± 13.8	0.11

Data are presented as mean ± standard deviation. P-values are presented for unpaired t-tests between tolerance groups. N, number of participants per group; MAP, mean arterial pressure; MCAv, middle cerebral artery velocity.

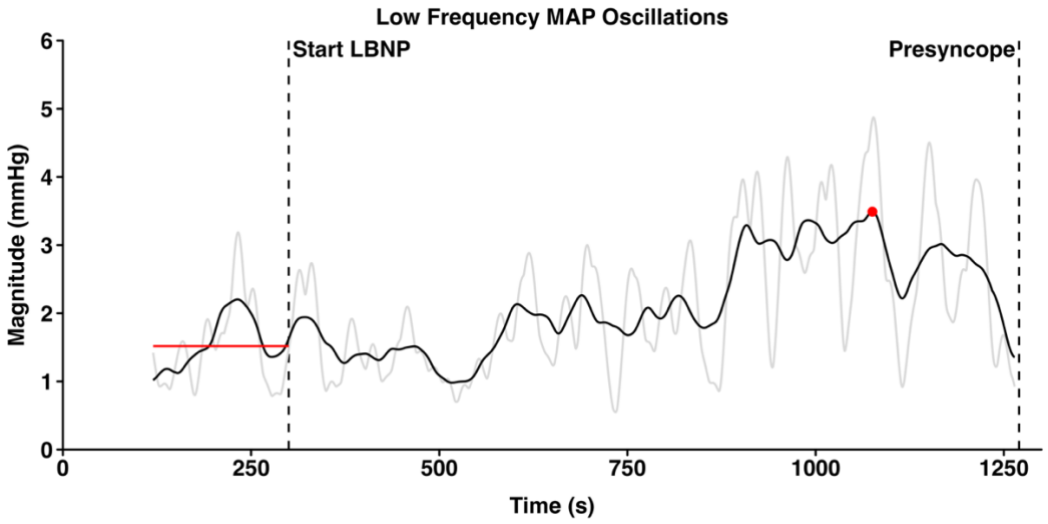

Figure 1. A representative example of the wavelet analysis.

The continuous wavelet function was performed on the resampled mean arterial pressure (MAP) signal (grey line) and subsequently smoothed with a 1-min moving average (dark line). The last 3-min of baseline (red horizontal line) was used as a reference to detect the maximum magnitude of oscillations during LBNP (red dot).

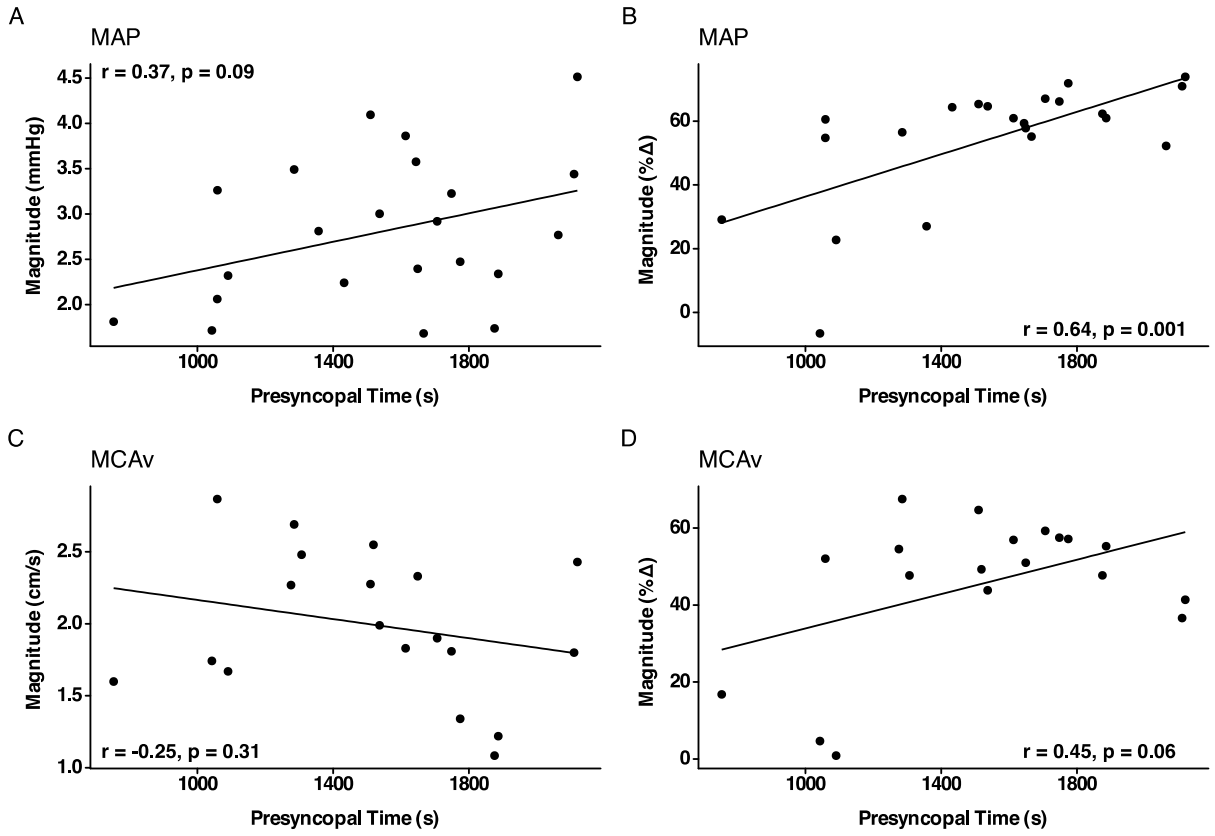

Figure 2. Correlations between presyncopal time and magnitude of oscillations.

Both absolute (panels A and C) and relative (panels B and D) values for mean arterial pressure (MAP) and middle cerebral artery velocity (MCAv). Exact Pearson's correlation (r) and p-values are reported.

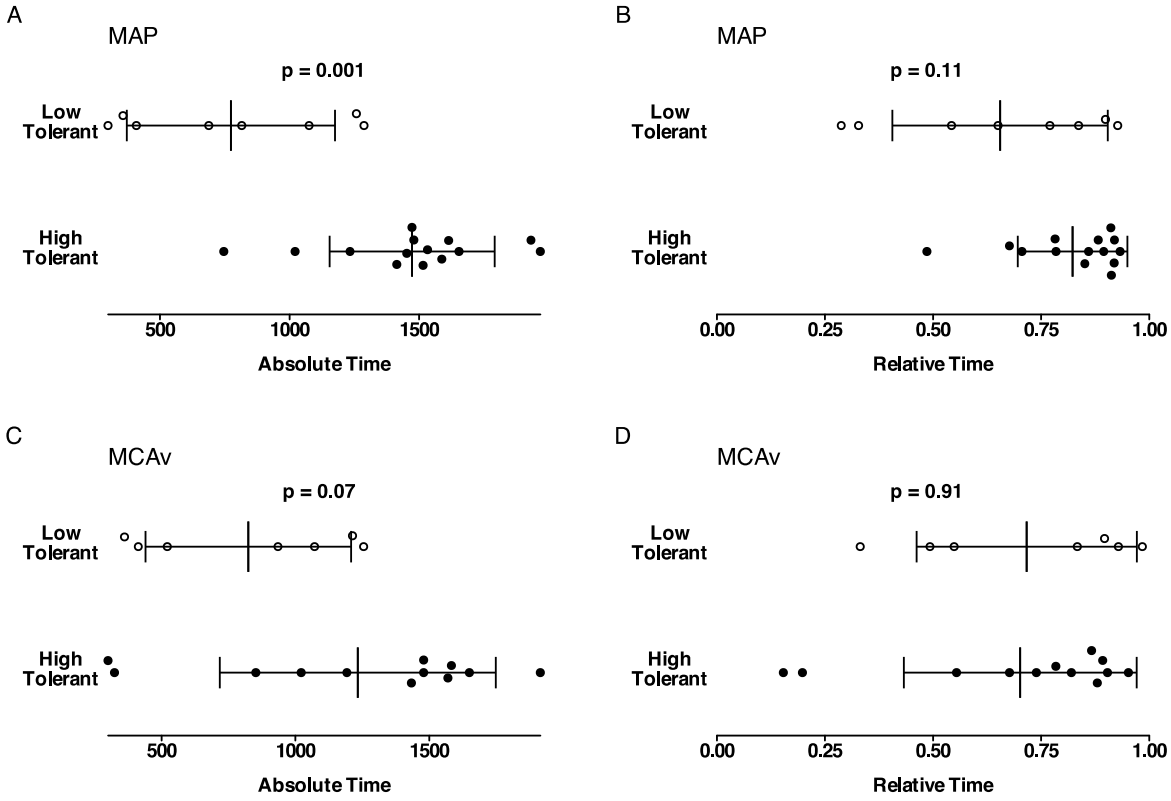

Figure 3. Absolute and relative timing of LF oscillations in arterial pressure and cerebral blood flow.

The absolute (panels A and C) and relative (panels B and D) time at which LF oscillations in mean arterial pressure (MAP) and middle cerebral artery velocity (MCAv) occurred for high and low tolerant participants. Data were compared with a Welch's t-test and the exact p-values are reported. Data are presented as mean ± standard deviation.

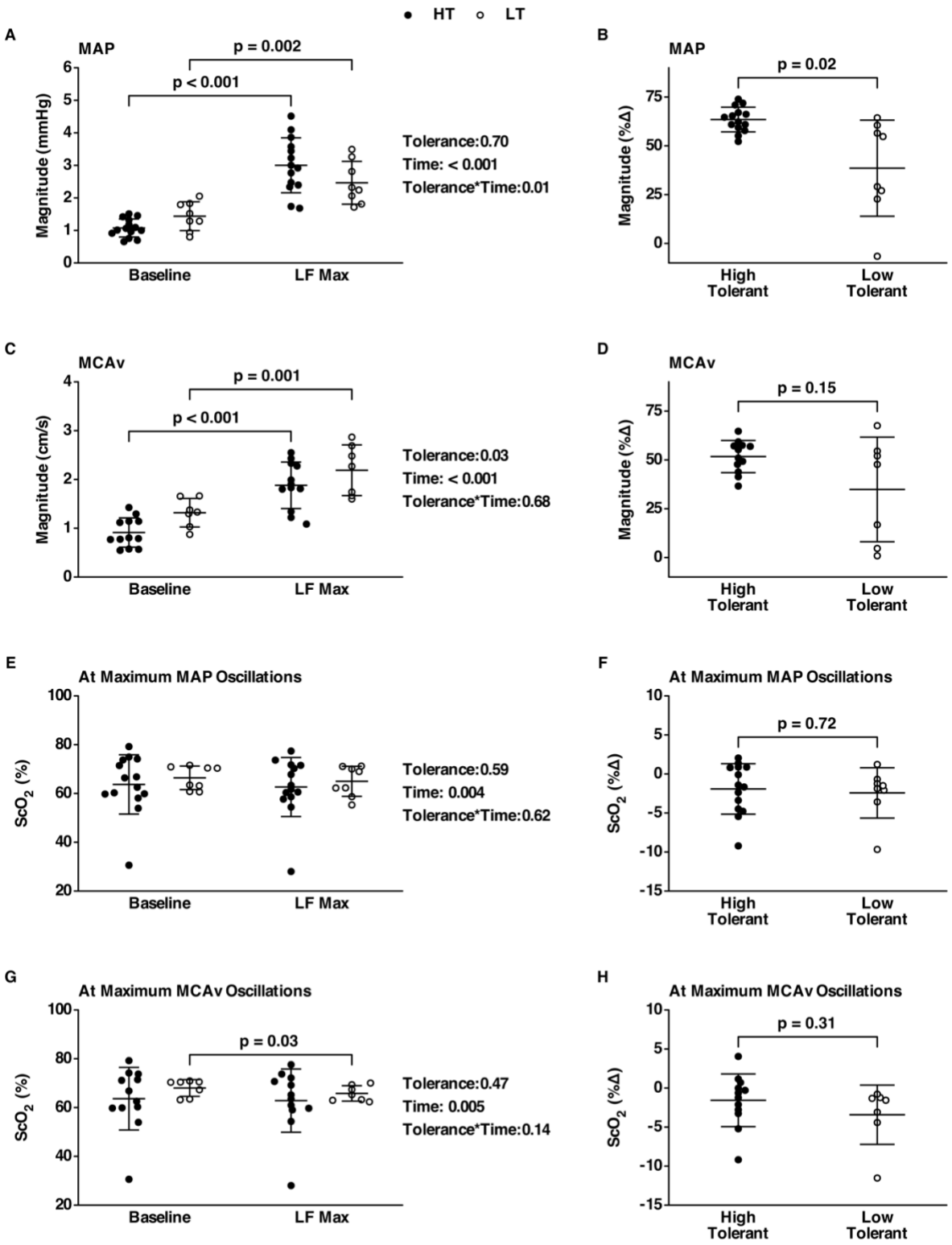
HT
LT
A
MAP
p = 0.002
p < 0.001
Magnitude (mmHg)
Baseline
LF Max
Tolerance:0.70
Time: < 0.001
Tolerance*Time:0.01
B
MAP
p = 0.02
Magnitude (%Δ)
High Tolerant
Low Tolerant
C
MCAv
p = 0.001
p < 0.001
Magnitude (cm/s)
Baseline
LF Max
Tolerance:0.03
Time: < 0.001
Tolerance*Time:0.68
D
MCAv
p = 0.15
Magnitude (%Δ)
High Tolerant
Low Tolerant
E
At Maximum MAP Oscillations
ScO2 (%)
Baseline
LF Max
Tolerance:0.59
Time: 0.004
Tolerance*Time:0.62
F
At Maximum MAP Oscillations
p = 0.72
ScO2 (%Δ)
High Tolerant
Low Tolerant
G
At Maximum MCAv Oscillations
p = 0.03
ScO2 (%)
Baseline
LF Max
Tolerance:0.47
Time: 0.005
Tolerance*Time:0.14
H
At Maximum MCAv Oscillations
p = 0.31
ScO2 (%Δ)
High Tolerant
Low Tolerant

Figure 4. The maximum magnitude of low frequency (LF) oscillations and influence on cerebral tissue oxygenation.

Mean arterial pressure (MAP; A – absolute values, B – relative values from baseline), middle cerebral artery velocity (MCAv; C – absolute values, D – relative values from baseline), cerebral tissue oxygen saturation (ScO_2) at maximum MAP oscillations (E – absolute values, F – relative values from baseline) and at maximum MCAv oscillations (G – absolute values, H – relative values from baseline). Absolute values were first compared using a linear mixed model for repeated measures. Specific pairwise comparisons between tolerance groups at each time point and within tolerance across time were performed and corrected using Holm's method (A, C, E, G). Relative changes were compared using Welch's t-test (B, D, F, H). HT, high tolerant; LT, low tolerant. Data are presented as mean ± standard deviation, and exact p-values are reported

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CHAPTER III

Peaks and Valleys: Oscillatory cerebral blood flow at high altitude protects cerebral tissue oxygenation

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Physiological Measurement 2021 Jun 29;42(6).

ABSTRACT

Introduction: Oscillatory patterns in arterial pressure and blood flow (at ~0.1 Hz) may protect tissue oxygenation during conditions of reduced cerebral perfusion and/or hypoxia. We hypothesized that inducing oscillations in arterial pressure and cerebral blood flow at 0.1 Hz would protect cerebral blood flow and cerebral tissue oxygen saturation during exposure to a combination of simulated hemorrhage and sustained hypobaric hypoxia. **Methods:** Eight healthy human subjects (4 male, 4 female; 30.1±7.6 y) participated in two experiments at high altitude (White Mountain, California, USA; altitude, 3800 m) following rapid ascent and 5-7 days of acclimatization: 1) static lower body negative pressure (LBNP, control condition) was used to induce central hypovolemia by reducing chamber pressure to -60 mmHg for 10-min **(0 Hz)**, and; 2) oscillatory LBNP where chamber pressure was reduced to -60 mmHg, then oscillated every 5-s between -30 mmHg and -90 mmHg for 10-min **(0.1 Hz)**. Measurements included arterial pressure, internal carotid artery (ICA) blood flow, middle cerebral artery velocity (MCAv), and cerebral tissue oxygen saturation (ScO_2). **Results:** Forced 0.1 Hz oscillations in mean arterial pressure and mean MCAv were accompanied by a protection of ScO_2 (0.1 Hz: -0.67±1.0 %; 0 Hz: -4.07±2.0 %; P = 0.01). However, the 0.1 Hz profile did not protect against reductions in ICA blood flow (0.1 Hz: -32.5±4.5 %; 0 Hz: -19.9±8.9 %; P = 0.24) or mean MCAv (0.1 Hz: -18.5±3.4 %; 0 Hz: -15.3±5.4 %; P = 0.16). **Conclusions:** Induced oscillatory arterial pressure and cerebral blood flow led to protection of ScO_2 during combined simulated hemorrhage and sustained hypoxia. This protection was not associated with the preservation of cerebral blood flow suggesting preservation of ScO_2 may be due to mechanisms occurring within the microvasculature.

INTRODUCTION

Adjustment of cerebral blood flow is a key mechanism for maintaining adequate oxygen delivery during challenges to cerebral oxygen availability. In addition to increases in absolute blood flow, the pattern of blood flow delivered to the brain may also influence the maintenance of oxygen within the brain tissues. Evidence for the role of oscillations in the maintenance of tissue oxygenation has slowly accumulated within the last 30 years (1-3).

Tsai and Intaglietta were the first to postulate, and then mathematically simulate, the role of vasomotion and oscillatory blood flow on the preservation of tissue oxygen delivery (4). Their early models demonstrated a protection of tissue oxygenation when vasomotion was active (1). This phenomenon was later confirmed through a series of mathematical simulations of oxygen transport through capillaries by Goldman & Popel (2). Using a model designed from the capillary network within a hamster cheek-pouch retractor muscle, vasomotion (and the resulting oscillatory blood flow) was simulated between 1.5-12 cycles per minute (0.025-0.2 Hz) in hypoxic tissues with and without myoglobin. Oscillatory blood flow was effective in improving tissue oxygen delivery when oscillations in blood flow were between 1.5-6 cycles per minute (0.025-0.1 Hz), with the most prominent effect observed when tissues lacked myoglobin, so had no internal oxygen stores (2). In a rat model of reduced muscle tissue perfusion via tourniquet aided constriction of the left femoral artery, the effects of spontaneously occurring vasomotion of about 2 cycles per minute (~0.03 Hz) were compared to when vasomotion was inhibited by calcium-channel blockade (5). When present, vasomotion preserved functional capillary density in the surrounding tissues, demonstrating a protection of perfusion to the tissue even when blood flow was reduced (5). In human models, oscillations in arterial pressure and cerebral blood flow around 0.1 Hz have been implicated in increased tolerance to central hypovolemia (3, 6-8).

During experimentally induced central hypovolemia (via application of lower body negative pressure, LBNP), preservation of brain tissue oxygenation is challenged by a reduction in cerebral blood flow (i.e., oxygen delivery) (9, 10). We previously reported the protection of cerebral tissue oxygen saturation by forcing 0.1 Hz oscillations in arterial pressure and cerebral blood flow with LBNP under normoxic conditions (3). However, oxygenation of the tissues can also be challenged under hypoxic conditions, including clinical conditions where gas exchange is impaired, and with exposure to high altitude.

Hypoxia stimulates cyclical vasomotion and may be an important compensatory response to the low oxygen conditions (4, 11). High altitude exposure imposes a chronic and systemic hypoxic challenge to the body, with reductions in the atmospheric partial pressure of oxygen and subsequent decreases in arterial oxygen saturation, oxygen content, and delivery. Salvi et al. reported an increase in vasomotion in the skin microvasculature of the human forearm centered around 0.1 Hz (measured by laser Doppler flux) during high altitude ascent up to 5050 m (12). However, the role of 0.1 Hz oscillatory arterial pressure and blood flow on brain tissue oxygenation under the combined stressors of hypoxia and hypovolemia has not been explored. Understanding these responses may assist in the development of potential therapies for clinical use. Generating oscillations in blood flow could be an effective treatment for clinical conditions such as hemorrhagic shock, stroke, and sepsis - all of which challenge the delivery of oxygen to metabolically active tissues due to reduced blood flow and/or reduced arterial oxygen content.

In this study, we examined the effects of inducing 0.1 Hz oscillations in arterial pressure and cerebral blood flow on cerebral tissue oxygenation during the combined stressors of central hypovolemia and sustained hypobaric hypoxia. We tested the hypothesis that inducing oscillations in arterial pressure and cerebral blood flow at 0.1 Hz would protect cerebral blood

flow and cerebral tissue oxygen saturation during exposure to a combination of simulated hemorrhage and sustained hypobaric hypoxia via ascent and partial acclimatization to high altitude.

METHODS

Ethical Approval

This study was conducted in accordance with the Canadian Government Tri-Council Policy on research with human participants, consistent with the Declaration of Helsinki, except for registration in a database. Ethical approval was received from the Mount Royal University Human Research Ethics Board (Protocol 101879), the University of Calgary Conjoint Health Research Ethics Board (Protocol REB18-0374), and the University of North Texas Health Science Center (Protocol 2019-110). This study was a part of a large high altitude research expedition to the Barcoft Laboratory on White Mountain in the Sierra Nevada Mountains, CA, USA in August 2019. However, the specific questions and participant recruitment for this study were determined *a priori*.

Participants

Young healthy, human participants were recruited for two experimental sessions, both conducted at high altitude (details to follow). Participants were briefed on the purpose, design, and risks of the study, and written informed consent was obtained. Prior to experimentation, participant height, weight, age, and sex were recorded. Female participants completed a urine pregnancy test to ensure they were not pregnant prior to each experimental session. It was not possible to control for menstrual cycle phase due to the nature of this expedition study. All

participants abstained from alcohol, exercise, caffeine, dietary supplements, and medications for at least 12 h before experimentation.

Instrumentation

Upon arrival for each experiment, participants laid supine in a LBNP chamber with their iliac crest in line with the chamber opening. They were sealed into the chamber around the waist with heavy-duty plastic, and a neoprene band. Participants were then instrumented for continuous ECG recordings in a lead II configuration (shielded leads, cable and amplifier; AD Instruments, Bella Vista, NSW, Australia), and continuous arterial pressure was monitored and recorded using finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands). Aortic diameter was measured (average of 2 measurements) using ultrasound (Terason uSmart 3300, Teratech, Burlington, MA, USA) with a 5 MHz curvilinear probe (5C2), which was subsequently used to correct the Modelflow® stroke volume measurements obtained from the Finometer prior to data collection.

Respiratory gases were monitored using an oral nasal cannula connected to a gas analyzer (ML206 Gas Analyzer; AD Instruments) and used to calculate respiratory rate, pressure of end-tidal CO_2 ($P_{ET}CO_2$), and O_2 ($P_{ET}O_2$) corrected for BTPS using atmospheric pressure. Arterial oxygen saturation (SpO_2) was continuously monitored via pulse oximetry (Nonin 7500 FO, Nonin Medical Inc, MN, USA).

Oxy-hemoglobin and deoxy-hemoglobin concentrations were measured via near infrared spectroscopy (OxiplexTS; ISS, Champaign-Urbana, IL, USA) in cerebral tissue over the right frontal lobe, and over the flexor carpi ulnaris muscle of the forearm (located by flexion of the fingers). Cerebral and muscle total hemoglobin concentration (THC; oxy-hemoglobin + deoxy-

hemoglobin), cerebral tissue oxygen saturation (ScO_2; (oxy-hemoglobin/THC)*100), and muscle tissue oxygen saturation (SmO_2) were then calculated.

Middle cerebral artery velocity (MCAv) was measured using transcranial Doppler ultrasound and a 2 MHz probe through a temporal window (ST3; Spencer Technologies, Seattle, WA, USA). Duplex Doppler ultrasound (Terason uSmart 3300, Teratech, Burlington, MA, USA) was used to measure internal carotid artery (ICA) diameter and velocity with a 15 MHz linear array probe (15L4 Smart Mark™). Two trained sonographers performed the measurements ensuring that repeated measures on each participant were performed by the same sonographer. A consistent ICA image was ensured between experiments by noting the position in reference to the carotid bifurcation, and the blood velocity profile at rest (13). Video recordings of the ultrasound measures were captured using screen recording software (Camtasia, Techsmith Corp, MI, USA) and stored as AVI files for later analysis (see "Data Analysis" section for details).

Hemoglobin (Hb) concentration was measured in participants the morning of each experimental day using finger capillary blood sampling and a hemoglobinometer (Hemocue Hemoglobin System, Hb201+ with microcuvettes; Ängelholm, Sweden).

Experimental Design

The combined stressors of hypobaric hypoxia (high-altitude exposure) and central hypovolemia (via LBNP) were used in order to assess the effects of oscillatory blood flow when both cerebral blood flow and cerebral tissue oxygen saturation are reduced.

High Altitude

Prior to exposure to altitude, all participants lived at or below an altitude of 1,045 m and had not traveled to high altitude within the 6 months before the study. All participants flew from Calgary, Canada (1,045 m) to Las Vegas, NV, USA (610 m). Less than 12-h after arrival in Las Vegas, participants rapidly ascended to 3800 m (Barcroft Laboratory, White Mountain, California) within 6 h. Participants acclimatized to this high-altitude environment for 4-5 days, and were subsequently tested on days 5, 6 and 7 of altitude exposure for this study (figure 1).

Lower Body Negative Pressure (LBNP)

After instrumentation, a 5-min baseline was recorded, and one of two 10-min LBNP protocols was initiated: 1) a non-oscillatory (0 Hz) condition where chamber pressure was lowered to -60 mmHg over 30-s and maintained at this pressure for 9.5-min, or 2) an oscillatory (0.1 Hz) condition where chamber pressure was lowered to -60 mmHg over 30-s, held at -60 mmHg for 30-s, and then oscillated at 0.1 Hz between -30 mmHg and -90 mmHg for 9-min with 5-s at each pressure (figure 1). The protocol was terminated if the participant's systolic arterial pressure fell below 80 mmHg, or at the participant's request due to symptoms of presyncope such as nausea, dizziness, lightheadedness, and loss of color vision. For the 0 Hz condition, if participants completed the 10-min stage of LBNP at -60 mmHg, the chamber was reduced to -70, -80, -90, and -100 mmHg for 5-min each until the onset of presyncope. The data collected for these additional steps are the focus of a separate manuscript and will not be reported here. A 10-min recovery period followed the completion of each LBNP protocol. The order of LBNP profiles was randomized and counterbalanced between experimental sessions. At least 24-h separated the two sessions.

Data Analysis

ECG, arterial pressure, stroke volume, MCAv, ScO_2, SmO_2, SpO_2, $P_{ET}CO_2$ and $P_{ET}O_2$ waveform data were continuously recorded at 1000 Hz (PowerLab/LabChart; AD Instruments) and stored for subsequent analysis. Specialized software (WinCPRS; Absolute Aliens, Turku, Finland) was used for beat-to-beat analysis of hemodynamic data in the time and frequency domains. The final 3-min of the baseline period was used for averaging of time domain measures, while the entire 5-min baseline period was used for frequency domain measures. During LBNP, time domain measures were averaged over the 1-min prior to LBNP release, while frequency domain measures were averaged over the 5-min prior to LBNP release.

R-waves were detected from the ECG recording and used for the calculation of heart rate, and for dividing all other waveform data into cardiac cycles for subsequent analysis. Beat-to-beat systolic and diastolic arterial pressures and cerebral blood velocities were then identified, and mean arterial pressure (MAP) and mean MCAv were calculated using the area under the curve. Cardiac output was calculated by multiplying heart rate by stroke volume, and systemic vascular resistance was calculated by dividing MAP by cardiac output.

Arterial oxygen content was calculated with the following equation:

$$CaO_2\ (mL \cdot dL^{-1}) = 1.36 \times [Hb](g \cdot dL^{-1}) \times SpO_2\ (\%)/100 + 0.003 \times P_{ET}O_2\ (mmHg)$$

where $P_{ET}O_2$ was used as a surrogate for PaO_2. Simultaneous diameter and blood velocity measurements in the ICA were obtained using specialized wall tracking software, as previously reported (14). A minimum of 10 consecutive cardiac cycles were used to average ICA blood

velocity and diameter during the last 5-min of each LBNP protocol, as per published guidelines (13). The anatomical location of the diameter measurements was matched between experimental sessions during analysis. These data were then used to calculate ICA blood flow as: $\pi(\frac{diameter}{2})^2 * \left(\frac{peak\ velocity}{2}\right) * 60$. Delivery of oxygen ($DO_2$) through the ICA was calculated as:

$$DO_2 = [ICA\ Flow\ (mL \cdot min^{-1}) \cdot CaO_2\ (mL \cdot dL^{-1})]/100.$$

The MCA conductance index and ICA conductance were calculated by dividing mean MCAv or ICA flow by MAP.

The oscillatory characteristics of MAP and MCAv were determined using the fast Fourier-transformation as previously described from our laboratory (15). Briefly, data were made equidistant by interpolating linearly and resampling at 5 Hz. Data were then passed through a low-pass impulse response filter using a cut-off frequency of 0.5 Hz. Fast Fourier-transformation was performed using a Hanning window and the Welch Method to compute power spectra. The frequency range used for low frequency (LF) power spectral analysis was 0.07-0.15 Hz. Spectral power is expressed as the integrated area within this range. Spectral power was also calculated as a point measurement at 0.1 Hz.

For each subject and each condition, the percent change from baseline was calculated for any given variable by taking the difference between baseline and LBNP values divided by the baseline value and multiplied by 100.

Statistical Analysis

Absolute data were analyzed using a linear mixed model for repeated measures with time point (baseline or hypovolemia) and LBNP condition (0 Hz or 0.1 Hz) as factors in the model

followed by Tukey post-hoc tests (JMP, SAS Institute, Cary, NC). The percent change between baseline and LBNP was calculated for the two protocols and compared using paired t-tests after testing for normality with the Shapiro-Wilk test to ensure normal distribution of data. Paired t-tests were also used to compare baseline hemoglobin concentration, chamber pressures, and LBNP experimental time between the two protocols. All data are presented as mean ± standard deviation unless otherwise stated. Exact P-values are reported for all comparisons.

RESULTS

Participants

Nine participants were initially recruited in the study, but one participant was unable to complete the experiments due to mild high-altitude illness. Eight individuals (4 males, 4 females) participated in both LBNP conditions (table 1). All participants completed the experiments on days 5-7 of high-altitude exposure, with at least 24-h between experiments.

LBNP Results

Average LBNP chamber pressure was slightly lower during the 0.1 Hz protocol by about 6 mmHg (0 Hz, -59.9 ± 0.4 vs 0.1 Hz, 66.6 ± 2.7 mmHg; $P < 0.01$). Six of the 8 participants completed the entire 0 Hz profile without becoming presyncopal, and seven participants completed the 0.1 Hz profile, with no difference in completion times (0 Hz, 880.5± 40.5 s vs. 0.1 Hz, 894.4 ± 15.8 s; $P=0.18$). Data from all 8 participants in both trials are included in the analysis. A representative figure of physiological signals acquired is shown in figure 2.

Cardiovascular Hemodynamic Responses

Hemoglobin concentrations measured from the morning of each experiment were not different (P=0.32; table 2). During the baseline period, prior to application of LBNP, there were no differences in any systemic hemodynamic variables between experimental conditions ("Cardiovascular" variables in table 2). When assessing the systemic hemodynamic responses to central hypovolemia with or without 0.1 Hz oscillations, no differences were observed in the responses of stroke volume, heart rate, MAP, or systemic vascular resistance, indicating a similar cardiovascular challenge between profiles (table 2, figure 3). Compared with the 0 Hz condition, $P_{ET}CO_2$ was generally higher for the 0.1 Hz condition (LBNP effect, P=0.04), accompanied by a lower $P_{ET}O_2$ (LBNP effect, P=0.03) and SpO_2 (LBNP effect, P=0.03), despite similar respiration rates ("Respiratory" variables in table 2). During LBNP for both conditions, SpO_2 increased, although there were no increases in respiration rate (table 2). SmO_2 decreased during the 0 Hz condition, but this decrease was attenuated during the 0.1 Hz profile (table 2, figure 3).

Cerebral Hemodynamic Responses

Baseline measurements of mean MCAv and ICA blood flow were no different between conditions ($P \geq 0.38$); figure 4, panels C & E). MCAv recordings were obtained in 7 participants during LBNP due to the loss of signal in one participant from headset movement upon onset of LBNP, and ICA blood flow recordings were obtained in 5 participants during LBNP with 2 recordings lost due to technical malfunctions and poor recording quality in 1 participant. ICA velocity decreased with LBNP (time effect, P<0.01) while a small decrease in diameter was observed only during the 0.1 Hz profile (table 2). MCAv and ICA blood flow decreased in both LBNP conditions, with no differences in responses between 0 or 0.1 Hz LBNP (figure 4, panels

C & E). CaO_2 was higher overall for the 0 Hz condition but increased with both LBNP profiles (figure 4, panel G). Unilateral DO_2 through the ICA decreased by a similar magnitude with both LBNP profiles (figure 4, panel I). Despite these similar responses in cerebral blood flow and oxygen delivery between profiles, reductions in ScO_2 were attenuated during the 0.1 Hz condition (figure 4, panels A & B). Importantly, this pattern of preserved ScO_2 during the 0.1 Hz condition was consistently observed for every participant.

Frequency Domain Responses

A relative increase in amplitude of oscillations was observed at 0.1 Hz for MAP during the 0.1 Hz condition, consistent with the experimental design (table 3). Oscillations in $P_{ET}CO_2$ were also explored as these may have contributed to oscillations in MCAv. Amplitude of $P_{ET}CO_2$ oscillations was higher overall for the 0.1 Hz vs. 0 Hz condition, with no difference over time (table 3).

DISCUSSION

The aim of this study was to assess the effects of 0.1 Hz hemodynamic oscillations on cerebral tissue oxygenation and cerebral blood flow during the combined stressors of simulated hemorrhage and sustained hypobaric hypoxia with high-altitude exposure. The main finding of our study is that oscillations in cerebral blood flow at 0.1 Hz protected against reductions in cerebral tissue oxygenation. Other important findings include: 1) hemodynamic oscillations did not protect against reductions in cerebral blood flow whether indexed by MCAv or ICA flow; and 2) the observed protection in cerebral oxygen saturation under the 0.1 Hz condition was not due to differences in arterial oxygen content or oxygen delivery.

The hemodynamic and tissue oxygenation responses to simulated and actual hemorrhage have been well documented (9, 16, 17). As the volume of blood loss increases, both cerebral blood flow and cerebral tissue oxygenation will gradually decrease. Initially, homeostatic mechanisms such as the baroreflex and cerebral autoregulation are engaged in order to maintain arterial pressure and cerebral blood flow, and there is a redistribution of blood volume towards vital organs (18) thus ensuring the delivery of oxygen to cerebral tissues. Eventually, however, these reflex compensatory responses cannot protect cerebral blood flow, leading to reduced oxygen delivery and tissue hypoxia (16). Increased extraction of oxygen from the blood into the tissues also compensates for this reduced delivery (19, 20), but tissue hypoxia will occur when this compensatory mechanism is exhausted with a persistent reduction in oxygen delivery. Reductions in oxygen delivery via decreases in oxygen content can also occur with hemorrhage due to decreases in arterial oxygen saturation and hemoglobin concentration, which become even more severe with pulmonary disorders (gas exchange limitations), anemia (reduced hemoglobin), or hypoxic conditions (hemoglobin saturation) (21).

Exposure to high altitude elicits a number of physiological responses to compensate for the hypoxic environment induced by the lower atmospheric PO_2. Initially, ventilation increases to attenuate the reduction in arterial oxygen content and oxygen delivery (22). Heart rate and cardiac output also increase with a chemoreflex-mediated elevation in sympathetic activity, further compensating for reductions in oxygen delivery (23). Interestingly, there is an acute reduction in plasma volume upon ascent to high altitude, which increases hematocrit and arterial hemoglobin concentration, and restores arterial oxygen content prior to erythropoietin-induced increases in total red blood cell volume (24, 25). This reduction in plasma volume and increase in hematocrit, however, may be detrimental during a hemorrhagic event at altitude due to

increased blood viscosity impeding blood flow. The compensatory cerebrovascular response to hypoxia is a vasodilation and subsequent increase in cerebral blood flow in order to maintain oxygen delivery to the cerebral tissues (26).

In our study, these acute compensatory responses to high altitude exposure and central hypovolemia must be considered. With the reduction in plasma volume and an increased workload placed on the heart to maintain cardiac output and blood flow to the brain with hypoxia, decreased central volume with hemorrhage will further stress the cardiovascular system. In addition, the effects of hypoxia on cerebrovascular regulatory mechanisms are not fully understood. Cerebral autoregulation, for example, may or may not be impaired under hypoxic conditions (27-31), which may further impair physiological compensation to hypovolemia. The additive effects of both hypoxia and hemorrhage on the reduction in cerebral blood flow and cerebral tissue oxygenation highlights the need to explore mechanisms and interventions that may provide benefit to the maintenance of tissue oxygenation during these combined physiological stressors.

Understanding the physiological role of oscillatory blood pressure and blood flow has been elusive. Many studies have sought to understand the mechanisms underlying hemodynamic oscillations at various frequencies, but most have developed mathematical models (4, 32-34), and only a few studies have sought experimental evidence to explain the physiological benefit of these responses. Early work using computational models suggested that oscillatory blood flow at low frequencies (less than 0.2 Hz) within the microvasculature could protect distal tissue oxygenation during reductions in flow (1, 2). This idea was further supported by an experimental study in a rat model of reduced perfusion to skeletal muscle via application of a tourniquet around the femoral artery. When endogenously occurring vasomotion was inhibited using

calcium-channel blockers during the occlusion, decreases in capillary perfusion in tissues distal to the occlusion were observed compared to when vasomotion (and therefore oscillatory blood flow) was present (5).

Data from the current study and a prior study from our laboratory (3) also indicate a protection of cerebral tissue oxygenation when oscillatory arterial pressure and cerebral blood flow are induced around 0.1 Hz during conditions of reduced cerebral perfusion and oxygen delivery. Unlike our prior study (3), stroke volume was not protected by oscillatory arterial pressure in the current investigation, and cerebral blood flow oscillations were not aided by oscillations in $P_{ET}CO_2$, indicating that these variables did not play a role in the protection of ScO_2. Similar to our prior study, however, there were no differences between LBNP profiles for other systemic cardiovascular responses such as mean arterial pressure, cardiac output, or systemic vascular resistance. The magnitude of the response in these variables was also similar to our prior work, except for cardiac output which exhibited a ~10% greater decrease at high altitude for both profiles compared with our previous study at low altitude. This finding highlights the greater physiological stress induced by the combined conditions of central hypovolemia plus hypoxia. We now demonstrate, in two independent studies, that during conditions of reduced cerebral blood flow, induced hemodynamic oscillations at 0.1 Hz protect against reductions in ScO_2 despite no protection in cerebral blood flow/velocity. Interestingly, hemodynamic oscillations in skin blood flow at 0.1 Hz measured with laser Doppler flux have also been observed during high altitude ascent and the associated hypobaric hypoxia (12). In a separate investigation connected to the current study, we also observed increased 0.1 Hz oscillations in arterial pressure with sustained exposure to high altitude compared with low altitude (unpublished observations). Given these findings, we propose that hemodynamic

oscillations are a compensatory mechanism through which tissue oxygenation is protected during states of hypoperfusion and/or hypoxia.

While the precise mechanisms for protection of tissue oxygenation have not been experimentally tested, some hypotheses have been presented. Intaglietta postulated that vasomotion creates a pump-like effect with brief but cyclical increases in both blood velocity and hematocrit down the vascular tree, aiding in the perfusion of more capillaries in hypoperfused tissue (4) – a phenomenon that Salvi et al. termed the "peripheral heart" (12). Hapuarachchi et al. suggested that the hemodynamic oscillations in arteriolar beds might have an oxygen sparing effect by preventing the diffusion of oxygen out of the arterioles (a naturally-occurring phenomenon) such that arterial blood arrives at the level of the capillary with a greater concentration of oxygen (35). Hemodynamic oscillations may also alter the distribution of red blood cells within the vessel lumen, where increases in blood velocity converge red blood cells to the center of the lumen and decreases in blood velocity allow for a more uniform distribution (36). This change in red blood cell orientation with oscillatory blood flow may alter oxygen extraction within the microvasculature and help account for the observed differences in tissue oxygenation. Further work is needed to identify and understand the physiological mechanisms underpinning protection of tissue oxygenation with oscillatory blood flow.

Methodological Considerations

There are a number of important considerations when interpreting the data from this study. First, experiments were conducted after 5 days at altitude. This exposure to the hypobaric hypoxia of altitude would allow for some compensatory adaptations to start occurring in order to increase arterial oxygen content; indeed, baseline arterial oxygen content was similar to low

altitude conditions in our subjects (unpublished observations). It is likely that testing in the acute phase of high-altitude exposure may have imposed a greater challenge to maintaining tissue oxygenation under the combined conditions of hypovolemia and hypoxia. Another consideration is the relatively small number of measurements we were able to obtain for ICA flow. While we recognize this limitation, ICA flow responses were similar to the MCA velocity responses (N=7).

Due to the nature of an expedition study, experiments were scheduled throughout the testing days, without control for circadian variation between participants (time of day was controlled for experiments within each participant), while blood samples for the measurement of hemoglobin concentration were collected in the morning for each individual. Accordingly, hemoglobin concentrations for some subjects were not time-synced with their experiments. However, it is unlikely that hemoglobin concentration varied in a physiologically meaningful way throughout the day of testing.

Another important consideration is the small sample size for some of our measurements. When measurements were successfully recorded, a maximum N of 8 was possible. Due to technical difficulties, we obtained MCA velocity recordings for an N=7, and N = 5 for ICA blood flow recordings. However, the repeated measures design, and the consistency of some of our key outcome variables (i.e., MCAv and ScO_2) with our prior work (3), provide added confidence in our conclusions from this small sample.

Lastly, the cerebral tissue oxygen saturation measurement has some important assumptions worth noting. The ScO_2 measurement is a mixed sample of oxy- and deoxy-hemoglobin predominantly from the venous blood (approximately 75% venous, 20% arterial, 5% capillary) (37). As such, ScO_2 is affected by both the delivery of oxygen to the tissue and oxygen extraction from the blood. Because indices of cerebral blood flow were no different between

experimental conditions, we assume that any changes in ScO_2 reflect changes in tissue oxygen extraction. The attenuated decrease in ScO_2 for the 0.1 Hz profile thus reflects a decrease in oxygen extraction which may be indicative of reduced metabolic consumption compared to the 0 Hz condition, or may be due to the oxygen sparing effect proposed by Hapuarachchi et al. (35). Further research is needed to address the underlying mechanisms contributing to this effect.

Translational Perspective

While recognizing there is much research to be done regarding the therapeutic potential of hemodynamic oscillations, the current findings continue to support potential clinical applications. Inducing oscillations at around 0.1 Hz in either arterial pressure or cerebral blood flow could be used to protect oxygenation of the tissues with reductions in perfusion such as during hemorrhage, sepsis, or during recovery from stroke. This approach could be especially useful in scenarios where access to advanced medical care is limited, such as extended transportation to a hospital, during high altitude/mountaineering expeditions, or for soldiers wounded on the battlefield. We clearly recognize that in such situations, LBNP is not a viable method for inducing hemodynamic oscillations. Other methods of delivery could be used, however, such as pneumatic cuffs that have been utilized by Hockin et al. during head-up tilt and LBNP (8, 38). These cuffs could easily be placed around the calf or thigh and rhythmically inflated and deflated at 0.1 Hz. It is plausible that engineering portable technology for therapeutic delivery of hemodynamic oscillations could be a relatively straight-forward task of repurposing this design.

In conclusion, our findings suggests that low frequency hemodynamic oscillations effectively prevented decreases in cerebral tissue oxygenation under combined central hypovolemia and hypobaric hypoxia, despite no effects on cerebral blood flow or oxygen delivery. Further research is needed to explore the microvascular and tissue responses to hemodynamic oscillations to gain further insight into the mechanisms underpinning these observations. Our current findings support the potential therapeutic use of hemodynamic oscillations in protecting vital organ oxygenation during challenges to blood flow and oxygen delivery.

ACKNOWLEDGEMENTS

We gratefully acknowledge our research participants for their willingness to contribute to this study, and to all other members of the White Mountain 2019 expedition team for their support. We also sincerely thank the staff at the Barcroft Laboratory for their generous service throughout our expedition.

FUNDING

Funding for this study was provided, in part, by an American Heart Association Grant-in-Aid (CAR; GRNT 17GRNT33671110), a Natural Sciences and Engineering Research Council of Canada Discovery Grant (TAD; RGPIN-2016-04915), a University of Calgary Research Grant Committee (RJAW), a NSERC Discovery grant (RJAW), and training fellowships awarded to GKA through a National Institutes of Health-supported Neurobiology of Aging Training Grant (T32 AG020494, Principal Investigator: N. Sumien) and an American Heart Association Predoctoral Fellowship (20PRE35210249), and to AJR through a Ruth L. Kirchstein NRSA F32

Postdoctoral Fellowship (1F32 HL144082-01A1). NGJ is a Parker B Francis Fellowship Recipient.

AUTHOR CONTRIBUTIONS

GKA, AJR, NJ, RJW, JB, TAD, and CAR contributed to the conception and design of this study. GKA, AJR, HJB, JB, BP, BRMB, TAD, and CAR participated in the acquisition and analysis of the data. All authors assisted in writing and revising the manuscript. All authors approved the final version of the manuscript.

Table 1. Participant Demographics

	Total Group (N=8)	Range	Males (N=4)	Females (N=4)
Age (y)	30.1 ± 7.6	22-42	26.0 ± 4.5	34.3 ± 8.3
Height (cm)	173.0 ± 13.8	150.0-188.0	184.8 ± 2.4	161.3 ± 8.3
Weight (kg)	72.4 ± 14.5	52.2-99.0	83.0 ± 11.6	61.9 ± 7.5
BMI (kg/m^2)	24.2 ± 4.2	18.1-31.1	24.3 ± 3.6	24.2 ± 5.4
Aortic Diameter (mm)	21.8 ± 0.8	19.2-25.5	23.8 ± 1.1	19.7 ± 0.5

Data are mean ± standard deviation.

Table 2. Time domain physiological responses at baseline and during LBNP

	Baseline		LBNP		P-values		
	0 Hz	0.1 Hz	0 Hz	0.1 Hz	Time	LBNP	Interaction
Cardiovascular							
Heart Rate (bpm)	79.9 ± 11.9	79.0 ± 10.2	110.6 ± 22.2 *	106.4 ± 19.8 *	<0.01	0.54	0.69
Stroke Volume (ml)	82.7 ± 18.6	82.8 ± 22.9	45.3 ± 16.8 *	48.2 ± 23.7 *	<0.01	0.72	0.75
Cardiac Output (l/min)	6.5 ± 1.2	6.5 ± 1.6	4.7 ± 1.2 *	4.7 ± 1.4 *	<0.01	0.98	0.99
Systolic Arterial Pressure (mmHg)	130.8 ± 11.1	127.2 ± 12.9	108.4 ± 10.5 *	105.1 ± 7.6 *	<0.01	0.29	0.97
Diastolic Arterial Pressure (mmHg)	75.8 ± 10.3	74.8 ± 10.6	73.4 ± 8.4	72.2 ± 8.1	0.16	0.53	0.94
Mean Arterial Pressure (mmHg)	96.8 ± 10.5	95.4 ± 11.5	86.1 ± 8.2 *	84.2 ± 6.4 *	<0.01	0.45	0.92
Systemic Vascular Resistance (mmHg/(l/min))	15.5 ± 3.8	16.0 ± 6.3	19.1 ± 4.9	19.6 ± 7.4	0.02	0.76	0.99
Cerebrovascular							
Cerebral Total Hemoglobin (μM)	47.1 ± 4.6	48.2 ± 7.2	45.7 ± 4.7	46.0 ± 8.2	0.24	0.44	0.97
Cerebral Oxygenated Hemoglobin (μM)	31.2 ± 4.5	32.2 ± 6.1	29.1 ± 4.4	30.4 ± 6.8	0.16	0.32	0.67
Cerebral Deoxygenated Hemoglobin (μM)	15.9 ± 1.4	16.0 ± 2.0	16.6 ± 1.5	15.6 ± 1.95	0.87	0.63	0.09
Systolic MCAv (cm/s)	81.1 ± 11.0	89.1 ± 17.2	66.1 ± 15.6 *	62.9 ± 17.1 *	<0.01	0.16	0.12
Diastolic MCAv (cm/s)	42.9 ± 8.7	46.9 ± 9.0	38.8 ± 10.6	35.4 ± 10.7 *	<0.01	0.57	0.18
MCA Conductance Index (cm/s/mmHg)	0.6 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.5 ± 0.2 *	0.01	0.33	0.15
ICA Diameter (cm)	0.57 ± 0.11	0.57 ± 0.10	0.57 ± 0.12	0.56 ± 0.12 *	0.05	0.46	0.06
Peak ICA Velocity (cm/s)	41.1 ± 6.9	38.6 ± 6.3	31.9 ± 9.6 *	29.3 ± 8.3 *	<0.01	0.55	0.98
ICA Conductance (ml/min/mmHg)	3.3 ± 1.2	3.2 ± 1.0	2.7 ± 0.9	2.5 ± 1.1	0.01	0.96	0.75
Peripheral Vascular							
SmO_2 (%)	75.3 ± 7.2	70.6 ± 5.9	66.3 ± 8.2 *	65.8 ± 4.2	<0.01	0.18	0.28
Muscle Total Hemoglobin (μM)	87.2 ± 22.0	83.4 ± 25.3	84.2 ± 24.5	80.8 ± 25.7	0.43	0.31	0.95
Muscle Oxygenated Hemoglobin (μM)	65.2 ± 15.3	59.2 ± 20.0	54.5 ± 12.3	53.1 ± 17.5	0.03	0.31	0.52
Muscle Deoxygenated Hemoglobin (μM)	22.0 ± 10.5	24.2 ± 7.7	29.7 ± 14.9 *	27.7 ± 9.2	<0.01	0.97	0.26
SpO_2 (%)	90.4 ± 2.4	88.2 ± 2.3	93.9 ± 3.8 *	92.9 ± 2.9 *	<0.01	0.03	0.41
Blood Hemoglobin Concentration (g/l)	162.3 ± 14.3	157.1 ± 22.1	-	-	0.32 (paired t-test)		

Respiratory							
$P_{ET}CO_2$ (mmHg)	25.1 ± 3.7	27.4 ± 1.7	19.4 ± 5.0	21.1 ± 3.5	<0.01	0.04	0.71
$P_{ET}O_2$ (mmHg)	60.5 ± 4.4	57.1 ± 2.9	69.7 ± 7.0 *	67.3 ± 5.8 *	<0.01	0.03	0.68
Respiration Rate (breaths/min)	15.3 ± 3.9	14.8 ± 4.1	15.1 ± 4.7	15.6 ± 6.4	0.69	0.98	0.51

ScO_2, cerebral tissue oxygen saturation; MCAv, middle cerebral artery velocity; ICA, internal carotid artery; SmO_2, muscle tissue oxygen saturation; SpO_2, arterial oxygen saturation; $P_{ET}CO_2$, end-tidal carbon dioxide; $P_{ET}O_2$, end-tidal oxygen. All data are presented as mean ± standard deviation. Data analyzed using a linear mixed model for repeated measures, with Tukey post-hoc tests, unless otherwise stated. * $0.0001 \leq P \leq 0.06$ vs. baseline within condition.

Table 3. Frequency domain physiological responses at baseline and during LBNP

	Baseline		LBNP		P-values		
	0 Hz	0.1 Hz	0 Hz	0.1 Hz	Time	LBNP	Interaction
Power – LF Range (0.07 – 0.15 Hz)							
Mean Arterial Pressure ($mmHg^2$)	4.9 ± 3.9	4.0 ± 1.8	11.5 ± 11.0	13.3 ± 11.25 *	<0.01	0.83	0.51
Mean Arterial Pressure (%Δ)	-	-	121.9 ± 119.9	248.7 ± 219.5		0.18 (paired t-test)	
Mean MCAv $(cm/s)^2$	2.2 ± 1.4	2.3 ± 1.3	2.9 ± 2.6	4.9 ± 3.1 *†	0.02	0.02	0.02
Mean MCAv (%Δ)	-	-	44.6 ± 129.0	203.1 ± 147.7		0.07 (paired t-test)	
$P_{ET}CO2$ (mmHg)	1.1 ± 0.7	2.0 ± 1.1 †	0.6 ± 0.4	1.7 ± 0.7 †	0.10	<0.01	0.71
Power – 0.1 Hz Peak							
Mean Arterial Pressure ($mmHg^2$)	145.5 ± 162.56	64.1 ± 52.68	160.0 ± 214.2	894.3 ± 1074.9 *†	0.04	0.10	0.04
Mean Arterial Pressure (%Δ)	-	-	20.5 ± 52.9	1462.3 ± 1412.5		0.02 (paired t-test)	
Mean MCAv $(cm/s)^2$	57.4 ± 54.0	38.2 ± 25.4	40.5 ± 42.3	348.1 ± 313.4 *†	0.02	0.02	0.01
Mean MCAv (%Δ)	-	-	104.8 ± 244.8	1486.9 ± 1362.2		0.07 (paired t-test)	

MCAv, middle cerebral artery velocity; $etCO_2$, end-tidal carbon dioxide. All data are presented as mean ± standard deviation. Data analyzed using a linear mixed model for repeated measures, with Tukey post-hoc tests, unless otherwise stated. * $0.005 \leq P \leq 0.04$ vs. baseline within condition. † $0.008 \leq P \leq 0.07$ vs. 0 Hz at that time point.

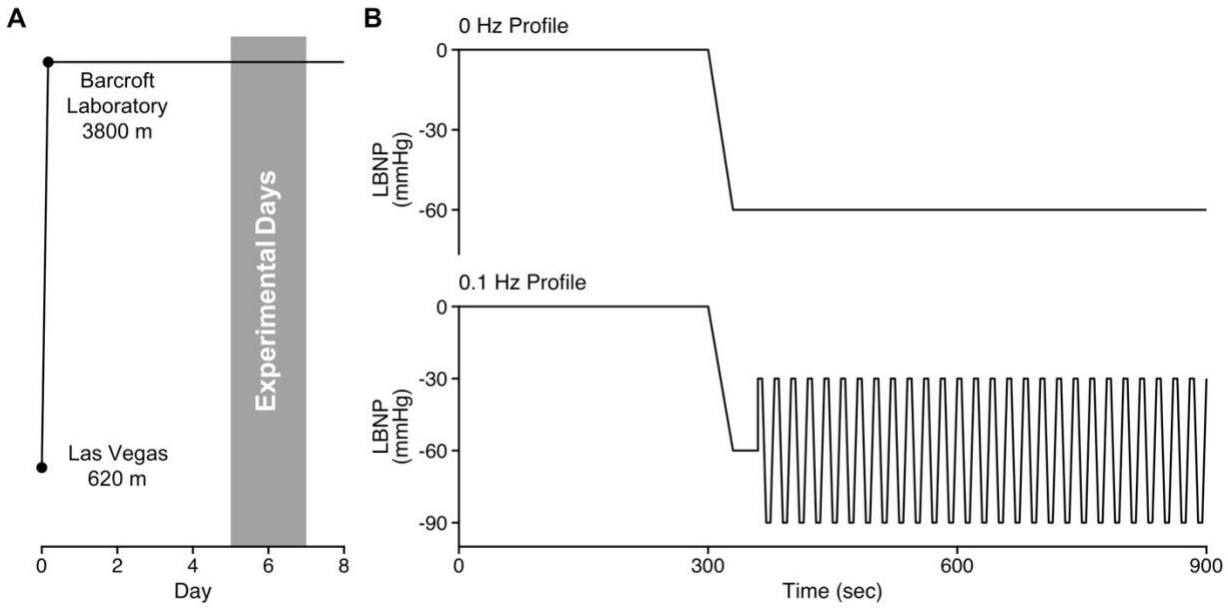

Figure 1. The high altitude ascent profile and LBNP profiles.

The ascent profile with an ascent of about 4 hours on day 0 followed by the timeline of experimentation (A). The representative tracings of the 0 Hz and 0.1 Hz lower body negative pressure (LBNP) profiles (B).

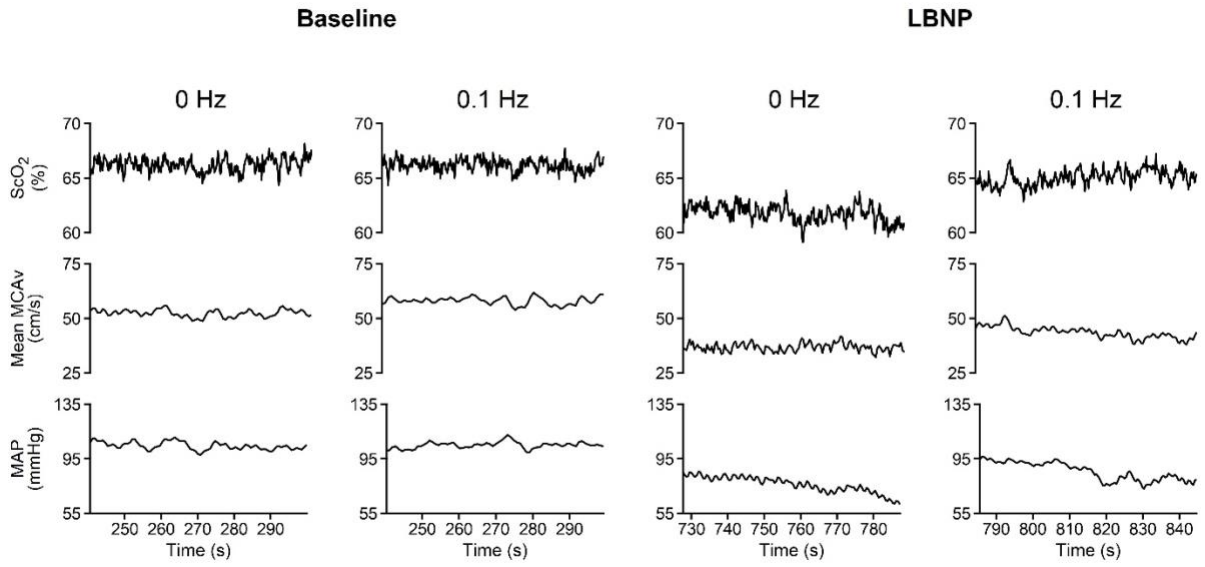

Figure 2. A representative tracing of physiological signals from one subject

Data are presented at baseline, and during the final minute of the 0 Hz and 0.1 Hz lower body negative pressure (LBNP) profiles. ScO_2, cerebral oxygen saturation; MCAv, middle cerebral artery velocity; MAP, mean arterial pressure.

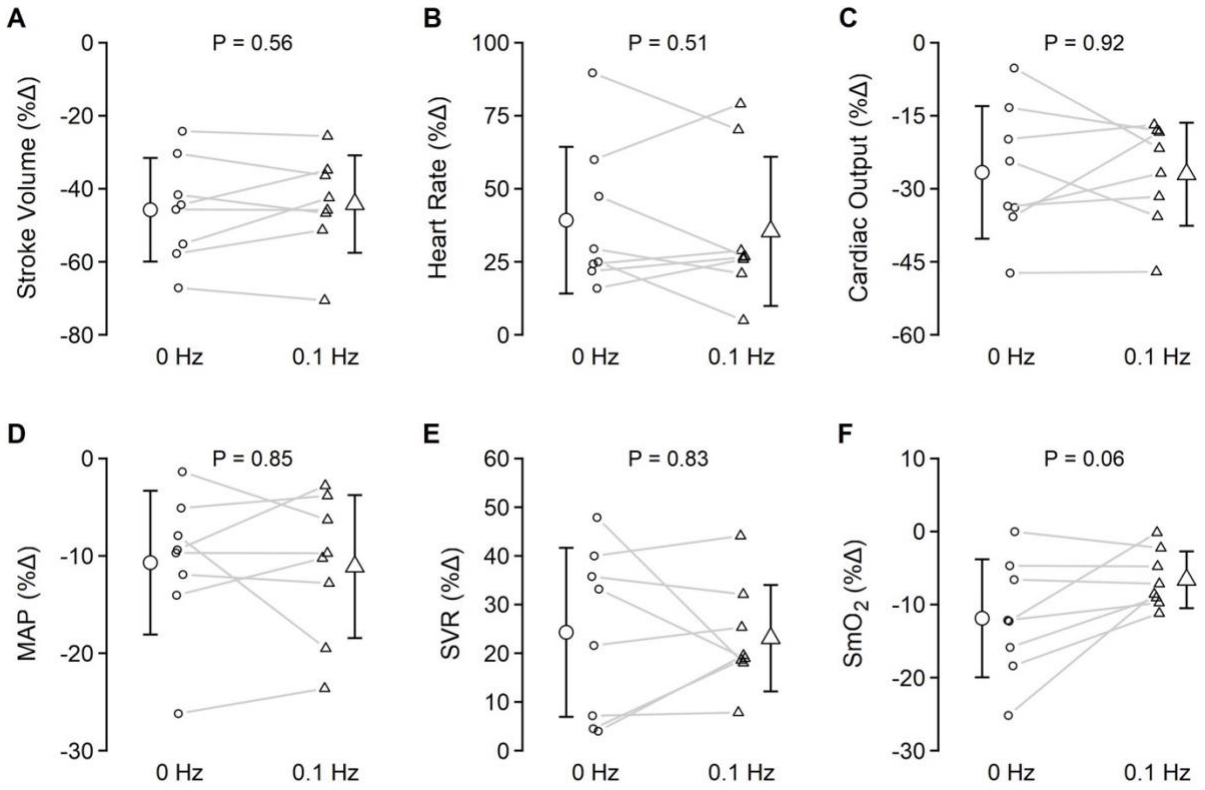

Figure 3. Relative systemic hemodynamic responses during 0 Hz and 0.1 Hz LBNP

MAP, mean arterial pressure; SVR, systemic vascular resistance; SmO_2, muscle tissue oxygen saturation. All data are presented as mean ± standard deviation. Data analyzed with paired t-tests. Exact p-values are reported in the figure.

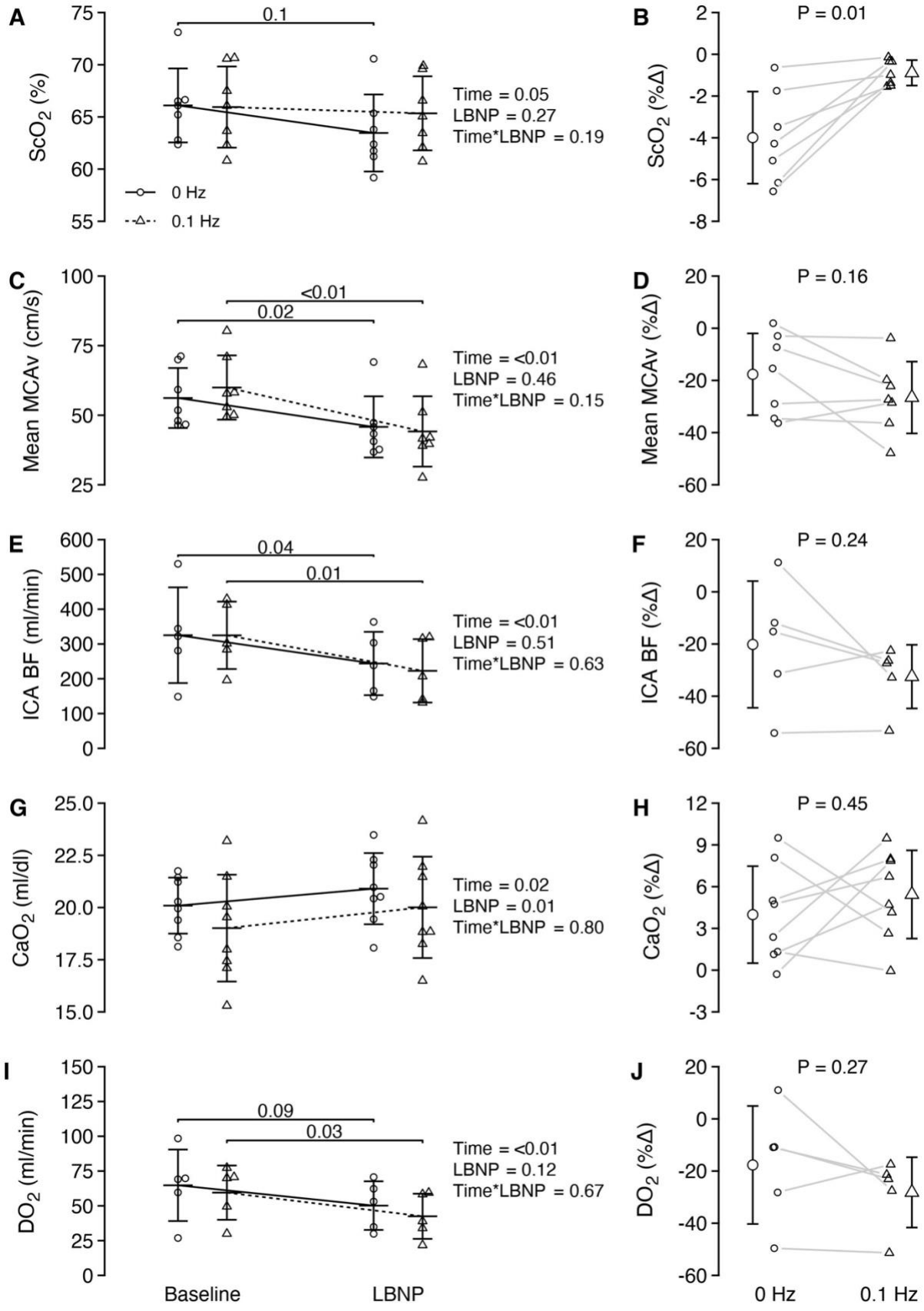
A
ScO2 (%)
0.1
Time = 0.05
LBNP = 0.27
Time*LBNP = 0.19
0 Hz
0.1 Hz
B
ScO2 (%Δ)
P = 0.01
C
Mean MCAv (cm/s)
<0.01
0.02
Time = <0.01
LBNP = 0.46
Time*LBNP = 0.15
D
Mean MCAv (%Δ)
P = 0.16
E
ICA BF (ml/min)
0.04
0.01
Time = <0.01
LBNP = 0.51
Time*LBNP = 0.63
F
ICA BF (%Δ)
P = 0.24
G
CaO2 (ml/dl)
Time = 0.02
LBNP = 0.01
Time*LBNP = 0.80
H
CaO2 (%Δ)
P = 0.45
I
DO2 (ml/min)
0.09
0.03
Time = <0.01
LBNP = 0.12
Time*LBNP = 0.67
J
DO2 (%Δ)
P = 0.27
Baseline
LBNP
0 Hz
0.1 Hz

Figure 4. Absolute and relative cerebral tissue oxygenation and cerebral hemodynamic responses during 0 Hz and 0.1 Hz LBNP

ScO_2, cerebral tissue oxygen saturation; MCAv, middle cerebral artery velocity; ICA BF, internal carotid artery blood flow; CaO_2, arterial oxygen content; DO_2, unilateral delivery of oxygen through the internal carotid artery. All data are presented as mean ± standard deviation. Absolute data were analyzed using a linear mixed model for repeated measures followed by Tukey post-hoc tests (panels A, C, E, G, I). Percent change values in the right column (panels B, D, F, H, J) were analyzed using paired t-tests. Exact p-values are reported in the figure.

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CHAPTER IV

Effect of Oscillatory Hemodynamics on the Cardiovascular Response to Simulated Hemorrhage with Isocapnia

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ABSTRACT

During conditions of cerebral hypoperfusion, we have demonstrated a protection of cerebral tissue oxygenation with oscillatory arterial pressure and cerebral blood flow at low frequencies (around 0.1 Hz and 0.05 Hz), despite no protection of cerebral blood flow or oxygen delivery. The influence of arterial PCO_2, a potent regulator of cerebral blood flow, has not previously been controlled. Hypocapnia resulting from LBNP contributes to the reduction in cerebral blood flow, and may be masking the effects of arterial pressure oscillations on cerebral blood flow. End-tidal clamping can be used to hold end-tidal CO_2 ($etCO_2$; an index of arterial PCO_2) at resting values

for subsequent assessment of the effect of arterial pressure oscillations on cerebral blood flow, without the simultaneous influence of hypocapnia. We hypothesized that with isocapnia, forced oscillations of arterial pressure and blood flow at 0.1 Hz and 0.05 Hz would improve internal carotid artery (ICA) blood flow responses compared to a non-oscillatory condition during simulated hemorrhage. Oscillatory arterial pressure and blood flow may also increase shear stress and the release of endothelial-derived vasoactive factors (such as nitric oxide and endothelin-1). We hypothesized that similar to blood flow, plasma concentrations of nitrite will increase and/or endothelin-1 will decrease between the oscillatory and non-oscillatory conditions. Simulated hemorrhage was induced via application of lower body negative pressure (LBNP). Eleven human participants (9 male, 2 female) underwent 3 LBNP profiles: a non-oscillatory condition (0 Hz) and two oscillatory conditions (0.1 Hz and 0.05 Hz). Resting $etCO_2$ and etO_2 were measured for 5-minutes during baseline, then $etCO_2$ and etO_2 were clamped at these values using dynamic end-tidal forcing for the rest of the experiment. Cerebral tissue oxygenation (ScO_2; measured via near-infrared spectroscopy), ICA blood flow (measured via duplex Doppler ultrasound) and middle cerebral artery velocity (MCAv; measured via transcranial Doppler ultrasound) were measured continuously, and compared between baseline and LBNP during each of the 3 profiles (via linear mixed model analysis). With clamped $etCO_2$, ICA flow did not decrease with LBNP (ANOVA $P = 0.93$) or differ between the 3 profiles (0 Hz: 2.17 ± 5.43 Δ%, 0.1 Hz: -0.44 ± 6.55 Δ%, 0.05 Hz: 0.23 ± 4.78 Δ%; $P = 0.56$). Similarly, ScO_2 did not decrease with LBNP (ANOVA $P = 0.21$) or differ between the 3 profiles (0 Hz: -2.55 ± 3.30 Δ%, 0.1 Hz: -1.58 ± 1.48 Δ%, 0.05 Hz: -0.23 ± 2.79 Δ%; $P = 0.13$). Plasma concentrations of nitrite did not change with LBNP overall (ANOVA $P = 0.46$) but did differ between profiles (0 Hz: 18.37 ± 21.14 Δ%, 0.1 Hz: -2.86 ± 21.30 Δ%, 0.05 Hz: 11.81 ± 12.76 Δ%; $P = 0.04$).

Plasma endothelin-1 increased with LBNP overall (ANOVA P = 0.03) but high variability in responses led to no differences between profiles (0 Hz: -11.3 ± 28.0 Δ%, 0.1 Hz: 34.1 ± 65.8 Δ%, 0.05 Hz: 65.5 ± 133.1 Δ%; P = 0.39). In conclusion, cerebral blood flow and cerebral oxygenation did not change during LBNP with isocapnia, regardless of induction of hemodynamic oscillations. Furthermore, forcing oscillations in arterial pressure and blood flow did not induce meaningful changes in circulating concentrations of nitrite or endothelin-1.

INTRODUCTION

Hemorrhage, or massive blood loss, challenges vital organ tissue perfusion and oxygenation, and can result in organ damage or death if left untreated. It is estimated that hemorrhage accounts for over 61,000 deaths in the United States of America per year, and 1.9 million deaths worldwide (1). Because hemorrhage is more frequent in younger populations, it accounts for over 85.6 million years of life lost per year worldwide (1). Interestingly, hemorrhage is also the leading cause of preventable deaths in the battlefield (2). These statistics highlight the need for improved therapies to treat hemorrhagic injuries.

Various experimental models of hemorrhage have been used, including animal models (3), human models with venesection (4, 5), and human models using lower body negative pressure (LBNP) (6-8). LBNP has been validated as a model of actual blood loss in animals (7) and humans (5, 9), and provides the benefit of inducing fundamental cardiovascular responses to hemorrhage without removal of blood volume. Using this technique, mechanisms that affect tolerance to blood loss have been explored (8, 10, 11). In 2011, Rickards et al. highlighted low frequency oscillations (around 0.1 Hz) in arterial pressure and cerebral blood flow as a potential mechanism influencing tolerance to hemorrhage (12).

Following these initial observations, our laboratory examined the effects of forcing oscillations in arterial pressure and cerebral blood flow on both tolerance to simulated hemorrhage (via LBNP) and on cerebral tissue oxygenation under conditions of both normoxia (13) and hypoxia (see Chapter III) (14). Under normoxic conditions, oscillating arterial pressure and cerebral blood flow at both 0.1 Hz and 0.05 Hz protected against reductions in cerebral oxygenation during LBNP, and improved tolerance to this stress (13). Interestingly, this effect occurred without a protection of middle cerebral artery velocity (MCAv), our index of cerebral blood flow. A similar protection of cerebral tissue oxygenation was also observed with oscillatory arterial pressure and blood flow in hypoxic conditions, but again, there was no protection of either intracranial flow (indexed via MCAv), or extracranial inflow (indexed by internal carotid artery (ICA) blood flow) (14).

Cerebral blood flow during LBNP is primarily regulated by cardiac output, arterial pressure and arterial PCO_2. In our previous studies, cardiac output, arterial pressure, and end-tidal CO_2 (etCO2; an index of arterial PCO_2) decreased under all conditions, with no differences in the magnitude of decreases between conditions (13, 14). Of these parameters, $etCO_2$ is arguably the most potent regulator of cerebral blood flow, but can also be experimentally controlled during LBNP. For example, the effect of clamping $etCO_2$ at baseline values (isocapnia) on the cerebral blood flow response to presyncopal LBNP was studied by Lewis et al. (15). Participants underwent both a free-breathing (poikilocapnic) and isocapnic LBNP protocol to presyncope. During the isocapnic trial, cerebral blood flow decreased but to a lesser magnitude than during the poikilocapnic trial. Because reductions in $PaCO_2$ contribute to the reduction in cerebral blood flow during LBNP, the aim of the current study is to control the hypocapnic response during LBNP to better assess whether hemodynamic oscillations protect

arterial pressure and cerebral blood flow without this additional stimulus. Interestingly, $etCO_2$ was oscillating at the same frequency as oscillatory LBNP in our previous study (13), which likely contributed to the oscillations in cerebral blood flow. By clamping $etCO_2$ at baseline values, this effect can also be removed and improve our ability to assess the direct effect of hemodynamic oscillations on the cardiovascular and cerebrovascular responses to simulated hemorrhage.

As hemodynamic oscillations could potentially create increases and decreases in shear stress, the production of endothelial-derived vasoactive mediators (such as nitric oxide and endothelin-1) could be altered, and play a role in the cardiovascular response to these oscillations. LBNP itself could also induce release of these mediators. For example, head-up tilt (a central hypovolemic stimulus) has been shown to increase the production of endothelin-1 in healthy participants (16). Understanding the balance between vasodilatory (via nitric oxide) and vasoconstrictive (via endothelin-1) mediator release may aid in understanding the cardiovascular responses to simulated hemorrhage with and without forced oscillations in arterial pressure and blood flow.

In the present study, we employ the use of end-tidal forcing (17) to clamp $etCO_2$ at baseline resting values during LBNP with and without forced hemodynamic oscillations. We hypothesized that forcing oscillations in arterial pressure and cerebral blood flow during simulated hemorrhage, while also clamping $etCO_2$ at eucapnic baseline values, will result in improved ICA blood flow responses compared to the non-oscillatory condition, and that cerebral tissue oxygenation will be protected compared to the non-oscillatory condition. We also hypothesized that circulating concentrations of nitric oxide will increase and/or endothelin-1 will

decrease during simulated hemorrhage with forced oscillations in arterial pressure and cerebral blood flow.

METHODS

Ethical approval

Ethical approval for this study was received from the North Texas Regional Institutional Review Board (Protocol #2019- 046), and all experimentation was performed at the University of North Texas Health Science Center.

Participants

Healthy human participants between ages 18-40 years were recruited for this study. Participants were excluded if there was any history of cardiovascular, respiratory, or neurological disease. Participants were briefed on the purpose, design, and risks of the study, and provided written, informed consent prior to inclusion. Prior to experimentation, participants completed a medical screening and familiarization session. General health history was recorded, followed by seated and standing blood pressure and 12-lead ECG measurements. Female participants completed a urine pregnancy test to ensure they were not pregnant. Participants were then familiarized with all the equipment to be used during experimentation.

Prior to each experiment, participants abstained from alcohol, caffeine, exercise, dietary supplements, and medication for 24 hours, and from food for at least 2 hours. All experiments were conducted in a temperature-controlled laboratory, and during the morning to control for the effect of circadian rhythm on cardiovascular responses. Female participants were tested during days 1-4 of their menstrual cycle (self-report) to account for hormonal fluctuations.

Instrumentation

Upon arrival for experimentation, the height, weight, and age of participants was recorded. Female participants also completed a urine pregnancy test prior to each experimental session to ensure they were still not pregnant. Participants were positioned into the LBNP chamber with their iliac crest in line with the chamber opening. A heavy-duty plastic liner and neoprene waist wrap were then used to create and air-tight seal between the participant and the chamber. ECG electrodes were placed in a lead-II configuration for continuous recording and subsequent R-wave detection (shielded leads, cable, and amplifier, AD Instruments, Bella Vista, NSW, Australia). Finger photoplethysmography was used for continuous recording of arterial pressure (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). ModelFlow® estimates of stroke volume were corrected by measuring aortic diameter at rest (average of two measures, probe: 3Sc, 4 MHz; GE Vivid T8, Chicago, IL). Cerebral blood velocities in the middle cerebral artery (MCAv) and posterior cerebral artery (PCAv) were measured through the right and left temporal windows, using 2 MHz probes fastened to an adjustable head frame (ST3, Spencer Technologies, Seattle, WA). Oxy-hemoglobin (Hb) and deoxy-Hb were measured, and cerebral tissue oxygenation was calculated (ScO_2; [oxy-Hb/(oxy-Hb + deoxy-Hb) * 100]) using a near-infrared spectroscopy probe (NIRS; OxiplexTS, ISS, Champaign-Urbana, IL) that was placed over the right temporal lobe. ICA measures of velocity and diameter were made using a 11 MHz linear array probe with an insonation angle of 60° (Phillips iE33, Andover, MA). Video recordings were captured at 30 Hz using an Epiphan capture card (DVI2USB 3.0, Epiphan Systems, Inc., Palo Alto, CA) and screen recording software (Camtasia, Techsmith Corporation, Okemos, MI) for later analysis. Respiratory gases ($etCO_2$; end-tidal O_2, etO_2) were captured

using a mouthpiece and a gas-sampling line connected to a gas analyzer (ML206 Gas Analyzer, AD Instruments, Bella Vista, NSW, Australia). Respiratory volumes were also measured using a pneumotachograph (Model 4830, 0-400 LPM, Hans Rudolph, Shawnee, KS) connected to a bacteriological filter and a two-way non-rebreathing valve (model 7900 series, Hans Rudolph, Shawnee, KS). The respiratory gas and volume signals were subsequently routed into a dynamic end-tidal forcing system for the clamping of $etCO_2$ and etO_2. This system controls breath-by-breath inspired fractions of O_2 and CO_2 in order to control end-tidal gas values independent of ventilation (17). Lastly, a 20-gauge flexible venous catheter was inserted in the antecubital fossa of the right forearm for repeated venous blood sampling.

Protocol

After instrumentation, a 5-min baseline period was completed to measure resting cardiovascular status, and to collect resting measures of $etCO_2$ and etO_2 which were entered into the dynamic end-tidal forcing software. The end-tidal forcing system was then connected to the inhalation port of a two-way non-rebreathing valve to clamp each individual participant's end-tidal gases to their resting values prior to the initiation of LBNP. Once clamping was achieved (within 1-2 minutes), participants then underwent one of three LBNP profiles, each separated by at least 48 h: 1) a 0 Hz condition where chamber pressure was gradually reduced to -60 mmHg for 30-s and subsequently held for an additional 10.5-min, 2) a 0.1 Hz condition where chamber pressure was gradually reduced to -60 mmHg over 30-s, held for another 30-s and then oscillated between -30 and -90 mmHg for 5-s at each pressure over 10-min, and 3) a 0.05 Hz condition where chamber pressure was gradually reduced to -60 mmHg over 30-s, held for another 30-s and then oscillated between -30 and -90 mmHg for 10-s at each pressure over 10-min. These

profiles were terminated early if participants reached presyncope, which we defined as a systolic arterial pressure of < 80 mmHg, or if the participant experienced symptoms such as nausea, lightheadedness, dizziness, or visual disturbances (such as grey out or tunnel vision). After release of chamber pressure, the dynamic end-tidal forcing system was removed, and participants rested for 10-min of recovery.

Blood Sample Collection & Analysis

A resting venous blood sample of 5 ml was drawn before application of LBNP, and another 5 ml sample was drawn at the end of LBNP for all three profiles. Blood samples were placed in a 6 ml EDTA collection tube and centrifuged at 2000 g for 15 min at 4°C. Plasma was immediately extracted into microcentrifuge tubes, snap frozen in liquid nitrogen, then placed in a -80°C freezer for storage. Plasma nitrite was analyzed using liquid chromatography with tandem mass spectrometry following a sample preparation optimized for human samples (18). Plasma concentrations of endothelin-1 were analyzed using a commercially available enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's directions (DET-100, R&D Systems, Minneapolis, MN).

Data Analysis

Continuous recordings of ECG, arterial pressure, stroke volume, LBNP chamber pressure, MCAv, PCAv, and respiratory gases and volumes were collected at 1000 Hz using PowerLab and LabChart 8 (AD Instruments, Bella Vista, NSW, Australia). These data were subsequently imported into and analyzed using a commercially available analysis package (Ensemble, Elucimed, Wellington, New Zealand). R-wave detection was used to identify cardiac

cycles, facilitating calculation of beat-to-beat metrics of mean arterial pressure, mean MCAv, and mean PCAv. End-tidal values for CO_2 and O_2 were extracted from the continuous breath-to-breath waveforms of PCO_2 and PO_2. The last 3-min of baseline and last 1-min of LBNP were used to calculate time domain averages of these signals. Frequency domain metrics of oscillatory power in mean arterial pressure, MCAv, and PCAv were calculated following spline interpolation of beat-to-beat measures and resampling at 4 Hz for spectral analysis. The Welch algorithm and a Hanning window were used for fast Fourier transform. Power spectral density within the low frequency range (LF, 0.07-0.15 Hz), very low frequency range (VLF, 0.02-0.07 Hz), and at the specific point frequencies of 0.1 Hz and 0.05 Hz were examined for the entire 5-min of baseline and the last 5-min of LBNP.

ICA diameter and velocity measures were extracted and down sampled to 1-s averages using specialized wall tracking software (Cardiovascular Suite, Quipu, Pisa, Italy). Current standards for measurement of the ICA velocity and diameter were followed, including matching anatomical location between experiments, measuring velocities 1.5-2 cm distal from the bifurcation, and collecting a minimum of 10-cardiac cycles of data for averaging (19). ICA blood flow was calculated as $\pi(\frac{diameter}{2})^2 * \left(\frac{peak\ velocity}{2}\right) * 60$. MCA conductance index and ICA conductance were calculated by dividing MCAv or ICA flow by mean arterial pressure. Resistance for both MCAv and ICA were calculated by dividing mean arterial pressure by MCAv or ICA flow.

Statistical Analysis

Both absolute and percent change from baseline data are presented. A two-way linear mixed model for repeated measures was used to analyze the absolute data, with LBNP profile (0

Hz, 0.1 Hz, 0.05 Hz) and time (baseline, LBNP) as the two factors. *A priori* post hoc tests were chosen to compare LBNP profiles at both baseline and during LBNP, and between baseline and LBNP within profile. These pair-wise comparisons were corrected for multiple comparisons with the Holm correction. A one-way linear mixed model was used to analyze the percent change from baseline across LBNP profiles (0 Hz, 0.1 Hz, 0.05 Hz), followed by Tukey post hoc tests. All statistical analysis was performed in R (R Core Team, 2021) with lme4 package (20) for linear mixed model analysis.

RESULTS

Participants

Eleven participants (9 males, 2 females; 29 ± 5 y, 172 ± 9 cm, 78 ± 18 kg) completed the 0 Hz and 0.1 Hz profiles, and 9 participants (8 males, 1 female) completed the 0.05 Hz profile. Due to signal recording error, 1 stroke volume recording was lost during the 0.1 Hz profile (N=10) and 2 were lost during the 0.05 Hz profile (N=7). Difficulties in signal acquisition (due to the dynamic movement of the participants during oscillatory LBNP, and respiratory mediated vessel movement) also resulted in a reduced number of recordings for ICA during the 0.05 Hz profile (N=7); MCAv during the 0 Hz (N=9), 0.1 Hz (N=9), and 0.05 Hz (N=6) profiles; and PCAv during the 0 Hz (N=8), 0.1 Hz (N=5), and 0.05 Hz (N=6) profiles.

LBNP

The average LBNP chamber pressure was similar (by design) during each of the three profiles (0 Hz, -62.3 ± 0.5 mmHg; 0.1 Hz, 58.3 ± 0.7 mmHg; 0.05 Hz, -59.2 ± 0.6 mmHg). Due to onset of presyncopal symptoms, 4 of the 11 (36%) participants did not complete the entire 0

Hz profile, while all participants completed the 0.1 Hz profile, and all 9 participants tested in the 0.05 Hz profile completed this profile.

Systemic Cardiovascular Responses

Stroke volume decreased during exposure to all three LBNP profiles (Table 1); however, this reduction was attenuated in both the 0.1 Hz and 0.05 Hz profiles (Figure 1A). Both pulse pressure and mean arterial pressure directly reflected these stroke volume responses (Figure 1C & 1E). The subsequent heart rate response was increased above baseline for all profiles (Table 1), but was increased to a greater extent in the 0 Hz profile compared to the oscillatory profiles (Figure 1B). Overall, systemic vascular resistance did not change across the three profiles (Table 1), but in relative terms, the increase in systemic vascular resistance was greater during the 0.1 Hz profile compared with the 0 Hz profile (Figure 1F). The compensatory increases in heart rate in response to the decreases in stroke volume resulted in similar cardiac output responses for all three profiles (Table 1; Figure 1D).

Cerebrovascular Responses

We successfully clamped both $etCO_2$ (Figure 2G) and etO_2 (Table 1) at resting baseline values. Subsequently, measures of ICA blood flow, MCAv, and PCAv did not change from baseline during LBNP and were no different between profiles (Figure 2, Table 1). As a result of this maintenance of cerebral blood flow during the hypovolemic stress of LBNP, ScO_2 did not change from baseline, and was not different between profiles (Figure 2). Interestingly, ICA conductance increased from baseline during the 0 Hz profile, and the relative increase was higher

compared to the 0.1 Hz profile (Figure 1G); however, MCAv conductance did not increase from baseline in the 0.1 Hz and 0.05 Hz profiles (Table 1; Figure 1H).

Blood sample analysis

While there was no overall increase in plasma nitrite from baseline to LBNP for any of the profiles (Table 1), there was a difference in the relative responses (Figure 1I), with an increase in the 0 Hz and 0.05 Hz profiles, but a decrease in the 0.1 H profile. While plasma endothelin-1 did increase with LBNP (Table 1), the relative change in plasma endothelin-1 concentrations did not differ between profiles, likely due to very high inter-participant variability (Table 1).

Frequency domain responses

As intended by the experimental design, mean arterial pressure oscillations in the LF range and at 0.1 Hz were increased during the 0.1 Hz profile (Table 2). Oscillations in mean arterial pressure in the VLF range and at 0.05 Hz were also increased during the 0.05 Hz profile (Table 2). This same response occurred for MCAv during the 0.1 Hz profile, but was only observed in the 0.05 Hz profile at the point measure of 0.05 Hz (Table 2). PCAv responses differed slightly in that LF oscillations increased from baseline for both the 0 Hz and 0.1 Hz profiles (Table 2), although oscillations at the point frequency of 0.1 Hz only increased with the 0.1 Hz profile. VLF oscillations for PCAv only showed an increase at the point measure of 0.05 Hz during the 0.05 Hz profile (Table 2).

DISCUSSION

The aim of the current study was to control the hypocapnic response during LBNP to then assess whether hemodynamic oscillations protected arterial pressure and cerebral blood flow. The main findings of this study are: 1) neither cerebral blood flow nor cerebral tissue oxygen saturation decreased with oscillatory or non-oscillatory LBNP when $etCO_2$ was clamped at baseline values; 2) subsequently, oscillating arterial pressure and cerebral blood flow did not increase cerebral blood flow measured by either ICA flow or MCAv; 3) mean arterial pressure and stroke volume were protected with oscillatory arterial pressure and blood flow/ and 4) circulating concentrations of nitrite and endothelin-1 did not meaningfully differ between the oscillatory and non-oscillatory LBNP profiles.

In this study, we sought to test whether oscillations in arterial pressure and blood flow could be a mechanism for increasing cerebral blood flow during simulated hemorrhage. Arterial partial pressure of CO_2 is a powerful regulator of cerebral blood flow (21, 22), and hypocapnia could potentially account for over 50% of the reduction in cerebral blood flow observed during LBNP (15). In our previous studies employing oscillatory arterial pressure and cerebral blood flow during LBNP, the hypocapnia induced by application of LBNP was not prevented, and $etCO_2$ decreased by the same magnitude for all conditions (13, 14). In our first study (13), we also observed an oscillatory pattern of the $etCO_2$ signal at the same frequencies as the oscillatory LBNP profiles, which could have been contributing to the oscillations in cerebral blood flow. To account for the known effects of hypocapnia on the decreases in cerebral blood flow during LBNP, and the oscillatory effect of $etCO_2$ with forced oscillations, we used dynamic end-tidal forcing to clamp $etCO_2$ and therefore $PaCO_2$ (17), at resting eucapnic values. Interestingly, all indices of cerebral blood flow (ICA flow, MCAv, and PCAv) did not change from baseline

despite reductions in both mean arterial pressure and stroke volume during each LBNP protocol. Subsequently, oscillating arterial pressure and cerebral blood flow did not increase our indices of cerebral blood flow compared to our control condition (0 Hz LBNP), and there was no change in cerebral tissue oxygenation. These findings highlight a potential use of hypercapnia as a therapeutic approach for preserving both cerebral blood flow and cerebral tissue oxygenation with reductions in central blood volume (e.g., hemorrhage, sepsis).

The effect of either CO_2 supplementation or end-tidal CO_2 clamping on tolerance to LBNP has been explored by several investigators. Howden et al. assessed the effectiveness of 5% CO_2 supplementation on tolerance to LBNP (23). CO_2 supplementation increased LBNP tolerance time, but the physiological mechanism for this response was not investigated. Lucas et al. also tested the viability of supplemental CO_2 as a means for improving tolerance to LBNP with the added physiological stress of heat (24). Participants in this study were exposed to two LBNP conditions to presyncope – heated with hypercapnia, or heated without hypercapnia. Despite increases in $etCO_2$ and MCAv, LBNP tolerance was not altered, which was contrary to the Howden et al. finding. In a similar study, also with the combined stress of LBNP plus heat, Shibasaki et al. also showed no improvement in LBNP tolerance, despite protection of volumetric flow through the ICA and vertebral artery at sub-maximal LBNP stages (25). Instead of supplemental CO_2, Lewis et al. clamped $etCO_2$ at resting values and compared MCAv and LBNP tolerance to a free-breathing condition (15). Again, tolerance was no different between these conditions despite an attenuated reduction in MCAv. In the current study, where we also clamped $etCO_2$, 7 of the 11 (64%) participants completed the 0 Hz profile. In comparison, in our previous study where $etCO_2$ was not clamped, and participants became hypocapnic, 7 of 14 (50%) participants completed the 0 Hz profile. There is no difference in these outcomes between

the eucapnic and hypocapnic profiles (P = 0.16; assessed via a chi-square test), further supporting the conclusion that CO_2 supplementation does not improve tolerance to LBNP, but does protect cerebral blood flow.

A consistent finding across all three of our studies assessing the effects of oscillatory hemodynamics during simulated hemorrhage, is the improvement in tolerance to this stress. In our first study in normoxic conditions, 7 of 14 (50%) participants completed the entire 0 Hz profile, 11 of 14 (79%) participants completed the 0.1 Hz protocol, and 13 of 14 (93%) participants completed the 0.05 Hz protocol (13). In our second study in hypoxic conditions, 6 of 8 (75%) participants completed the 0 Hz condition, and 7 of 8 (88%) participants completed the 0.1 Hz profile. In our current study, 7 of 11 (64%) participants completed the 0 Hz profile, and all participants completed the 0.1 Hz profile (11/11, 100%) and 0.05 Hz profile (9/9, 100%). Other studies have also demonstrated improvement in tolerance to central hypovolemia by inducing oscillatory hemodynamics. Tolerance time to combined head-up tilt and LBNP was increased in a study by Lucas et al. who used a slow breathing protocol (6 breaths/min, or 0.1 Hz) to induce hemodynamic oscillations (26). Similarly, tolerance to combined head-up tilt and LBNP was increased in a study by Hockin et al. who employed the use of intermittent calf compression with a 15-s cycle (0.07 Hz) (27). Taken together, low frequency (around 0.05-0.1 Hz) hemodynamic oscillations appear to show promise in improving tolerance to these hypovolemic stimuli.

With both 0.1 Hz and 0.05 Hz oscillations, stroke volume was protected, similar to our prior study in normoxic conditions (13). This protection of stroke volume resulted in an attenuation in the compensatory heart rate increase with these two profiles compared with the 0 Hz profile, and no difference in the subsequent cardiac output responses across profiles. Despite

the similar cardiac output and systemic vascular resistance responses, however, mean arterial pressure was protected in both the oscillatory LBNP profiles compared to the 0 Hz profile. This finding differs from our previous studies, where there was no difference in the reduction in mean arterial pressure between profiles (13, 14). This finding is likely explained by 1) the separation of LBNP profiles onto different days in our current study vs. our previous study conducted in normoxic conditions (13), and; 2) testing in normoxic vs. hypoxic conditions (14). In our normoxic study, all LBNP profiles (0 Hz, 0.05 Hz, and 0.1 Hz) were run within the same experimental session, with a randomized order. This approach resulted in an elevation in sympathetic activity during recovery, which did not return to baseline levels (indexed by circulating norepinephrine) (13). This sympathoexcitation could have resulted in a "priming" of the baroreflex, and protection of arterial pressure in all subsequent profiles, regardless of the forced hemodynamic oscillations. Similarly, sympathetic activity was most likely elevated in our second study conducted at high altitude (14), due to the effects of hypoxia on the sympathetic nervous system (28). As endogenously generated hemodynamic oscillations at ~0.1 Hz in humans are the result of sympathetic activity (29), forcing oscillations around this frequency may augment sympathetic activity, resulting in a protection of mean arterial pressure compared to the non-oscillatory condition. Interestingly, due to the greater decrease in arterial pressure during the 0 Hz profile, ICA conductance increased (likely due to vasodilation distal to the ICA) compared to the 0.1 Hz profile to maintain similar blood flow to the brain.

Plasma concentrations of nitrite did not differ meaningfully between profiles. While the percent change in nitrite concentration differed between the 0 Hz and 0.1 Hz profiles, the magnitude of change was no more than what might be seen in daily fluctuations in basal nitrite plasma concentrations (30). Plasma endothelin-1 concentrations did not differ between profiles

but did demonstrate an effect with LBNP (Table 1). This is in line with a previous observation from Kaufmann et al. who reported an increase in plasma endothelin-1 during head-up tilt in healthy participants (16). Together, however, hemodynamic oscillations did not appear to exert their effects through release of these vasoactive mediators.

Methodological Considerations

Vertebral artery blood flow was not assessed, so the effect of forced arterial pressure and blood flow oscillations on volumetric blood flow into the posterior portions of the brain were not examined. However, PCAv was also maintained at baseline values, following the same pattern of response as both the ICA blood flow and MCAv measures, so we assume vertebral artery blood flow would show a similar response.

The number of female participants was low in this study, so it makes generalizing the findings to both sexes more difficult. However, the two female participants had similar cardiovascular responses as their male counterparts. Future work should seek to clarify whether the cardiovascular responses to forcing hemodynamic oscillations during simulated hemorrhage are altered by sex. Additionally, the influence of the menstrual cycle could be explored as hormonal changes across the menstrual cycle may change vascular responsiveness to forced hemodynamic oscillations.

Conclusions

In conclusion, neither cerebral blood flow nor cerebral oxygenation decreased during any LBNP profile due to the induced state of eucapnia. Forcing hemodynamic oscillations did protect stroke volume and mean arterial pressure, and also improved tolerance to the simulated hemorrhage protocols. This improvement in tolerance was not associated with protection of

cerebral blood flow, cerebral tissue oxygenation, or changes in plasma concentrations of nitrite or endothelin-1. Future studies should explore mechanisms that may play a role in protecting mean arterial pressure such as changes to sympathetic activity and/or neurovascular transduction.

Table 1. Time Domain responses to oscillatory LBNP

		Time						
	Profile	Baseline	Late LBNP	Profile	Time	Profile*Time	%Δ from Baseline	P-value
Cardiovascular Responses								
Stroke Volume (ml)	0 Hz	76.4 ± 15.5	41.3 ± 9.1*	< 0.001	< 0.001	0.07	-45.5 ± 10.0	< 0.001
	0.1 Hz	80.7 ± 14.4	55.2 ± 9.6*†				-31.4 ± 7.2 †	
	0.05 Hz	82.6 ± 18.9	59.0 ± 15.5*†				-28.6 ± 7.6 †	
Heart Rate (bpm)	0 Hz	66.1 ± 9.7	105.8 ± 16.7*	< 0.001	< 0.001	< 0.001	61.1 ± 21.0	< 0.001
	0.1 Hz	66.7 ± 8.8	83.0 ± 10.7*†				24.7 ± 9.1 †	
	0.05 Hz	65.1 ± 7.8	82.1 ± 14.8*†				26.2 ± 17.2 †	
Pulse Pressure	0 Hz	56.9 ± 5.3	37.7 ± 7.4*	0.003	< 0.001	0.03	-33.7 ± 11.2	0.003
(mmHg)	0.1 Hz	59.3 ± 7.2	45.8 ± 9.9*†				-23.3 ± 10.4 †	
	0.05 Hz	56.3 ± 9.5	45.7 ± 8.7*†				-19.0 ± 6.1 †	
Mean Arterial	0 Hz	98.5 ± 7.1	85.0 ± 10.4*	< 0.001	< 0.001	0.10	-13.7 ± 8.7	0.01
Pressure (mmHg)	0.1 Hz	104.3 ± 6.4†	97.7 ± 9.9*†				-6.4 ± 6.6 †	
	0.05 Hz	98.7 ± 8.4	90.4 ± 10.7*†				-8.5 ± 5.9 †	
Cardiac Output	0 Hz	5.1 ± 1.4	4.3 ± 1.1*	0.12	< 0.001	0.81	-12.9 ± 15.4	0.91
(l/min)	0.1 Hz	5.3 ± 0.9	4.6 ± 0.8*				-13.3 ± 12.9	
	0.05 Hz	5.4 ± 1.3	4.9 ± 1.2				-9.7 ± 6.3	
Systemic Vascular	0 Hz	20.8 ± 5.8	21.0 ± 6.9	0.78	0.20	0.66	1.5 ± 18.0	0.05
Resistance	0.1 Hz	20.0 ± 4.2	22.0 ± 5.6				9.5 ± 15.1 †	
(mmHg·min·ml^{-1})	0.05 Hz	19.3 ± 5.7	20.3 ± 7.8				3.7 ± 11.1	
Cerebrovascular Responses								
Peak ICA Velocity	0 Hz	43.1 ± 4.9	44.1 ± 5.4	0.18	0.66	0.89	2.6 ± 7.4	0.78
(cm/s)	0.1 Hz	41.5 ± 5.1	41.6 ± 5.2				0.6 ± 8.8	
	0.05 Hz	43.2 ± 4.2	43.3 ± 4.2				0.5 ± 5.8	
ICA Diameter (cm)	0 Hz	0.52 ± 0.05	0.52 ± 0.06	0.68	0.75	0.93	-0.12 ± 2.17	0.90
	0.1 Hz	0.52 ± 0.05	0.52 ± 0.05				-0.43 ± 1.96	
	0.05 Hz	0.49 ± 0.03	0.49 ± 0.03				-0.09 ± 2.05	
ICA Conductance	0 Hz	2.8 ± 0.7	3.3 ± 0.8*	0.001	0.004	0.24	19.5 ± 13.2	0.03
(ml/min/mmHg)	0.1 Hz	2.6 ± 0.6	2.7 ± 0.6†				6.9 ± 11.5 †	
	0.05 Hz	2.6 ± 0.6	2.8 ± 0.6				9.1 ± 6.3	
	0 Hz	0.38 ± 0.11	0.32 ± 0.08*	0.004	0.001	0.35	-15.28 ± 10.15	0.04

ICA Resistance (mmHg/ml/min)	0.1 Hz 0.05 Hz	0.41 ± 0.08 0.41 ± 0.09	0.38 ± 0.08† 0.37 ± 0.08				-5.54 ± 9.19 † -8.08 ± 5.26	
MCA Resistance Index (mmHg/cm/s)	0 Hz 0.1 Hz 0.05 Hz	1.7 ± 0.4 1.9 ± 0.4 1.7 ± 0.5	1.4 ± 0.3 1.8 ± 0.4† 1.6 ± 0.4	< 0.001	0.01	0.60	-12.7 ± 5.5 -5.8 ± 8.5 -5.2 ± 3.8 †	0.05
MCA CVC Index (cm/s/mmHg)	0 Hz 0.1 Hz 0.05 Hz	0.63 ± 0.13 0.56 ± 0.11 0.61 ± 0.13	0.72 ± 0.14 0.60 ± 0.11† 0.64 ± 0.13	< 0.001	0.02	0.44	14.5 ± 7.38 7.7 ± 10.7 4.1 ± 2.5 †	0.06
PCAv (cm/s)	0 Hz 0.1 Hz 0.05 Hz	37.7 ± 8.4 43.4 ± 4.7 40.3 ± 8.2	35.7 ± 7.2 43.3 ± 4.2 38.7 ± 6.9	0.11	0.33	0.83	-4.0 ± 12.7 -0.2 ± 5.5 -3.5 ± 5.3	0.44
PCA Resistance Index (mmHg/cm/s)	0 Hz 0.1 Hz 0.05 Hz	2.80 ± 0.84 2.45 ± 0.30 2.59 ± 0.57	2.40 ± 0.60 2.29 ± 0.27 2.49 ± 0.40	0.89	0.04	0.40	-12.97 ± 7.92 -6.11 ± 11.73 -2.72 ± 5.98	0.17
PCA CVC Index (cm/s/mmHg)	0 Hz 0.1 Hz 0.05 Hz	0.38 ± 0.10 0.41 ± 0.05 0.40 ± 0.08	0.44 ± 0.10 0.44 ± 0.05 0.41 ± 0.06	0.82	0.05	0.41	16.17 ± 12.70 7.79 ± 14.20 3.10 ± 6.51	0.22
Total Cerebral Hb Concentration (μM)	0 Hz 0.1 Hz 0.05 Hz	44.1 ± 9.7 45.6 ± 10.5 42.7 ± 11.7	43.7 ± 9.7 45.9 ± 10.4 43.1 ± 11.6	0.31	0.94	0.95	-0.8 ± 3.3 0.8 ± 3.4 0.9 ± 2.5	0.35
Cerebral Oxy Hb (μM)	0 Hz 0.1 Hz 0.05 Hz	29.8 ± 8.3 30.9 ± 8.7 28.8 ± 8.5	29.0 ± 8.5 30.7 ± 8.8 28.9 ± 8.3	0.37	0.73	0.90	-3.3 ± 5.9 -0.8 ± 4.0 0.7 ± 5.1	0.20
Cerebral Deoxy Hb (μM)	0 Hz 0.1 Hz 0.05 Hz	14.3 ± 3.3 14.7 ± 3.5 14.0 ± 3.9	14.8 ± 3.2 15.2 ± 3.4 14.2 ± 4.1	0.06	0.07	0.81	4.0 ± 5.1 3.9 ± 4.1 1.1 ± 4.2	0.15
Respiratory Responses								
Respiratory Rate (breaths/min)	0 Hz 0.1 Hz 0.05 Hz	12.6 ± 3.6 13.7 ± 4.6 13.3 ± 3.7	16.5 ± 7.1* 14.6 ± 5.7 14.4 ± 6.4	0.69	0.01	0.13	30.7 ± 32.7 10.4 ± 31.3 6.3 ± 28.8	0.15
etO_2 (mmHg)	0 Hz 0.1 Hz 0.05 Hz	101.5 ± 12.2 101.9 ± 11.3 102.4 ± 8.9	102.3 ± 10.5 104.5 ± 10.0 104.5 ± 9.8	0.62	0.13	0.83	1.1 ± 3.4 2.7 ± 4.2 2.1 ± 3.9	0.53
Plasma Samples								
Nitrite (μM)	0 Hz 0.1 Hz	3.0 ± 0.9 3.6 ± 1.6	3.5 ± 0.6 3.3 ± 0.7	0.05	0.46	0.21	18.4 ± 21.1 -2.7 ± 21.3 †	0.04

	0.05 Hz	2.8 ± 0.5	3.0 ± 0.4				11.8 ± 12.8	
Endothelin-1	0 Hz	1.39 ± 0.43	1.45 ± 0.30	0.56	0.03	0.30	11.31 ± 27.96	0.39
(pg/ml)	0.1 Hz	1.26 ± 0.18	1.66 ± 0.80				34.07 ± 65.78	
	0.05 Hz	1.28 ± 0.30	2.03 ± 1.45				65.45 ± 133.07	

ICA, internal carotid artery; MCAv, middle cerebral artery velocity; CVC index, cerebrovascular conductance index; PCAv, posterior cerebral artery velocity; Hb, hemoglobin; etO_2, end-tidal O_2. Absolute data were analyzed using a two-way linear mixed model, followed by *a priori* planned post-hoc tests between profiles at baseline and LBNP, and within profile between baseline and LBNP, with a Holm correction for multiple comparisons. Percent change from baseline data were analyzed using a one-way linear mixed model followed by Tukey post hoc tests. Data are presented as mean ± standard deviation. *P vs. baseline ≤ 0.06, †P vs. 0 Hz ≤ 0.07, §P vs. 0.1 Hz ≤ 0.07

Table 2. Frequency Domain Responses to Oscillatory LBNP

		Time						
	Profile	Baseline	LBNP	Profile	Time	Profile*Time	%Δ from Baseline	P-value
MAP LF ($mmHg^2$)	0 Hz	9.4 ± 7.1	25.3 ± 17.2	0.02	< 0.001	0.04	187.6 ± 176.7	0.01
	0.1 Hz	13.0 ± 19.4	46.4 ± 27.6 *†				582.2 ± 545.7 †	
	0.05 Hz	10.6 ± 9.9	20.6 ± 19.5§				135.5 ± 148.5 §	
MAP at 0.1 Hz ($mmHg^2$)	0 Hz	96.9 ± 92.0	392.9 ± 409.9	< 0.001	< 0.001	< 0.001	394.2 ± 481.8	0.03
	0.1 Hz	274.9 ± 637.9	2222.6 ± 1714.2 *†				3132.6 ± 4163.2†	
	0.05 Hz	130.6 ± 209.1	320.0 ± 256.7 §				313.0 ± 361.3 §	
MAP VLF ($mmHg^2$)	0 Hz	12.6 ± 4.9	14.7 ± 12.6	0.01	0.02	0.04	18.8 ± 93.4	0.09
	0.1 Hz	10.7 ± 4.4	14.0 ± 8.5				39.1 ± 80.2	
	0.05 Hz	14.6 ± 4.9	46.2 ± 49.2 *†§				231.0 ± 364.2	
MAP at 0.05 Hz ($mmHg^2$)	0 Hz	137.7 ± 75.7	157.9 ± 140.2	0.003	0.01	0.005	43.9 ± 134.5	0.003
	0.1 Hz	110.7 ± 75.6	231.3 ± 175.1				167.8 ± 254.3	
	0.05 Hz	169.4 ± 70.9	2104.0 ± 2442.5 *†§				1329.1 ± 1472.3 †§	
MCAv LF $(cm/s)^2$	0 Hz	4.0 ± 2.5	13.0 ± 13.4	0.87	< 0.001	0.39	196.0 ± 193.3	0.03
	0.1 Hz	3.5 ± 1.9	15.5 ± 10.2 *				358.8 ± 194.0	
	0.05 Hz	5.1 ± 3.2	9.8 ± 5.2				125.8 ± 112.9 §	
MCAv at 0.1 Hz $(cm/s)^2$	0 Hz	44.6 ± 26.6	241.5 ± 245.7	0.03	< 0.001	0.02	415.3 ± 488.2	0.002
	0.1 Hz	44.1 ± 33.1	739.6 ± 661.9 *†				2070.7 ± 1461.3 †	
	0.05 Hz	44.5 ± 19.5	174.6 ± 143.9 §				308.9 ± 307.7 §	
MCAv VLF $(cm/s)^2$	0 Hz	8.8 ± 5.1	8.6 ± 5.1	0.24	0.16	0.51	14.9 ± 62.3	0.44
	0.1 Hz	7.8 ± 5.0	11.0 ± 7.0				72.0 ± 99.7	
	0.05 Hz	9.5 ± 5.4	14.5 ± 14.6				92.9 ± 200.9	
MCAv at 0.05 Hz $(cm/s)^2$	0 Hz	66.5 ± 35.5	72.0 ± 46.2	0.01	0.03	0.04	38.9 ± 125.3	0.01
	0.1 Hz	85.7 ± 65.1	94.7 ± 105.0				32.6 ± 87.7 †§	
	0.05 Hz	95.8 ± 67.4	309.7 ± 296.9 *†§				298.7 ± 309.2	
PCAv LF $(cm/s)^2$	0 Hz	1.8 ± 1.5	4.5 ± 3.7 *	0.29	< 0.001	0.13	175.2 ± 231.7	0.70
	0.1 Hz	2.1 ± 0.2	7.1 ± 2.6 *				232.8 ± 111.9	
	0.05 Hz	2.1 ± 1.6	4.1 ± 1.9				141.4 ± 143.1	
	0 Hz	15.2 ± 7.7	78.0 ± 81.0	0.003	< 0.001	< 0.001	362.5 ± 444.2	0.004

PCAv at 0.1 Hz $(cm/s)^2$	0.1 Hz	18.6 ± 9.9	312.0 ± 177.2 *†				2201.7 ± 1716.6 †	
	0.05 Hz	20.1 ± 11.2	83.5 ± 81.2 §				241.6 ± 166.7 §	
PCAv VLF $(cm/s)^2$	0 Hz	3.2 ± 2.0	2.8 ± 1.9	0.29	0.95	0.53	9.0 ± 92.6	0.95
	0.1 Hz	4.1 ± 1.7	5.1 ± 3.2				21.6 ± 48.4	
	0.05 Hz	4.2 ± 2.8	3.7 ± 1.5				13.5 ± 51.9	
PCAv at 0.05 Hz $(cm/s)^2$	0 Hz	22.8 ± 20.9	22.9 ± 13.0	0.004	0.004	0.02	44.2 ± 98.4	0.13
	0.1 Hz	44.6 ± 24.1	74.9 ± 52.7				165.1 ± 289.7	
	0.05 Hz	32.1 ± 25.5	115.0 ± 70.5 *†				410.7 ± 488.3	

MAP, mean arterial pressure; LF, low frequency; VLF, very low frequency; MCAv, middle cerebral artery velocity; PCAv, posterior cerebral artery velocity. Absolute data were analyzed using a two-way linear mixed model, followed by *a priori* planned post-hoc tests between profiles at baseline and LBNP and within profile between baseline and LBNP, with a Holm correction for multiple comparisons. Percent change from baseline data were analyzed using a one-way linear mixed model followed by Tukey post hoc tests. Data are presented as mean ± standard deviation. *P vs. baseline ≤ 0.04, †P vs. 0 Hz ≤ 0.07, §P vs. 0.1 Hz ≤ 0.05

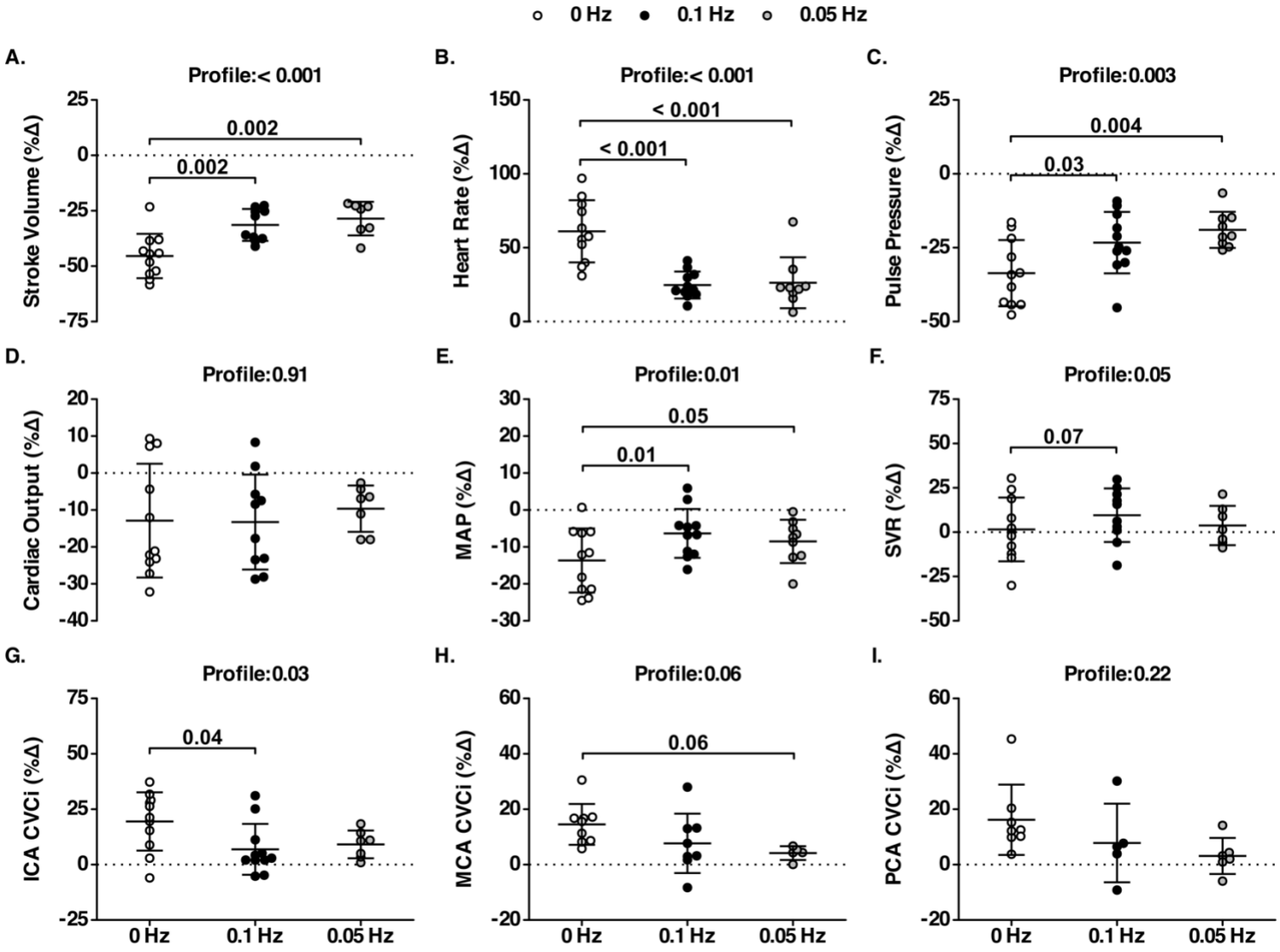
0 Hz
0.1 Hz
0.05 Hz
A.
Profile:< 0.001
Stroke Volume (%Δ)
0.002
0.002
B.
Profile:< 0.001
Heart Rate (%Δ)
< 0.001
< 0.001
C.
Profile:0.003
Pulse Pressure (%Δ)
0.004
0.03
D.
Profile:0.91
Cardiac Output (%Δ)
E.
Profile:0.01
MAP (%Δ)
0.05
0.01
F.
Profile:0.05
SVR (%Δ)
0.07
G.
Profile:0.03
ICA CVCi (%Δ)
0.04
H.
Profile:0.06
MCA CVCi (%Δ)
0.06
I.
Profile:0.22
PCA CVCi (%Δ)
0 Hz
0.1 Hz
0.05 Hz

Figure 1. Systemic Cardiovascular Responses to Oscillatory LBNP

MAP, mean arterial pressure; SVR, systemic vascular resistance; ICA CVCi, cerebrovascular conductance index for the internal carotid artery; MCA CVCi, cerebrovascular conductance index for the middle cerebral artery; PCA CVCi, cerebrovascular conductance index for the posterior cerebral artery. Data were analyzed using a one-way linear mixed model, followed by Tukey post hoc tests. Data are presented as mean ± standard deviation. Exact p-values are reported.

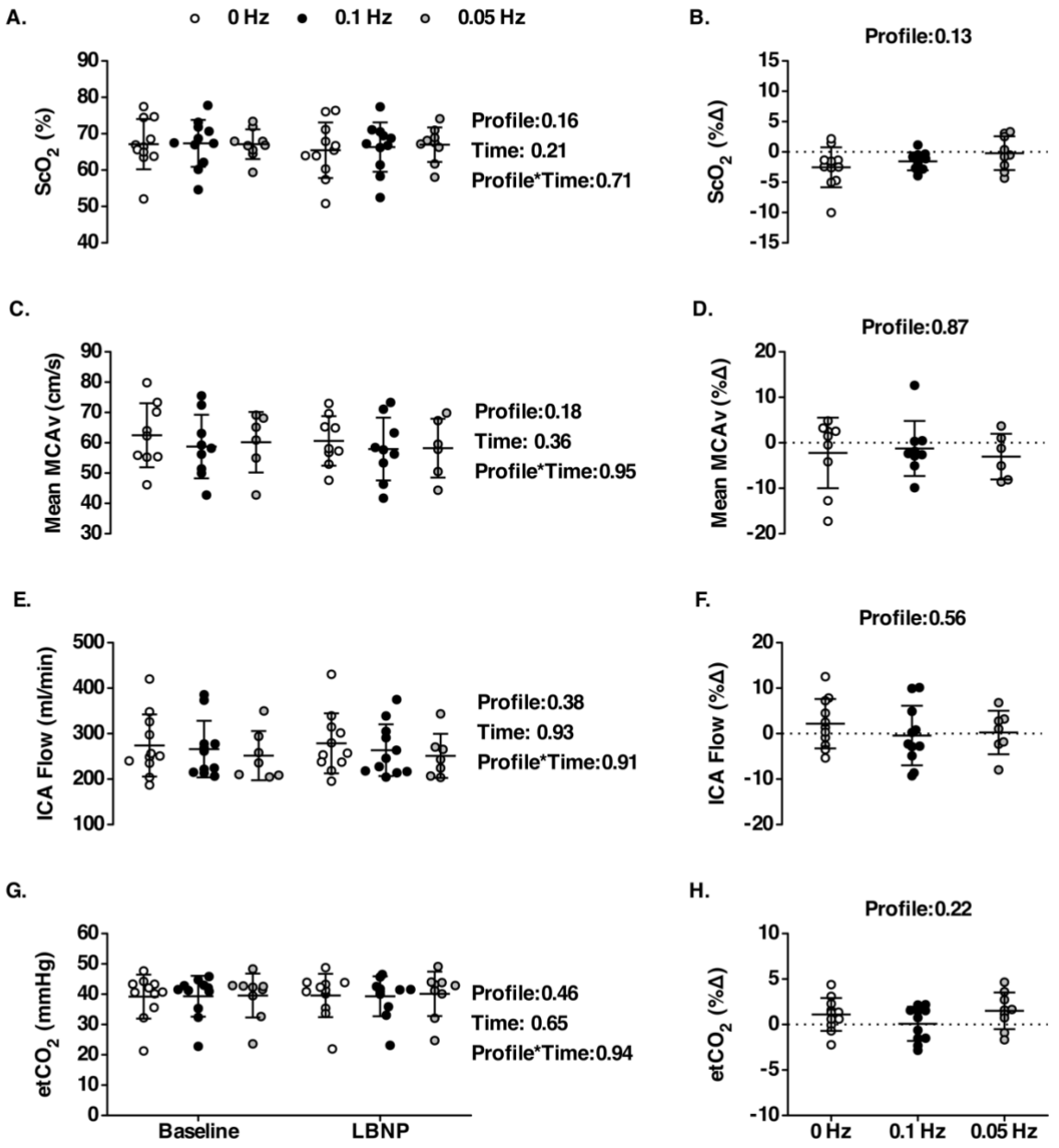
A.
0 Hz
0.1 Hz
0.05 Hz
ScO2 (%)
Profile:0.16
Time: 0.21
Profile*Time:0.71
B.
Profile:0.13
ScO2 (%Δ)
C.
Mean MCAv (cm/s)
Profile:0.18
Time: 0.36
Profile*Time:0.95
D.
Profile:0.87
Mean MCAv (%Δ)
E.
ICA Flow (ml/min)
Profile:0.38
Time: 0.93
Profile*Time:0.91
F.
Profile:0.56
ICA Flow (%Δ)
G.
etCO2 (mmHg)
Profile:0.46
Time: 0.65
Profile*Time:0.94
Baseline
LBNP
H.
Profile:0.22
etCO2 (%Δ)
0 Hz
0.1 Hz
0.05 Hz

Figure 2. Cerebral Responses to oscillatory LBNP with clamped $etCO_2$.

Absolute responses are shown in panels A, C, E, and G, while relative (% Δ from baseline) data are show in panels B, D, F, and H. ScO_2, cerebral tissue oxygenation; MCAv, middle cerebral artery velocity; ICA, internal carotid artery; $etCO_2$, end-tidal CO_2. Absolute data were analyzed using a two-way linear mixed model, followed by *a priori* planned post-hoc tests between profiles at baseline and LBNP and within profile between baseline and LBNP, with a Holm correction for multiple comparisons. Percent change from baseline data were analyzed using a one-way linear mixed model followed by Tukey post hoc tests. Data are presented as mean ± standard deviation. Exact p-values are presented.

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CHAPTER V

Sex Differences in Response to Forced Hemodynamic Oscillations During Simulated Hemorrhage

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ABSTRACT

Hemorrhage continues to be a leading cause of preventable death from trauma. Examination of the physiological responses to hemorrhage and survival statistics in patients, indicates that females have better recovery outcomes, including a survival advantage. However, further research is needed to determine if development of therapeutic interventions to treat hemorrhagic

injuries should be sex-specific based on differences in physiological responses. Oscillatory arterial pressure and blood flow (~0.1 Hz) may be a potential therapeutic technique for protecting tissue oxygenation during hemorrhage. This therapeutic approach has not yet been examined with a focus on potential sex differences. We hypothesized that tissue oxygenation will decrease by a similar magnitude for both males and females in response to simulated hemorrhage using lower body negative pressure (LBNP), and that forced hemodynamic oscillations at 0.1 Hz will equally protect tissue oxygenation for both sexes. Seven (7) females and 15 males underwent two LBNP conditions: 1) a non-oscillatory (0 Hz) control condition and 2) a 0.1 Hz oscillatory condition. Cerebral and peripheral muscle tissue oxygenation were measured continuously using near-infrared spectroscopy (NIRS). Cerebral tissue oxygenation was reduced by a similar magnitude for females and males in both profiles (0 Hz: females, -3.8 ± 5.2 % vs. males, -4.2 ± 3.7 %; 0.1 Hz: females, -2.9 ± 3.7 % vs. males, -4.3 ± 2.6 %; ANOVA: Profile, $p = 0.63$; Sex, $p = 0.55$). Muscle tissue oxygenation, however, decreased to a greater extent in males during the 0 Hz profile (females, -7.1 ± 3.3 % vs. males, -14.3 ± 4.6 %, $p = 0.02$), but this response in males was attenuated in the 0.1 Hz profile (0 Hz, -14.3 ± 4.6 % vs. 0.1 Hz, -9.5 ± 4.1 %, $p = 0.08$). This greater decrease in muscle tissue oxygenation during the 0 Hz condition in males vs. females, and subsequent restoration of muscle tissue oxygenation in the 0.1 Hz condition highlights the potential need for sex-specific research in the treatment of hemorrhage.

INTRODUCTION

Hemorrhage, or massive blood loss, accounts for more than 60,000 deaths per year in the United States of America, and 1.9 million deaths worldwide (1). Hemorrhage continues to be a leading cause of preventable death due to traumatic injury in both the civilian and military

settings (2, 3). In the military setting, most of these deaths occur in males due to a greater number of males in active duty (4). In the civilian setting, twice as many men die from trauma-induced hemorrhage as women (2); however, it is important to note that sex-specific causes of hemorrhage also exist, such as pregnancy complications where 23% of deaths related to maternal disorders are due to hemorrhage (1).

In addition to these epidemiological differences between the sexes, there are also physiological differences in response to hemorrhagic injuries. Females are generally reported as having better recovery outcomes following trauma (including hemorrhage), and are less susceptible to post-traumatic infection (i.e. sepsis) and multi-organ failure (5). For example, in a retrospective study of patients admitted with traumatic injuries, plasma lactate concentrations, a marker of inadequate tissue perfusion, was lower in pre-menopausal women compared to men of a similar age (6). These beneficial outcomes are commonly linked to the protective effects of estrogen which regulates, in part, the inflammatory and heat-shock protein response during traumatic hemorrhage (7). Further understanding sex differences in the physiological responses to hemorrhage is essential for subsequent development of therapeutic approaches.

Lower body negative pressure (LBNP) has been validated as a human model of simulated hemorrhage, with induction of progressive central hypovolemia (8, 9). Studies examining tolerance and physiological responses to LBNP between the sexes have yielded equivocal results. While some studies have indicated that females have a lower tolerance to LBNP and impaired physiological compensatory responses (10, 11), others have shown no difference between males and females in either tolerance or compensatory physiological responses (12, 13).

Of the mechanisms that contribute to higher tolerance to simulated hemorrhage with LBNP, oscillatory arterial pressure and cerebral blood flow at around 0.1 Hz (10-s cycle) has

been implicated as a possible compensatory mechanism (14). When comparing the endogenous generation of arterial pressure oscillations (between 0.04-0.15 Hz) in response to a maximal LBNP stimulus, both men and women exhibit increases in the amplitude of oscillations over the course of the LBNP exposure, with no differences between the sexes at any given stage (13). Furthermore, cerebral tissue oxygenation (13) and muscle tissue oxygenation (unpublished data) in these same participants exhibited similar decreases, with no difference between men and women. We have recently employed hypovolemic oscillatory LBNP to force hemodynamic oscillations at the target frequency of 0.1 Hz, as a potential therapy to treat hemorrhagic injuries (15, 16). In these studies, we have demonstrated a protection of cerebral and muscle tissue oxygenation with 0.1 Hz oscillations, despite similar reductions in arterial pressure and cerebral blood flow (15, 16); however, it is not yet known if there is sex-specific effect in these responses. In this retrospective study, we hypothesized that cerebral and peripheral muscle tissue oxygenation will decrease similarly for both males and females in response to the hypovolemia induced by LBNP, and that forced hemodynamic oscillations at 0.1 Hz will equally protect tissue oxygenation for both sexes.

METHODS

Data utilized for this retrospective study represents the amalgamation of data from two prior IRB-approved studies (15, 16) at the University of North Texas Health Science Center in Fort Worth, TX, USA (IRB #2016-049 and IRB #2019-110). However, the research questions proposed in this study are unique and independent of these two prior publications. IRB #2016-049 was conducted at the University of North Texas Health Science Center in Fort Worth, TX, while IRB #2019-110 was conducted at Mount Royal University in Calgary, Alberta, Canada

(Mount Royal University Human Research Ethics Board Protocol 101879; University of Calgary Conjoint Health Research Ethics Board Protocol REB18-0374), as part of baseline testing for a high-altitude research expedition (see reference (16) for details). Each participant provided written informed consent prior to being enrolled in these studies.

Participants

All participants were free from known cardiovascular, neurological, and respiratory diseases. Participants in the IRB #2016-049 study abstained from alcohol, exercise, medications, and caffeine 24 hours prior to experimentation, whereas participants in the IRB #2019-110 study abstained from these items 12 hours prior to experimentation. Females who participated in the IRB #2016-049 study were tested during the early follicular phase of their menstrual cycle (days 1-4), and only one of these participants used a form of hormonal medication (hormonal vaginal ring). Due to the logistical constraints of the high-altitude expedition, menstrual cycle phase was not controlled in female participants tested in the IRB #2019-110 study, and all were using some form of hormonal medication: 3 participants were using an intrauterine device, and had either amenorrhea or oligomenorrhea; 1 participant was using oral hormonal medication.

Instrumentation

Participants were positioned into an LBNP chamber with their waist in line with the opening of the chamber. Three ECG electrodes were then placed in a lead II configuration (shielded leads, cable and amplifier; AD Instruments, Bella Vista, NSW, Australia). A neoprene waist band was secured around a heavy-duty plastic liner at the level of the iliac crest to create a tight seal around the participant. A near-infrared spectroscopy probe was placed on the forehead

and secured using a black self-adhering wrap for measures of cerebral tissue oxygenation (ScO_2), while a muscle tissue oxygenation (SmO_2) probe was placed on the forearm over the flexor carpi ulnaris muscle (OxiplexTS; ISS, Champaign–Urbana, IL, USA). Two 2 MHz transcranial Doppler ultrasound probes were then placed on either side of the head over the temporal windows using adjustable headgear (ST3; Spencer Technologies, Seattle, WA, USA). These probes were positioned to measure middle cerebral artery velocity (MCAv). Arterial pressure and was continuously measured using finger photoplethysmography with the finger cuff placed around the middle finger (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands), and stroke volume was measured and recorded by the Finometer from this waveform. Finally, an oral/nasal cannula connected to a gas analyzer was used to measure end-tidal CO_2 ($etCO_2$) and O_2 (etO_2) as well as respiration rate (ML206 Gas Analyzer; AD Instruments).

Experimental Design

After instrumentation, breathing was monitored for 2-min. If respiration rate was lower than 10 breaths per min, the participant's breathing was paced at 10 breaths per min using a metronome. If respiration rate was above 10 breaths per min, the participant was paced at their spontaneous rate. Following a 5-min baseline period, all participants underwent two conditions of LBNP in randomized order: 1) a 0 Hz profile where chamber pressure was gradually reduced over 30 s to -60 mmHg and subsequently maintained at this pressure for 9.5 min; and 2) a 0.1 Hz profile where chamber pressure was reduced to -60 mmHg over 30 s, held at -60 mmHg for 30 sec, then oscillated between -30 mmHg for 5-sec and -90 mmHg for 5-sec repeatedly for 9.5 min. Participants in the IRB #2016-049 study underwent these two LBNP profiles as well as a third oscillatory profile (0.05 Hz) within the same experimental session in a randomized order;

each profile was separated by 5-min. Data from the 0.05 Hz profile is not included in the current analysis. Testing for this protocol was always performed in the morning. Participants in the IRB #2019-110 study underwent the 0 Hz and 0.1 Hz profiles in randomized order on separate days. Time of day for this protocol was fixed within participants, but not between participants. For all participants, the LBNP chamber was turned off if the following presyncopal criteria were met: a reduction in systolic arterial pressure below 80 mmHg, or the onset of presyncopal symptoms such as lightheadedness, dizziness, nausea, or visual disturbances such as blurred vision or grey out. A 10-min recovery period concluded each experimental session.

Data Analysis

Continuous measurements were collected at 1000 Hz using PowerLab and LabChart 8 (AD Instruments, Dunedin, New Zealand). Data were then imported into a specialized analysis software for offline analysis (WinCPRS, Absolute Aliens, Finland). R-wave detection was performed to create cardiac cycle windows in the arterial pressure and cerebral artery blood velocity waveforms. Heart rate was calculated from the R-R interval waveform. Beat-to-beat metrics of mean arterial pressure and mean MCAv were calculated as the area under the curve within each cardiac cycle. Cardiac output was calculated by multiplying heart rate by stroke volume, and systemic vascular resistance was calculated by dividing mean arterial pressure by cardiac output. Cerebral vascular resistance index was calculated by dividing mean arterial pressure by mean MCAv. For all time domain variables, baseline averages were calculated from the final 3-min of the 5-min baseline period for all experiments in the IRB #2019-110 study. In the IRB #2016-049 study, the entire 5-min of baseline was averaged and used for 10 of 28 experiments; however, the other 5-min “baseline” periods were also the recovery period between

profiles, so the final 1-min of this period was averaged for baseline. For measurements made during LBNP, the final 1-min of the profile was averaged for all experiments.

Frequency domain analysis was also performed for measures of power spectral density in mean arterial pressure and mean MCAv. Briefly, data were linearly interpolated and resampled at 5 Hz, then filtered using a finite low-pass impulse response filter with a cut-off frequency of 0.5 Hz. The fast Fourier transform with a Hanning window, and the Welch method was then used for power spectral density analysis. The frequency range of interest for this analysis was the low frequency range of 0.07-0.15 Hz. Power spectral density was measured as the area under the curve within this frequency range. A point estimate of spectral power was also made at 0.1 Hz in mean arterial pressure and mean MCAv. A minimum of 4-min was used for frequency domain analysis during all baseline periods and at the end of LBNP.

Statistical Analysis

All data are presented as mean ± standard deviation (SD). A three-factor (sex, time, LBNP profile) linear mixed model was used to analyze all absolute data. Post-hoc testing was performed on the estimated marginal means for *a priori* comparisons of interest, including between the sexes within time and profile, between profiles within time and sex, and between time within sex and profile. Percent change values were also compared using a two-factor (sex and LBNP profile) linear mixed model. Post-hoc testing was performed on the estimated marginal means for *a priori* comparisons of interest between sexes within profile, and between profiles within sex. Post-hoc tests for both linear mixed models were corrected for multiple comparisons using the Holm correction method.

RESULTS

Fifteen males and 7 females were included in the final analysis. There was no difference in age between the sexes; however as expected, male participants were taller and weighed more than the female participants (Table 1).

Baseline Resting Comparisons

No differences were observed between the sexes at baseline for heart rate, mean arterial pressure, or stroke volume index (Table 1). No differences were observed in MCAv or respiration rate despite males having a higher $etCO_2$ at rest (Table 1). ScO_2 was lower for females compared to males, while SmO_2 was no different between the sexes (Table 1).

Systemic and Cerebrovascular Responses to LBNP

Stroke volume decreased to a greater extent in the 0 Hz vs. 0.1 Hz profile, and corresponded with a higher heart rate response in both males and females (Table 2; Figure 1). This resulted in no differences in cardiac output or mean arterial pressure responses between profiles and between sexes (Table 2, Figure 1). MCAv was not recorded in 1 male participant due to technically difficulties. Mean MCAv decreased similarly in response to LBNP for both sexes (Figure 2, Table 2). Respiration rate was no different across time, sex, or profile. End-tidal CO_2 decreased, while end-tidal O_2 increased with LBNP regardless of profile, with no differences between sexes.

Tissue Oxygenation Responses to LBNP

In both sexes, ScO_2 decreased with LBNP, and was higher overall in the 0.1 Hz condition (Table 2, Profile and Time main effect terms). However, ScO_2 decreased by a similar magnitude from baseline in both male and female participants (Figure 3).

SmO_2 signal quality was insufficient for 2 females and 6 males. SmO_2 decreased for both sexes in both profiles; however, males experienced a greater decrease in SmO_2 during the 0 Hz profile compared to females, which was then attenuated in the 0.1 Hz profile (Table 2, Figure 1). Muscle total hemoglobin concentration was lower in females but decreased by a similar magnitude to males in both profiles (Table 3). When examining the muscle oxyhemoglobin response, males had a greater decrease in muscle oxyhemoglobin in response to LBNP which may indicate a greater overall oxygen extraction compared to females (Table 3).

Frequency Domain Measures

Oscillatory power increased for arterial pressure in response to LBNP (Table 2; Time factor $P<0.001$). However, the relative increase in oscillatory power was only observed in mean arterial pressure when measured at the point frequency of 0.1 Hz. A similar response was observed for mean MCAv, with a greater relative increase in oscillatory power during the 0.1 Hz profile. However, post hoc testing showed that these increases only occurred for males and not females, although these responses exhibit very high variability (Table 3).

DISCUSSION

The aim of this study was to examine the tissue oxygenation responses between males and females in response to forced 0.1 Hz hemodynamic oscillations during simulated hemorrhage. The main findings from this study are: 1) there was no effect of sex on the cerebral

tissue oxygenation responses to oscillatory LBNP, 2) males exhibited greater decreases in muscle tissue oxygenation during the 0 Hz profile, which was then protected with 0.1 Hz oscillations, and 4) there was no effect of sex on the cerebral and systemic hemodynamic responses to oscillatory LBNP.

Cerebral Tissue Oxygenation Responses

Cerebral tissue oxygenation is known to decrease during exposure to LBNP in response to decreases in central blood volume and cerebral blood flow delivery (17, 18). Our prior studies have assessed the effect of applying 0.1 Hz oscillations in arterial pressure and cerebral blood flow on cerebral tissue oxygenation during central hypovolemia (15, 16). In these studies, we demonstrated that forcing 0.1 Hz oscillations in arterial pressure and cerebral blood flow during central hypovolemia protected against reductions in cerebral and muscle tissue oxygenation. However, the effect of sex on these responses had not been examined. In the current study, there were no differences in the cerebral tissue oxygenation responses between males and females to either profile. This finding is not surprising, however, as MCAv responses, our index of cerebral blood flow and oxygen delivery, were also similar between the sexes. Another key factor that may impact cerebral tissue oxygenation is tissue metabolism. A recent study demonstrated similar resting cerebral metabolic rate of oxygen between men and women using positron emission tomography and single-photon emission computed tomography (19), but this has not been assessed during central hypovolemia such as with LBNP (either static or oscillatory profiles).

Muscle Tissue Oxygenation Responses

While there was no difference in cerebral tissue oxygen responses between the sexes, males experienced a greater decrease in muscle tissue oxygenation for the 0 Hz profile, which was subsequently attenuated in the 0.1 Hz profile. While we are not aware of studies comparing the effect of sex on muscle tissue oxygenation responses to LBNP, some research has been conducted using vascular occlusion protocols. Keller and Kennedy explored the role of sex on the rate of oxygen desaturation measured via NIRS with 5-min of forearm vascular occlusion and during handgrip to fatigue (20). After matching for strength, males exhibited a greater rate of oxygen desaturation than females, which is likely related to greater oxygen extraction from the blood (20). The authors speculated that this difference could be based on skeletal muscle fiber type composition (21), mitochondrial properties (20), or density of capillaries around the muscle fibers (22). In relation to our findings, assuming oxygen delivery (i.e., blood flow) to the tissue is similar, greater mitochondrial oxidative efficiency in women compared to men (23) may facilitate less oxygen extraction from the blood into the muscle tissue to meet metabolic demand. Further research is needed to examine tissue oxygenation responses between the sexes during states of hypoperfusion, which in turn, could inform development of clinical treatments that are sex specific.

Other cardiovascular responses (stroke volume, heart rate, cardiac output, mean arterial pressure, systemic vascular resistance) matched those previously reported in our study at low altitude (15). As these systemic hemodynamic parameters were similar between the sexes, this does not account for the differences observed in muscle tissue oxygenation. Further investigations should include local measurements to the peripheral tissue, such as blood flow and

oxygen delivery, to understand the mechanisms driving the difference in muscle tissue oxygenation responses with oscillatory LBNP.

Methodological Considerations

The data for this study were pooled from two prior studies with some differences in the respective experimental designs. As participants in the IRB #2016-049 study underwent all LBNP profiles within the same experimental session, there may have been carry-over effects of the first profile on the subsequent profiles. While the randomization of the profiles should account for any order effects, the use of the 5-min recovery period as a baseline period for some experiments may not have been enough time for all variables to return to a true resting baseline state. To account for this, the last 60 s of the 5-min recovery was used as a baseline value to provide more time for a return to resting values. Using this approach, tissue oxygenation, the main outcome of interest for this study, did return to baseline values within this time frame.

A second important consideration is the control of menstrual cycle phase in only one of the studies (IRB #2016-049), which may have resulted in different hormonal profiles between female participants. Interestingly, however, three of the four females in the study without menstrual phase control (IRB #2019-110) were using an IUD, with two of these participants reporting amenorrhea, while the third participant had oligomenorrhea. The other female participant in this study was using oral contraceptives, and was not menstruating at the time of the experiments, so may have also exhibited low circulating ovarian hormone concentrations. This suggests that female participants may have had relatively low circulating concentrations of estrogen and progesterone, despite a lack of menstrual cycle control, although blood samples were not obtained for definitive confirmation of hormone concentrations.

Conclusion

In conclusion, we hypothesized that cerebral and peripheral muscle tissue oxygenation would decrease similarly for both males and females in response to LBNP, and that forced hemodynamic oscillations at 0.1 Hz would protect tissue oxygenation for both sexes. Interestingly, while most cardiovascular responses were similar between males and females, the greater reduction in muscle tissue oxygenation with simulated hemorrhage in male participants was attenuated with application of 0.1 Hz hemodynamic oscillations. Further research is needed to elucidate the mechanisms associated with this interesting finding, and highlights the need to understand sex-specific responses to hemorrhage for the development of individualized therapeutic approaches.

Table 1. Resting Participant Characteristics

	F	M	P-value
N	7	15	.
Age (y)	31 ± 8	26 ± 3	0.19
Height (cm)	162.4 ± 7.5	180.5 ± 5.3	< 0.001
Weight (kg)	63.5 ± 8.7	82.7 ± 13.7	< 0.001
Body Surface Area (m^2)	1.7 ± 0.1	2.0 ± 0.2	< 0.001
Heart Rate (bpm)	65.9 ± 6.6	63.0 ± 11.8	0.47
Stroke Volume Index (ml/m^2)	51.4 ± 8.2	51.8 ± 8.2	0.91
Mean Arterial Pressure (mmHg)	93.5 ± 4.9	89.3 ± 8.2	0.15
Mean MCAv (cm/s)	56.8 ± 11.4	52.0 ± 10.3	0.36
ScO_2 (%)	60.3 ± 11.0	71.4 ± 5.2	0.04
SmO_2 (%)	66.4 ± 5.8	71.0 ± 5.8	0.15
$etCO_2$ (mmHg)	33.3 ± 1.8	37.0 ± 3.0	0.002
Respiration Rate (breaths/min)	15.1 ± 3.7	13.9 ± 3.6	0.49

Data are presented as mean ± standard deviation. ScO_2, cerebral tissue oxygen saturation; SmO_2, muscle tissue oxygen saturation; $etCO_2$, end-tidal CO_2. Baseline values between females and males were compared with an unpaired t-test. Exact p-values are reported.

Table 2. Absolute Responses to Oscillatory LBNP Between Males and Females

		Baseline		LBNP								
		0 Hz	0.1 Hz	0 Hz	0.1 Hz	Sex	Profile	Time	Sex: Profile	Sex: Time	Profile: Time	Sex: Profile: Time
Heart Rate (bpm)	F	62.4 ± 10.4	67.2 ± 4.7	103.5 ± 20.9	78.9 ± 9.3	0.46	< 0.001	< 0.001	0.35	0.64	< 0.001	0.05
	M	61.5 ± 13.0	62.0 ± 12.6	92.7 ± 15.6	79.9 ± 14.8							
Mean Arterial Pressure (mmHg)	F	95.6 ± 5.1	91.8 ± 4.4	81.5 ± 10.7	84.6 ± 6.2	0.52	0.46	< 0.001	0.31	0.62	0.17	0.13
	M	89.7 ± 8.7	92.0 ± 7.8	80.5 ± 11.2	82.4 ± 9.2							
Pulse Pressure (mmHg)	F	57.0 ± 7.3	53.3 ± 7.1	33.7 ± 8.0	39.6 ± 7.3	0.47	0.25	< 0.001	0.81	0.46	0.01	0.23
	M	56.4 ± 6.4	56.2 ± 7.4	37.8 ± 10.0	41.3 ± 5.9							
Stroke Volume Index (ml/m^2)	F	53.2 ± 6.5	50.7 ± 5.9	25.4 ± 7.8	34.3 ± 6.8	0.42	0.03	< 0.001	0.78	0.56	< 0.001	0.35
	M	53.9 ± 8.7	53.2 ± 9.4	30.1 ± 7.8	35.8 ± 7.2							
Cardiac Output Index (ml/min/m^2)	F	3.3 ± 0.7	3.4 ± 0.4	2.5 ± 0.6	2.7 ± 0.6	0.99	0.25	< 0.001	0.60	0.08	0.55	0.93
	M	3.2 ± 0.4	3.2 ± 0.5	2.7 ± 0.4	2.8 ± 0.4							
SVR Index (mmHg/L/min)/m^2	F	30.1 ± 7.4	27.3 ± 3.5	34.1 ± 12.8	32.8 ± 8.5	0.47	0.46	0.01	0.31	0.18	0.99	0.53
	M	28.1 ± 3.8	29.2 ± 5.1	30.5 ± 6.6	30.0 ± 5.3							
Mean MCA Velocity (cm/s)	F	58.7 ± 12.5	59.4 ± 11.5	47.8 ± 13.9	49.4 ± 13.5	0.28	0.24	< 0.001	0.94	0.30	0.75	0.46
	M	51.0 ± 10.2	53.5 ± 11.4	43.8 ± 11.0	44.0 ± 12.6							
Cerebral Vascular Resistance Index (cm/s/mmHg)	F	1.7 ± 0.5	1.6 ± 0.5	1.9 ± 0.8	1.8 ± 0.5	0.58	0.55	0.001	0.41	0.69	0.50	0.91
	M	1.8 ± 0.3	1.8 ± 0.3	1.9 ± 0.5	2.0 ± 0.5							
$etCO_2$ (mmHg)	F	33.5 ± 4.1	33.7 ± 1.1	25.7 ± 7.6	27.7 ± 6.0	0.25	0.71	< 0.001	0.37	0.99	0.95	0.33
	M	36.2 ± 5.0	36.5 ± 4.5	30.1 ± 6.7	28.8 ± 8.5							
etO_2 (mmHg)	F	109.7 ± 5.0	112.1 ± 3.9	123.4 ± 12.7	118.9 ± 8.1	0.54	0.78	< 0.001	0.84	0.65	0.25	0.69
	M	108.0 ± 7.5	109.5 ± 6.9	117.9 ± 11.4	116.1 ± 12.2							
Respiration Rate (breaths/min)	F	15.2 ± 3.3	15.8 ± 4.6	16.0 ± 4.0	14.4 ± 3.8	0.25	0.16	0.17	0.83	0.51	0.26	0.09
	M	14.3 ± 3.5	13.4 ± 3.5	13.3 ± 3.8	12.9 ± 3.5							
ScO_2 (%)	F	60.4 ± 12.0	61.8 ± 11.7	58.5 ± 13.1	60.3 ± 12.6	0.01	0.003	< 0.001	0.50	0.11	0.85	0.81
	M	71.2 ± 5.6	72.2 ± 5.2	68.1 ± 4.7	69.1 ± 5.4							
Cerebral dHb (µg)	F	16.5 ± 4.1	15.8 ± 4.5	17.2 ± 4.4	16.7 ± 4.6	0.22	0.02	< 0.001	0.68	0.26	0.80	0.53
	M	13.9 ± 2.8	13.7 ± 2.9	15.4 ± 3.5	14.8 ± 3.3							
Cerebral HbO_2 (µg)	F	26.1 ± 9.0	25.2 ± 7.4	25.2 ± 9.1	25.5 ± 7.8	0.04	0.74	0.04	0.43	0.09	0.79	0.52
	M	35.3 ± 10.4	36.3 ± 9.6	33.1 ± 7.5	33.5 ± 7.9							
Cerebral Total Hb (µg)	F	42.5 ± 8.7	41.0 ± 7.5	42.5 ± 8.7	42.3 ± 7.2	0.16	0.67	0.68	0.38	0.19	0.86	0.42
	M	49.2 ± 11.7	49.9 ± 11.1	48.5 ± 10.1	48.3 ± 9.9							
SmO_2 (%)	F	67.3 ± 6.8	68.2 ± 8.0	62.4 ± 6.0	63.6 ± 9.3	0.49	0.004	< 0.001	0.20	0.004	0.16	0.21
	M	71.7 ± 6.0	72.7 ± 5.9	61.5 ± 6.9	65.9 ± 6.6							
Muscle dHb (µg)	F	20.0 ± 6.3	18.2 ± 7.5	22.7 ± 6.4	23.5 ± 10.1	0.15	0.03	< 0.001	0.14	0.04	0.87	0.10
	M	24.5 ± 6.6	23.0 ± 6.1	32.5 ± 8.8	28.9 ± 7.7							
Muscle HbO_2 (µg)	F	40.9 ± 9.3	39.5 ± 12.2	37.7 ± 9.1	39.5 ± 6.2	0.003	0.32	< 0.001	0.41	0.001	0.05	0.73
	M	61.8 ± 10.0	61.3 ± 9.6	51.1 ± 7.7	55.2 ± 8.2							

Muscle Total Hb (µg)	F	60.9 ± 12.3	57.7 ± 16.9	60.4 ± 13.2	63.0 ± 12.3	0.002	0.67	0.78	0.89	0.07	0.08	0.46
	M	87.2 ± 12.4	85.4 ± 11.3	84.3 ± 11.9	84.9 ± 11.3							
Mean Arterial Pressure LF ($mmHg^2$)	F	1.0 ± 0.3	2.1 ± 1.0	4.9 ± 2.6	7.5 ± 9.2	0.70	0.31	< 0.001	0.53	0.68	0.36	0.79
	M	2.3 ± 3.0	1.5 ± 1.2	6.6 ± 7.2	8.3 ± 8.0							
Mean Arterial Pressure 0.1 Hz ($mmHg^2$)	F	18.6 ± 8.1	25.3 ± 14.4	61.5 ± 43.6	729.2 ± 1345.6	0.99	0.02	0.01	0.84	0.93	0.02	0.91
	M	39.1 ± 86.1	22.1 ± 22.0	88.7 ± 104.7	677.0 ± 697.0							
Mean MCA Velocity LF $(cm/s)^2$	F	1.0 ± 0.8	2.7 ± 2.8	2.5 ± 2.4	4.7 ± 4.3	0.09	0.01	0.002	0.08	0.45	0.39	0.79
	M	0.9 ± 0.9	0.9 ± 0.5	1.5 ± 0.8	2.4 ± 1.6							
Mean MCA Velocity 0.1 Hz $(cm/s)^2$	F	10.4 ± 10.7	36.2 ± 28.3	43.3 ± 39.3	326.2 ± 586.5	0.43	0.03	0.02	0.47	0.53	0.05	0.67
	M	16.0 ± 26.0	10.7 ± 10.4	27.1 ± 30.9	187.4 ± 216.0							

SVR, systemic vascular resistance; MCA, middle cerebral artery; $etCO_2$, end-tidal CO_2; etO_2, end-tidal O_2; ScO_2, cerebral tissue oxygenation; dHb, deoxygenated hemoglobin; HbO_2, oxygenated hemoglobin; SmO_2, muscle tissue oxygenation; LF, low frequency. Data were analyzed using a linear mixed model with three factors. For simplicity, pairwise comparisons are not presented in the table. Reference the ANOVA p-values (presented here) and the results from the relative changes for detailed comparisons. Data are presented as mean ± standard deviation. Exact p-values are reported.

Table 3. Relative (%Δ from baseline) Responses to Oscillatory LBNP Between Males and Females

	0 Hz		0.1 Hz				
(%Δ)	F	M	F	M	Sex	Profile	Sex:Profile
Pulse Pressure	-40.7 ± 14.2	-33.2 ± 15.0	-26.0 ± 8.1 *	-25.9 ± 10.5	0.46	< 0.001	0.21
Cerebral Vascular Resistance Index	7.1 ± 12.5	6.0 ± 15.9	13.3 ± 13.8	10.8 ± 19.0	0.80	0.06	0.82
etO_2	12.2 ± 6.4	9.3 ± 10.3	6.0 ± 4.8	6.2 ± 11.3	0.83	0.10	0.57
Respiration Rate	4.8 ± 7.0	-6.9 ± 12.1 †	-7.3 ± 10.0 *	-3.5 ± 10.4	0.32	0.15	0.02
Cerebral dHb	4.6 ± 4.8	10.7 ± 8.1	6.4 ± 6.6	8.3 ± 5.0	0.15	0.86	0.23
Cerebral HbO_2	-4.1 ± 6.7	-5.0 ± 7.0	0.7 ± 6.6	-7.0 ± 5.4 †	0.14	0.28	0.01
Cerebral Total Hb	-0.2 ± 1.4	-0.9 ± 4.2	3.4 ± 5.1	-2.9 ± 3.5 †	0.04	0.40	0.01
Muscle dHb	14.7 ± 9.4	33.2 ± 13.9	29.9 ± 27.6	25.5 ± 9.1	0.25	0.54	0.07
Muscle HbO_2	-8.1 ± 4.5	-17.0 ± 5.9	6.8 ± 31.6	-9.6 ± 6.1	0.03	0.05	0.50
Muscle Total Hb	-1.2 ± 2.1	-3.3 ± 4.3	14.4 ± 30.5	-0.6 ± 3.7	0.11	0.08	0.20
Mean Arterial Pressure LF	433 ± 399	424.1 ± 683.0	334.7 ± 480.5	690 ± 837	0.58	0.59	0.25
Mean Arterial Pressure 0.1 Hz	259 ± 294	1144 ± 2042.2	2804 ± 3728	5206 ± 4678 *	0.23	0.01	0.50
MCA Mean Velocity LF	133 ± 96	170 ± 282	117 ± 236	264 ± 324	0.49	0.46	0.30
MCA Mean Velocity 0.1 Hz	398 ± 460	753 ± 1260	717.3 ± 1211	2176 ± 1890 *	0.16	0.05	0.19

etO_2, end-tidal O_2; dHb, deoxygenated hemoglobin; HbO_2, oxygenated hemoglobin; LF, low frequency. Data were analyzed using a linear mixed model with two factors. Post hoc tests were determined *a priori* to compare between sexes during lower body negative pressure (LBNP) profile and between LBNP profiles within sex. The Holm correction was used to correct for multiple comparisons. Data are presented as mean ± standard deviation. Exact p-values are reported. *P ≤ 0.08 vs profile within sex. †P = 0.08 vs sex within profile.

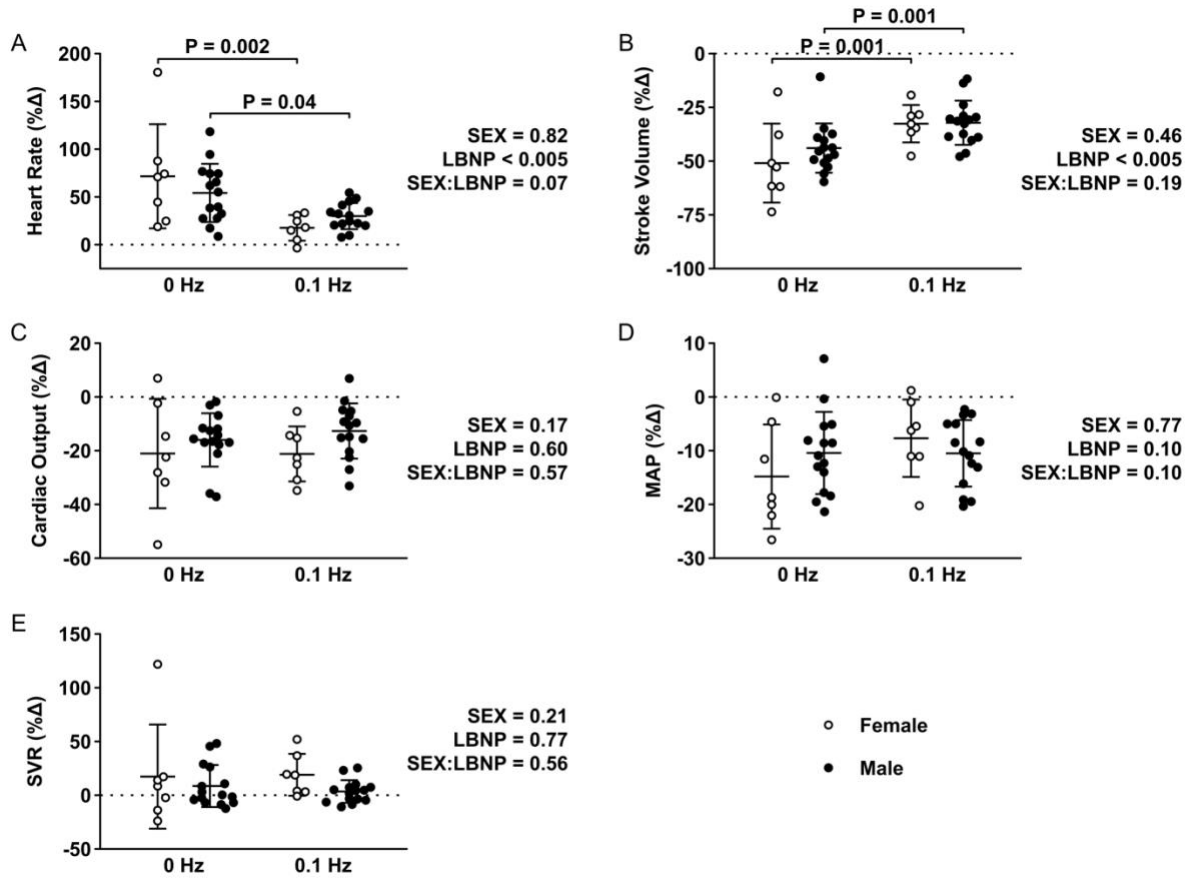

Figure 1. Systemic cardiovascular response to oscillatory LBNP between the sexes

MAP, mean arterial pressure; SVR, systemic vascular resistance; data were analyzed using a two-way linear mixed model. Post hoc comparisons between sexes at each time point and within sex across time points were determined *a priori* and corrected using the Holm correction. Data are presented as mean ± standard deviation. Exact p-values are presented in the figure.

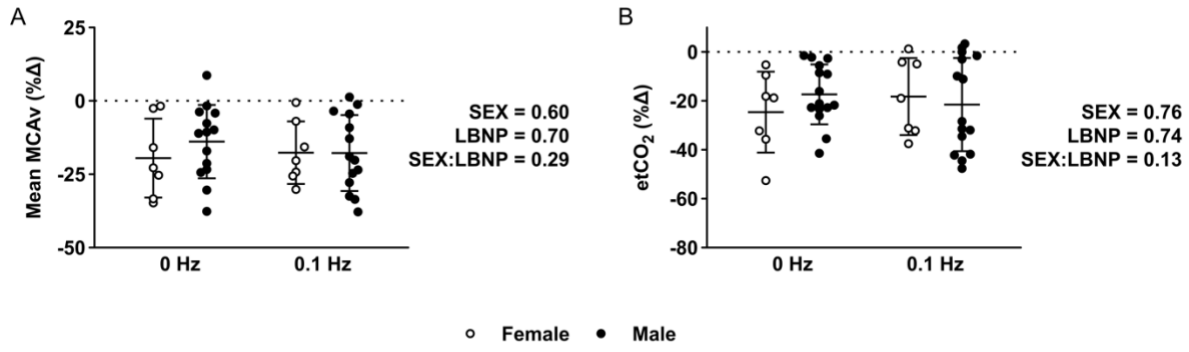

Figure 2. Middle cerebral artery velocity (MCAv) and end-tidal CO_2 responses to oscillatory LBNP between the sexes

Data were analyzed using a two-way linear mixed model. Post hoc comparisons between sexes at each time point and within sex across time points were determined *a priori* and corrected using the Holm correction. Data are presented as mean ± standard deviation. Exact p-values are presented in the figure.

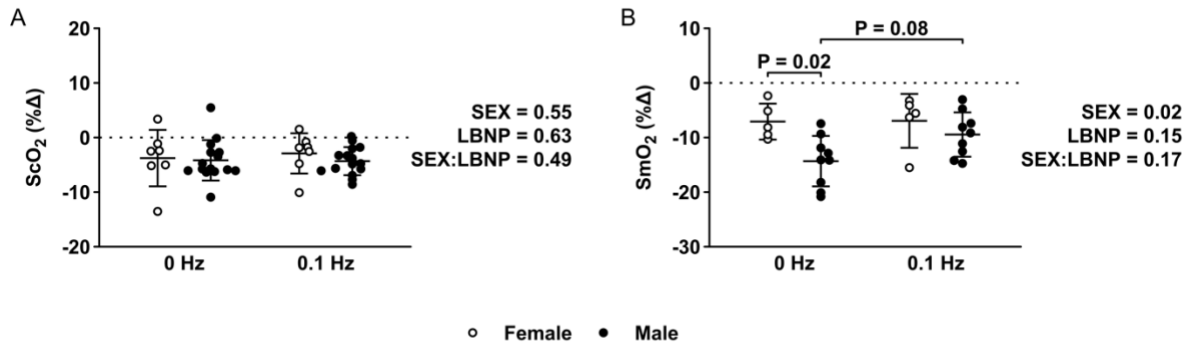

Figure 3. Tissue oxygenation responses to oscillatory LBNP between the sexes

ScO_2, cerebral tissue oxygenation; SmO_2, muscle tissue oxygenation; data were analyzed using a two-way linear mixed model. Post hoc comparisons between sexes at each time point and within sex across time points were determined *a priori* and corrected using the Holm correction. Data are presented as mean ± standard deviation. Exact p-values are presented in the figure.

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CHAPTER VI

The Effects of Sustained Hypoxia on Characteristics of Forced 0.1 Hz Oscillations in Arterial Pressure during Simulated Hemorrhage

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ABSTRACT

Forcing oscillations in arterial pressure and cerebral blood flow at 0.1 Hz during simulated hemorrhage protects cerebral oxygenation at both low and high altitude. Arterial pressure oscillations at 0.1 Hz are endogenously driven by rhythmic fluctuations in sympathetic nerve activity. As hypobaric hypoxia increases basal sympathetic activity, we hypothesized that the

amplitude of forced oscillations in arterial pressure and cerebral blood flow during simulated hemorrhage would be greater at high altitude compared to low altitude. Eight healthy human participants (4 female, 4 male) underwent a hypovolemic oscillatory lower body negative pressure (OLBNP) protocol, where chamber pressure reduced to -60 mmHg then oscillated every 5-s between -30 mmHg and -90 mmHg over 10-min (0.1 Hz). This protocol was performed at both low altitude (LA; Calgary, Alberta, Canada; 1045 m) and high altitude (HA; White Mountain, California, USA; 3800 m). Mean arterial pressure (MAP), mean middle cerebral artery velocity (MCAv), and cerebral tissue oxygen saturation (ScO_2) were recorded continuously. Frequency analysis (via continuous wavelet transform) was used to quantify oscillations in MAP and mean MCAv at ~0.1 Hz. Baseline amplitude of oscillations were greater at HA than LA for MAP (1.9 ± 0.6 mmHg vs. 1.2 ± 0.5 mmHg; P = 0.005) but not for mean MCAv (0.9 ± 0.4 cm/s vs. 1.1 ± 0.3 cm/s; P = 0.34). Oscillatory amplitudes increased with 0.1 Hz OLBNP and altitude for MAP (ANOVA main effect, OLBNP: P < 0.001, Altitude: P = 0.007) and mean MCAv (ANOVA main effect, OLBNP: P = 0.002, Altitude: P = 0.008). Amplitude of oscillations during OLBNP were greater at HA vs. LA for both MAP (4.0 ± 2.1 mmHg vs. 2.6 ± 1.4 mmHg, P = 0.05) and mean MCAv (2.4 ± 1.1 cm/s vs. 0.9 ± 0.4 cm/s; P = 0.01). The relative (%Δ) decrease in ScO_2 was not different between HA and LA (-0.63 ± 0.92 % vs. -2.56 ± 2.61 %, P = 0.11). Oscillatory amplitudes at 0.1 Hz in both MAP and mean MCAv increased during OLBNP at high altitude. This effect may be due, in part, to the sympathoexcitatory stimulus of hypobaric hypoxia. However, the greater amplitudes of oscillations did not alter the protection of cerebral tissue oxygenation in this environment.

INTRODUCTION

Most deaths due to trauma occur prior to a patient reaching the hospital (1), and a large proportion of these deaths occur due to hemorrhage (2). Accordingly, detection and treatment of hemorrhagic injuries is highly time sensitive. Access to definitive healthcare is challenged when trauma occurs in extreme environments; for example, about 75% of communities in Alaska are not connected to a hospital via roadway (3). Current recommendations for wilderness and environmental trauma follow military guidelines developed in the Afghanistan and Iraq wars, and recommend the treatment of massive hemorrhage first (4, 5). This is important because many outdoor recreational activities (such as skiing, mountaineering, riding snow mobiles) involve a higher risk of injury, including hemorrhage, and are often compounded by environmental factors like high altitude.

Baseline physiological status and reflex responses are affected by environmental factors such as high altitude, where oxygen delivery to the vital organs can be challenged. Acute exposure to high altitude (hypobaric hypoxia) initially causes an increase in ventilation to compensate for the reduced partial pressure of oxygen (6). This is accompanied by increases in sympathetic activity, heart rate, and cardiac output to ensure adequate oxygen delivery to the tissues (7). Plasma volume decreases over the course of a few days (8, 9), which increases hematocrit and subsequently arterial oxygen content, but also leads to an increase in blood viscosity. The increases in sympathetic activity, heart rate and cardiac output could potentially be beneficial in the face of hemorrhage at high altitude as these responses match the reflex responses to hemorrhage. However, the decreased plasma volume (and therefore circulating blood volume) coupled with the decrease in oxygen saturation during acute altitude exposure

could exacerbate the decrease in tissue oxygenation during hemorrhage, and decrease the chances of survival. For example, in a study of acute hypoxia exposure (48 h of simulated altitude at 4000 m) followed by hemorrhage in rats, survival was decreased compared to a control group not exposed to hypoxia (10).

In addition to the cardiovascular compensatory responses, another possible compensatory mechanism to tissue hypoxia is increased oscillatory arterial pressure and cerebral blood flow. Hemodynamic oscillations around 0.1 Hz have been implicated in higher tolerance to simulated hemorrhage (via application of lower body negative pressure) (11). We have also previously demonstrated that forcing oscillations in mean arterial pressure and cerebral blood flow at 0.1 Hz during simulated hemorrhage improves tolerance (12), and protects cerebral tissue oxygenation at both low and high altitude (12, 13). This effect may be due to cyclical increases in red blood cell velocity and hematocrit in the microcirculation (14) which could then maintain functional capillary density (15) and improve tissue oxygenation (16, 17).

With acute exposure to hypobaric hypoxia, oscillations in mean arterial pressure and cerebral blood flow around 0.1 Hz increase at rest (18, 19). High altitude ascent also increased oscillations at this frequency in human skin microcirculation (measured via laser Doppler flux) (20). Hypoxia has previously been shown to increase oscillations in the microcirculation (21). For example, Bertuglia et al. subjected hamsters to hypoxic gas mixtures of 8, 11, and 15% O_2 and measured the effect on arteriolar diameter oscillations (22). Hypoxia increased the amplitude of these oscillations in all three hypoxic conditions. Increases in hemodynamic oscillations may be a result of increased sympathetic activity which is known to increase with exposure to hypoxia (23). This is because sympathetic neural activity is a known mechanism for inducing arterial pressure oscillations at around 0.1 Hz in humans (24). It should be noted, however, that

increases in oscillations around 0.1 Hz in arterial pressure and cerebral blood flow have not always been observed with exposure to high altitude (25, 26).

In our laboratory, we are exploring the use of forcing arterial pressure and cerebral blood flow oscillations as a potential therapy for protecting tissue oxygenation. Given the combination of physiological responses to high altitude (increased sympathetic activity, decreased plasma volume and decreased arterial oxygen saturation), comparing the effectiveness of forcing hemodynamic oscillations at low and high altitude is important to aid in this therapeutic development. As exposure to the hypobaric hypoxia of high altitude increases basal sympathetic activity, we hypothesized that the amplitude of forced oscillations in arterial pressure and cerebral blood flow during simulated hemorrhage will be greater at high altitude compared to low altitude. Also given the importance of implementing timely therapeutics to treat hemorrhage, we explored how quickly forcing oscillations (via application of oscillatory lower body negative pressure, LBNP) translated to physiological oscillations in arterial pressure and cerebral blood flow between low and high-altitude conditions. As sympathetic activity is increased at high altitude and arterial pressure oscillations at 0.1 Hz are caused by sympathetic activity, we hypothesized that oscillations in arterial pressure and cerebral blood flow would occur more quickly at high altitude compared to low altitude.

METHODS

This study was approved by the Mount Royal University Human Research Ethics Board (Protocol 101879), the University of Calgary Conjoint Health Research Ethics Board (Protocol REB18-0374), and the University of North Texas Health Science Center (Protocol 2019-110). The study was conducted in accordance with the Canadian Government Tri-Council Policy on

research with human participants, consistent with the Declaration of Helsinki, except for registration in a database. This study was a part of a large high altitude research expedition conducted in Calgary, Alberta, Canada, and the Barcoft Laboratory on White Mountain in the Sierra Nevada Mountains, CA, USA in August 2019. Some results from this expedition have been published elsewhere (13, 27). However, the specific questions addressed for the current study are novel and have not been published.

Participants

Participants were informed about the overall design and purpose of the study, including any risks, and they gave written informed consent prior to participation. Baseline demographics including age, height, weight, and sex were documented. Female participants took a urine pregnancy test prior to each experimental session to ensure they were not pregnant. Controlling menstrual cycle phase for each experiment was not possible due to the logistics of a research expedition, but cycle phase was recorded. All female participants were using some form of hormone medication (N=1 daily estrogen/progesterone pill, N=3 intrauterine device).

Instrumentation

Participants were positioned supine into a LBNP chamber. After aligning the participant's waist with the opening of the chamber, heavy-duty plastic attached to the chamber, and a neoprene waist band were used to create an air-tight seal. ECG electrodes were then placed in a lead II configuration (shielded leads, cable and amplifier; AD Instruments, Bella Vista, NSW, Australia) for continuous monitoring, and arterial pressure and stroke volume were measured via finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam,

The Netherlands). Aortic diameter was measured using a 5 MHz curvilinear ultrasound probe (Terason uSmart 3300, Teratech, Burlington, MA, USA) to correct the ModelFlow® estimates of stroke volume prior to the beginning of data collection.

Middle cerebral artery velocity (MCAv) was monitored via transcranial Doppler ultrasound using a 2 MHz probe over the temporal window (ST3; Spencer Technologies, Seattle, WA, USA). Respiratory rate and end-tidal CO_2 ($EtCO_2$) were measured from respiratory gases using an oral-nasal cannula connected to a gas analyzer (ML206 Gas Analyzer; AD Instruments). Internal carotid Artery (ICA) diameter and velocity were monitored using duplex Doppler ultrasound (Terason uSmart 3300, Teratech, Burlington, MA, USA) with a 15 MHz linear array probe (15L4 Smart Mark™). Recordings of the ICA were captured via screen recording software (Camtasia, Techsmith Corp, MI, USA) and stored as AVI files for offline analysis. Cerebral tissue oxygen saturation (ScO_2) was monitored continuously using near-infrared spectroscopy (OxiplexTS; ISS, Champaign-Urbana, IL, USA) with the probe placed over the right frontal lobe. Efforts were made to measure MCAv and ScO_2 on the same side for each participant.

Experimental Design

Participants lived at or below 1045 m, and had not been exposed to high altitude stress for at least 6 months before the study. Each participant completed the same experimental conditions at low altitude (1045 m; Calgary, Canada) and at high-altitude (3800 m; Barcroft Laboratory, White Mountain, California, USA). Travel to high altitude consisted of flying from Calgary, Alberta, Canada to Las Vegas, NV, USA (610 m) and, less than 12 hours later, driving to the Barcroft Laboratory within 6 hours. Following a 4-day acclimatization period, participants were tested on days 5-7. After a 5-min baseline period, participants were exposed to an

oscillatory LBNP profile. Chamber pressure was decreased over 30-sec until -60 mmHg, then held at this pressure for a further 30-s. Thereafter, chamber pressure was manually oscillated between -30 mmHg and -90 mmHg with 5-s at each pressure for 9-min, or until onset of presyncopal signs or symptoms. Presyncope was defined as a reduction in systolic arterial pressure below 80 mmHg, or the onset of presyncopal symptoms such as nausea, light-headedness, dizziness, or visual disturbances such as grey out. After chamber pressure was released, there was a 10-min recovery period.

Data Analysis

Continuous data for ECG, arterial pressure, MCAv, EtCO2, and ScO_2 were recorded at 1000 Hz (PowerLab/LabChart; AD Instruments). R-wave detection for the binning and calculation of cardiovascular signals was performed using specialized software (WinCPRS; Absolute Aliens, Turku, Finland). Beat-to-beat signals of heart rate, mean arterial pressure (MAP) and mean MCAv were extracted and subsequently imported into MATLAB (Mathworks, Inc., Natick, MA). These signals were then re-sampled at 10 Hz with cubic spline interpolation followed by high pass filtering with a fifth-order Butterworth filter and a cut-off frequency of 0.005 Hz, and a low pass fifth-order Butterworth filter with a cut-off frequency of 0.8 Hz. The continuous wavelet transform was calculated from these signals using the cwt() function from MATLAB with the Morlet wavelet. The magnitude of oscillations was extracted at 0.1 Hz. Baseline averages for time and frequency domain signals were calculated over the final 3-min of baseline. LBNP measures for time domain signals were averaged over the last 1-min of LBNP, and frequency domain signals were averaged over the last 3-min of LBNP (both designated as "Late LBNP"). In order to evaluate the timing of induction of arterial pressure and cerebral blood

flow oscillations, 1-min averages of oscillatory amplitude for MAP and mean MCAv were calculated from the continuous wavelet transform output throughout the LBNP protocol up until minute 8; this was the last common time point for all participants. Changes from baseline were calculated as the difference between baseline and LBNP values divided by baseline values and multiplied by 100.

ICA velocity and diameter were analyzed continuously using automated wall-tracking software (28). Care was taken to ensure the matching of anatomical location for repeated measures, and a minimum of 10 cardiac cycles was used to calculate mean values (29). ICA flow was subsequently calculated using the following equation: $\pi \cdot \left(\frac{Diameter}{2}\right)^2 \cdot \left(\frac{peak\ velocity}{2}\right) \cdot 60$. Cardiac output was calculated by multiplying heart rate by stroke volume, and systemic vascular resistance was calculated by dividing MAP by cardiac output. MCA conductance and ICA conductance were calculated by dividing mean MCAv or ICA flow by MAP.

Statistical Analysis

Two-factor linear mixed models for repeated measures were used to analyze all outcome variables over time and between altitudes. Post-hoc tests were performed on pre-determined comparisons of interest (differences from baseline within time, and between altitude at each time point) and corrected using Holm's correction. Baseline hemodynamic data, and relative changes from baseline between high and low altitude conditions were analyzed using paired t-tests. All analyses were conducted in R. Unless otherwise stated, all data are presented as mean ± standard deviation (SD), and exact P values are reported for all comparisons.

RESULTS

Baseline Hemodynamic Status

Eight participants (4 M, 4 F; 30 ± 8 y; 173 ± 14 cm; 73 ± 14 kg) underwent oscillatory LBNP at low and high altitude. Exposure to high altitude increased heart rate, and ICA velocity, ICA diameter, and ICA flow at rest (Table 1). A small increase in respiratory rate was also associated with a decrease in $EtCO_2$ (Table 1). Baseline amplitude of 0.1 Hz oscillations in MAP was also higher at high altitude (Table 1). There was no difference in any other resting parameter between altitude conditions.

Responses to oscillatory LBNP

When comparing the amplitude of oscillations during 0.1 Hz LBNP, oscillations for MAP increased from baseline during LBNP under both altitude conditions, but were higher at high altitude than low altitude (Figure 1A). However, the relative change from baseline in amplitude of MAP oscillations was similar between high and low altitude (P=0.38, Figure 1B). The amplitude of MCAv 0.1 Hz oscillations only increased at high altitude (Figure 1C), and there was a greater relative change from baseline at high altitude vs. low altitude (P=0.09; Figure 1D).

Figure 2 shows the amplitude of 0.1 Hz oscillations in MAP and mean MCAv at baseline, and across the first 8 minutes of OLBNP for both altitude conditions. MAP oscillations increased above baseline at minute 2 of OLBNP at low altitude, and minute 3 at high altitude (Figure 2A). MCAv oscillations did not increase above baseline with OLBNP at low altitude but did demonstrate a sustained increase from minute 6 of OLBNP at high altitude (Figure 2B).

Heart rate, as expected, was higher at high altitude and exhibited a greater response to OLBNP at high altitude compared to low altitude (Table 2). This greater heart rate response was

associated with greater decreases in arterial pressure and stroke volume observed during OLBNP at high altitude (Table 2); cardiac output followed a similar pattern of response. Interestingly, mean MCAv and MCAv conductance were similar at rest between altitudes, and decreased by a similar magnitude in response to OLBNP (Table 2). ICA flow and conductance were higher at rest with high altitude, but did not statistically differ as they decreased during LBNP at both altitudes (both P=0.14 for % Δ from baseline; Table 2). This resulted in an overall effect of LBNP (time factor) on ScO_2, however, the absolute and relative responses between high and low altitude did not differ (Table 2). $EtCO_2$ was lower at high altitude but decreased by a similar magnitude during LBNP for both altitude conditions (Table 2).

DISCUSSION

In this study, we tested the hypotheses that 1) the amplitude of forced 0.1 Hz oscillations in arterial pressure and cerebral blood flow during simulated hemorrhage would be greater at high altitude compared to low altitude, and 2) oscillations in arterial pressure would occur sooner at high altitude compared to low altitude. The main findings from this study include: 1) baseline amplitude of 0.1 Hz MAP oscillations, but not MCAv oscillations, increased with altitude; 2) the relative increase in MAP oscillations during 0.1 Hz OLBNP was similar for both altitudes, but MCAv oscillations only increased at high altitude, and; 3) the onset of the increase in oscillations from baseline generated by 0.1 Hz LBNP was relatively similar for MAP at low and high altitude, but only increased for MCAv at high altitude and late into the LBNP protocol.

The increase in baseline amplitude of MAP 0.1 Hz oscillations is consistent with previous reports from acute normobaric hypoxia studies. For example, Iwasaki et al. exposed 15 human participants to a step-wise hypoxia protocol of 21%, 19%, 17%, and 15% inspired O_2 (10-min at

each step) (18). The amplitude of MAP oscillations around 0.1 Hz increased from baseline with 17% and 15% inspired O_2. Similar to our study, however, there was no increase in the amplitude of 0.1 Hz MCAv oscillations. For comparison, arterial oxygen saturation was about 93% at the 15% O_2 step in the Iwasaki et al. study and 89% in our study participants at high altitude (13, 18). Subudhi et al. exposed participants to a more extreme hypoxic gas stimulus of 12% inspired O_2, and reported an increase in the amplitude of 0.1 Hz oscillations for both MAP and mean MCAv (19).

Our findings, and these reports utilizing acute normobaric hypoxia, are in contrast with two studies examining oscillatory hemodynamics during high altitude ascent. Ainslie et al. reported no increase in oscillatory amplitude around 0.1 Hz for either MAP or MCAv during a high-altitude ascent from 1,400 m to 5,400 m (measured 1-2 days after arrival) (25). It should be noted, however, that with an acute hypoxic stimulus (4-5 min of 12% and 10% O_2) in these same participants (also reported by these authors), no increase in MAP or MCAv oscillations was observed either. This may indicate that this group of participants had a reduced oscillatory hemodynamic response to hypoxia, which may be related to suppressed sympathoexcitation. Similarly, Smirl et al. reported no statistical increase in oscillatory amplitude around 0.1 Hz for either MAP or MCAv on day 2 or week 2 following ascent from sea level to 5050 m (26); although there was doubling of the average oscillatory amplitudes in both MAP and MCAv. It may be that high variability in these responses led to a lack of statistical differences. In this same study, squat-to-stand maneuvers were utilized to drive arterial pressure oscillations and cerebral blood flow oscillations at 0.1 Hz for both sea level and high-altitude conditions. The amplitudes of oscillations in MAP and MCAv at 0.1 Hz during this maneuver were similar at sea level and day 2 of high altitude, but decreased compared to sea level at week 2 of high altitude. This

contrasts with our study where high altitude increased the amplitude of forced oscillations in MAP and MCAv at 0.1 Hz. While speculative, this could be because of the added stress of hypovolemia during oscillatory LBNP protocol used in our study. The reduced plasma volume at high altitude, in addition to the central hypovolemia induced with LBNP, may have augmented the sympathetic response, leading to an increase in the amplitude of arterial pressure oscillations compared with the squat-stand test.

Despite the lack of increase in "macrocirculatory" oscillations of MAP and mean MCAv in these previous studies, it is still possible that microcirculatory oscillations existed, similar to those reported by Salvi et al. in response to high altitude ascent (20). These investigators demonstrated increases in oscillations around 0.1 Hz in the skin microcirculation, measured via laser Doppler flux, within the 24 h of reaching 5050 m (ascent from 1350 m to 3400 m in 2 days, and from 3400 m to 5050 m in 5 days). Taken together, it is possible that the amplitude of 0.1 Hz hemodynamic oscillations is related to the magnitude of the hypoxic stimulus, and the degree of physiological compensation that has occurred with acclimatization to chronic hypoxia, such as with ascent to high altitude.

From a therapeutic standpoint, inducing hemodynamic oscillations at 0.1 Hz during simulated hemorrhage has been shown to protect cerebral tissue oxygenation at both low and high altitudes (12, 13). In this study, the relative increase in amplitude of 0.1 Hz MAP oscillations was similar between low and high altitudes (Figure 1), despite the higher magnitude of baseline 0.1 Hz MAP oscillations at high altitude. The increase of baseline oscillations in MAP is likely related to an increase in sympathetic activity from high altitude exposure (23, 30), and the influence of sympathetic activity on arterial pressure oscillations (24). Interestingly, MCAv 0.1 Hz oscillations did not increase with 0.1 Hz LBNP at low altitude, and only increased

late in the 0.1 Hz LBNP protocol at high altitude. This could be due to a buffering effect by cerebral autoregulation at low altitude, which could subsequently be "impaired" at high altitude (19, 31). However, studies are mixed on whether high altitude impairs cerebral autoregulation (19, 25, 31) or not (26). It is possible that if there is a reduced buffering capacity for cerebral autoregulation at high altitude, this may be compensatory so as to allow cerebral blood flow oscillations to protect the cerebral tissues from hypoxia (i.e., to improve oxygenation). Ultimately, cerebral tissue oxygenation responses were similar at low and high altitude. This occurred despite the added physiological challenges presented by high-altitude exposure such as decreased plasma volume, increased blood viscosity, and decreased oxygen saturation. In our earlier study, we compared the cerebral tissue oxygenation responses to simulated hemorrhage (via LBNP) with or without oscillations at high altitude, and observed a protection in cerebral tissue oxygenation with oscillations in arterial pressure and cerebral blood flow (13). Our current finding that the protection of cerebral tissue oxygenation was of a similar magnitude between low and high altitudes, highlights that forcing hemodynamic oscillations may be an effective potential therapy for treating conditions of cerebral hypoperfusion in a variety of environments.

When considering the timing of these forced 0.1 Hz oscillations, the amplitude of MAP oscillations increased early in the LBNP protocol at both high and low altitude, while the amplitude of MCAv oscillations only increased late into the LBNP protocol, and only at high altitude. Therapeutically, forcing these hemodynamic oscillations may be more beneficial if they occur quickly following initiation of the oscillatory stimulus. Given the similar timing of the response for oscillations in MAP, forcing oscillations at high or low altitude seems to be equally effective. It is possible, however, that a faster response could be elicited by increasing the magnitude of the oscillatory stimulus, such as increasing the amplitude of oscillatory LBNP.

Alternative techniques could also be used to induce oscillatory arterial pressure and cerebral blood flow, such as pneumatic leg cuffs (32). Changing the magnitude of oscillations induced via variations in leg cuff pressures and timing, could also increase the response of the subsequent hemodynamic oscillations. The total hemodynamic response, however, will also rely on the interplay with other physiological mechanisms, such as increased heart rate, sympathetic activity, and increased cerebral blood flow. For example, in our current study, an increase in MCAv oscillations at 0.1 Hz was only observed when heart rate and sympathetic activity (although not directly measured) were most elevated and cerebral blood flow was reduced at the end of LBNP during the high-altitude condition.

Methodological Considerations

There are some important methodological considerations regarding the study design. First, due to the nature of scheduling experiments for a research expedition, we could not control for menstrual cycle phase for our female participants. However, all female participants were using a form of hormonal medication (N=1 daily estrogen/progesterone pill, N=3 intrauterine device), with only 1 female participant reporting menstruation during the high-altitude condition. Additionally, participants using intrauterine devices reported either complete cessation of menstruation, or lengthy time intervals between menstruation. These factors could indicate relatively stable concentrations of estrogen and progesterone for the two experiments, although this is speculative as we did not measure circulating hormone concentrations at the time of this study. A second consideration for this expedition study is the relatively small number of participants. However, this concern is somewhat mitigated with the repeated measures design of the study which requires a smaller number of participants compared with two separate groups of

individuals. Finally, technical difficulties resulted in the loss of ICA flow data for three participants. However, the ICA flow responses were similar to the MCAv responses (N=6), and the repeated measures design also allows for greater confidence in our results.

Conclusions

Resting amplitudes of 0.1 Hz oscillatory arterial pressure increased with high altitude ascent, most likely due to the increase in sympathetic activity. Forcing arterial pressure oscillations and cerebral blood flow (only at high altitude) at 0.1 Hz during simulated hemorrhage resulted in a similar cerebral oxygenation response between high and low altitudes. Oscillations in cerebral blood flow may have only been present at high altitude due to reduced cerebral autoregulation. The timing of the generation of arterial pressure oscillations did not differ between high and low altitude. This indicates that forcing oscillations in arterial pressure and blood flow could be a quick and effective therapy for protecting cerebral tissue oxygenation at both low and high altitudes.

Table 1. Baseline hemodynamic status between low and high altitude

	Low Altitude	High Altitude	P-value
Heart Rate (bpm)	65.8 ± 11.5	79.0 ± 10.2	0.003
Stroke Volume (ml)	91.4 ± 15.1	82.8 ± 22.9	0.25
MAP (mmHg)	94.0 ± 7.0	95.4 ± 11.5	0.66
Cardiac Output (ml/min)	6.0 ± 1.2	6.5 ± 1.6	0.31
SVR (mmHg·min·ml^{-1})	16.2 ± 2.7	16.0 ± 6.3	0.89
Mean MCAv (cm/s)	58.8 ± 8.6	60.0 ± 11.5	0.62
MCAv Conductance (cm·s^{-1}·mmHg^{-1})	0.62 ± 0.11	0.62 ± 0.14	0.84
ICA Velocity (cm/s)	33.5 ± 8.1	40.1 ± 6.0	0.07
ICA Diameter (cm)	0.56 ± 0.12	0.57 ± 0.12	0.003
ICA Blood Flow (ml/min)	234.7 ± 49.2	310.8 ± 105.8	0.07
ICA Conductance (ml·min^{-1}·mmHg^{-1})	2.5 ± 0.6	3.4 ± 1.2	0.10
$EtCO_2$ (mmHg)	35.5 ± 2.3	27.4 ± 1.7	< 0.001
Respiration Rate (breaths/min)	13.1 ± 3.4	14.8 ± 4.1	0.08
ScO_2 (%)	66.5 ± 2.9	66.5 ± 4.0	0.98
Cerebral THC (μM)	46.0 ± 8.0	48.8 ± 8.2	0.48
Cerebral Deoxy Hb (μM)	15.6 ± 3.0	16.3 ± 2.2	0.43
Cerebral Oxy Hb (μM)	30.4 ± 5.6	32.5 ± 6.8	0.50
MAP power at 0.1 Hz (mmHg)	1.2 ± 0.5	1.9 ± 0.6	0.005
MCAv power at 0.1 Hz (cm/s)	0.93 ± 0.35	1.14 ± 0.32	0.34

MAP, mean arterial pressure; SVR, systemic vascular resistance; MCAv, middle cerebral artery velocity; ICA, internal carotid artery; $EtCO_2$, end-tidal CO_2; ScO_2, cerebral tissue oxygenation; THC, total hemoglobin concentration; Hb, hemoglobin. All data are presented as mean ± standard deviation and were compared using paired t-tests.

Table 2. Time domain responses to OLBNP at low altitude (LA) and high altitude (HA)

		Time Point		ANOVA P-values				
	Altitude	**Baseline**	**Late LBNP**	**Altitude**	**Time**	**Altitude*Time**	**%Δ**	**P-value**
Heart Rate (bpm)	LA	65.8 ± 11.5	79.3 ± 18.0*				20.6 ± 18.3	
	HA	79.0 ± 10.2†	106.4 ± 19.8*†	< 0.001	< 0.001	0.07	35.4 ± 25.5	0.08
Stroke Volume (ml)	LA	91.4 ± 15.1	63.2 ± 20.2*				-31.7 ± 12.3	
	HA	82.8 ± 22.9	48.2 ± 23.7*†	0.01	< 0.001	0.40	-44.2 ± 13.4	0.002
MAP (mmHg)	LA	94.0 ± 7.0	89.1 ± 7.6				-5.2 ± 5.7	
	HA	95.4 ± 11.5	84.2 ± 6.4*	0.34	< 0.001	0.10	-11.1 ± 7.3	0.09
Cardiac Output (ml/min)	LA	6.0 ± 1.2	4.9 ± 1.4*				-18.4 ± 15.1	
	HA	6.5 ± 1.6	4.7 ± 1.4*	0.54	< 0.001	0.31	-27.0 ± 10.6	0.08
SVR ($mmHg \cdot min \cdot ml^{-1}$)	LA	16.2 ± 2.7	19.4 ± 5.0				19.1 ± 19.4	
	HA	16.0 ± 6.3	19.6 ± 7.4	0.99	0.02	0.86	23.1 ± 10.9	0.40
Mean MCAv (cm/s)	LA	58.8 ± 8.6	45.5 ± 12.0*				-23.0 ± 12.1	
	HA	60.0 ± 11.5	44.2 ± 12.6*	0.97	< 0.001	0.56	-26.5 ± 13.8	0.39
MCA Conductance ($cm \cdot s^{-1} \cdot mmHg^{-1}$)	LA	0.62 ± 0.11	0.51 ± 0.16*				-17.9 ± 12.8	
	HA	0.62 ± 0.14	0.53 ± 0.18*	0.65	< 0.001	0.83	-16.2 ± 14.3	0.80
ICA Velocity (cm/s)	LA	33.5 ± 8.1	30.6 ± 4.9				-4.6 ± 26.5	
	HA	40.1 ± 6.0†	31.9 ± 6.1*	0.04	0.01	0.14	-20.7 ± 3.6	0.28
ICA Diameter (cm)	LA	0.56 ± 0.12	0.54 ± 0.12				-2.8 ± 2.3	
	HA	0.57 ± 0.12	0.55 ± 0.14*	0.04	0.002	0.45	-4.8 ± 4.5	0.39
ICA Flow (ml/min)	LA	234.7 ± 49.2	219.4 ± 103.0				-9.8 ± 25.9	
	HA	310.8 ± 105.8†	226.1 ± 87.7*	0.02	0.01	0.04	-28.3 ± 4.2	0.14

ICA Conductance ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	LA	2.5 ± 0.6	2.6 ± 1.2				-3.0 ± 27.7	
	HA	3.4 ± 1.2†	2.7 ± 1.1	0.04	0.15	0.12	-20.4 ± 10.0	0.14
$EtCO_2$ (mmHg)	LA	35.5 ± 2.3	25.3 ± 6.5*				-28.6 ± 17.8	
	HA	27.4 ± 1.7†	21.1 ± 3.5*†	< 0.001	< 0.001	0.10	-23.3 ± 9.3	0.22
Respiration Rate (breaths/min)	LA	13.1 ± 3.4	12.1 ± 3.0				-6.6 ± 10.6	
	HA	14.8 ± 4.1	15.6 ± 6.4†	0.01	0.92	0.31	4.3 ± 22.2	0.18
ScO_2 (%)	LA	66.5 ± 2.9	64.8 ± 2.9				-2.6 ± 2.6	
	HA	66.5 ± 4.0	66.1 ± 3.9	0.24	0.07	0.26	-0.6 ± 0.9	0.11
Cerebral THC (µM)	LA	46.0 ± 8.0	46.0 ± 6.0				0.9 ± 7.2	
	HA	48.8 ± 8.2	46.8 ± 8.7	0.47	0.69	0.68	-4.0 ± 8.9	0.13
Cerebral Deoxy Hb (µM)	LA	15.6 ± 3.0	16.5 ± 2.7				6.3 ± 6.8	
	HA	16.3 ± 2.2	15.7 ± 2.1	0.91	0.79	0.25	-3.0 ± 9.3	0.05
Cerebral Oxy Hb (µM)	LA	30.4 ± 5.6	29.5 ± 3.7				-1.7 ± 8.5	
	HA	32.5 ± 6.8	31.1 ± 7.2	0.33	0.54	0.87	-4.5 ± 8.9	0.35

MAP, mean arterial pressure; SVR, systemic vascular resistance; MCAv, middle cerebral artery velocity; ICA, internal carotid artery; EtCO2, end-tidal CO_2; ScO_2, cerebral tissue oxygenation; THC, total hemoglobin concentration; Hb, hemoglobin. All data are presented as mean ± standard deviation. A two-factor linear mixed model was used to compare absolute data with Holm-corrected post hoc tests. Percent changes from baseline were compared using paired t-tests. *P ≤ 0.08 vs baseline, †P ≤ 0.08 vs low altitude.

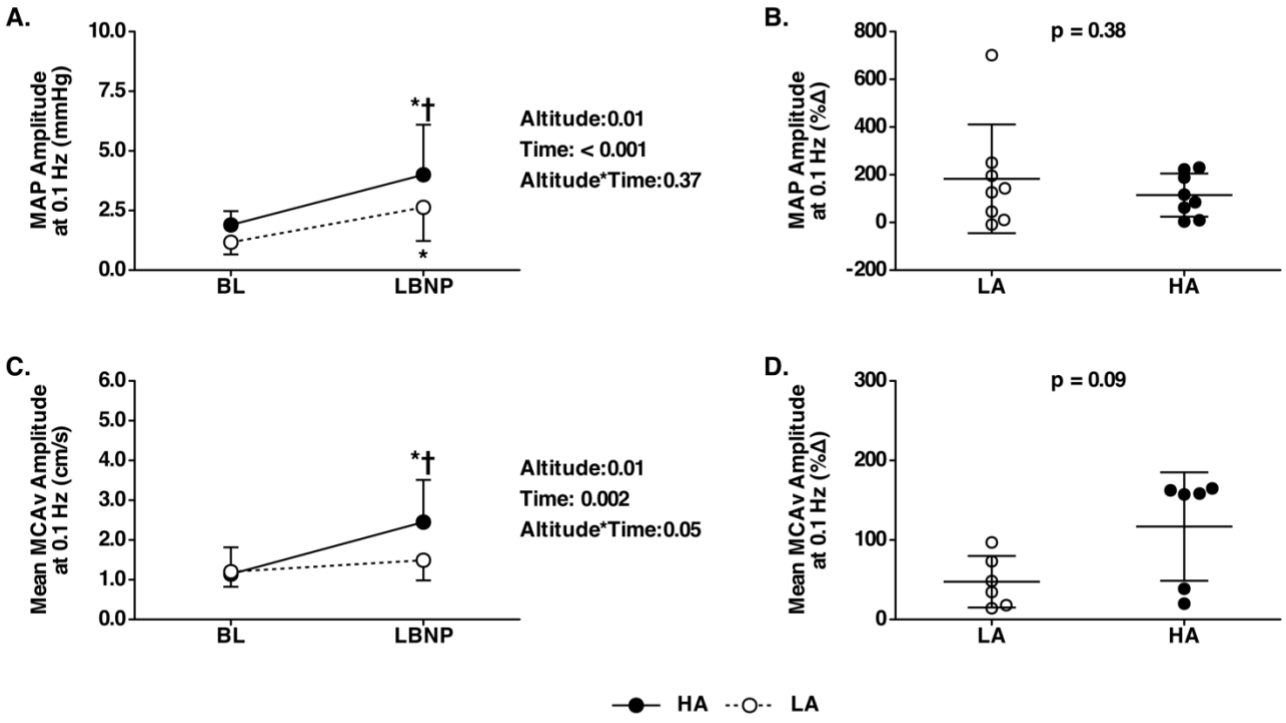

Figure 1. Amplitude of hemodynamic oscillations at high (HA) and low altitude (LA) in response to oscillatory LBNP.

The absolute response in MAP (Panel A) and MCAv (Panel C) were analyzed using a linear mixed model for repeated measures with Holm-corrected post-hoc tests. Post-hoc testing was limited to comparisons between baseline and each time point within altitude during OLBNP, and between altitudes at each time point. The relative response from baseline for both MAP (Panel B) and MCAv (Panel D) were analyzed using paired t-tests. Data are presented as mean ± standard deviation. * $P \leq 0.02$ vs. baseline values within altitude condition; † $P \leq 0.02$ vs low altitude within time.

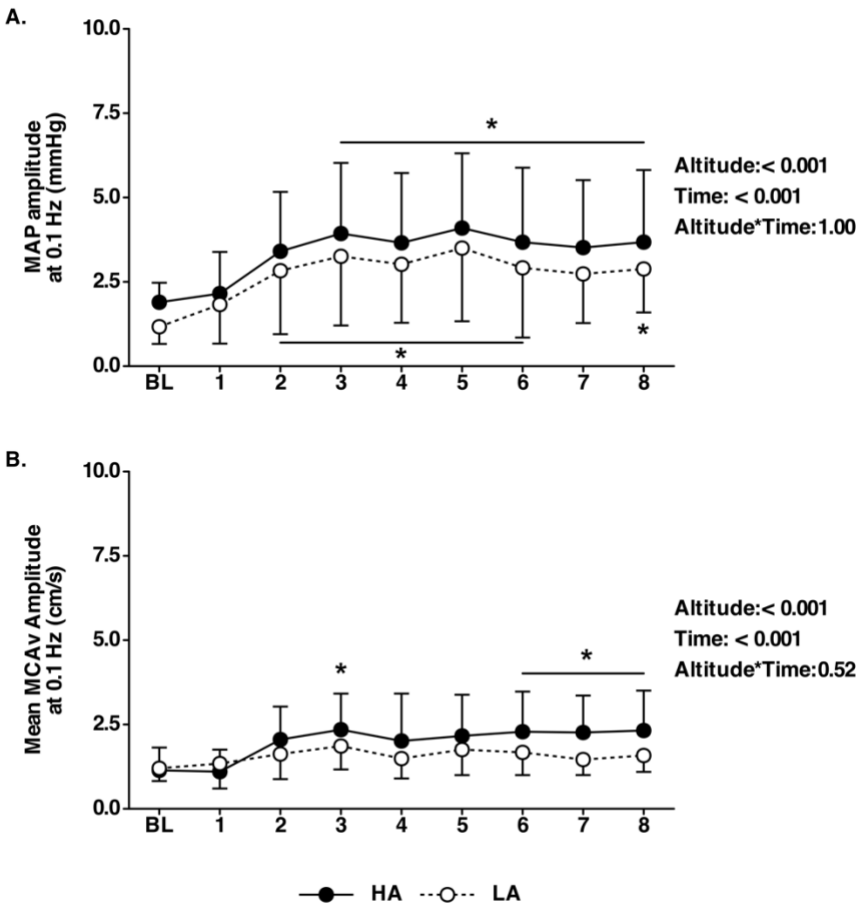

Figure 2. Amplitude of hemodynamic oscillations over time.

The amplitude of oscillations at 0.1 Hz for both mean arterial pressure (MAP; panel A) and middle cerebral artery velocity (MCAv; panel B) during each minute of oscillatory lower body negative pressure at high (HA) and low (LA) altitude. Data are presented as mean ± standard deviation. Data were analyzed using a linear mixed model for repeated measures with Holm-corrected post-hoc tests. Post-hoc testing was limited to comparisons between baseline and each time point within altitude during OLBNP, and between altitudes at each time point. * $P \leq 0.07$ vs. baseline values within altitude condition.

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CHAPTER VII

DISCUSSION

The overall aim of this dissertation was to characterize the physiological role and potential therapeutic applications of hemodynamic oscillations. The goal of Specific Aim 1 was to determine whether the characteristics of endogenous low frequency hemodynamic oscillations influences tolerance to simulated hemorrhage. The goal of Specific Aim 2 was to assess the effect of forced hemodynamic oscillations during simulated hemorrhage on cerebral blood flow and tissue oxygenation, with consideration of arterial blood gases and sex.

Specific Aim 1

When using the continuous wavelet transform, we observed that greater increases in the magnitude of low frequency oscillations in arterial pressure and cerebral blood flow were related to greater tolerance to the central hypovolemia induced by LBNP, but the relative time profiles for when the maximum magnitude of oscillations occurred was not related to tolerance. These findings suggest that development of therapies which can effectively increase low frequency oscillations in arterial pressure or blood flow of an individual could potentially aid increasing tolerance to hypovolemic conditions such as blood loss.

Specific Aim 2

The four studies that addressed Specific Aim 2 of this dissertation focused on the role of arterial blood gases and biological sex on the physiological responses to forced hemodynamic oscillations.

In the first study at high altitude, cerebral tissue oxygen saturation was protected with forced hemodynamic oscillations during the combination of simulated hemorrhage and hypobaric hypoxia. This protection was not due to increases in cerebral blood flow (indexed by both MCAv or ICA blood flow), or oxygen delivery to the brain. These findings highlight the need to explore the microvascular responses to forcing hemodynamic oscillations, as research in this area may be able to better elucidate the mechanisms responsible for the protection of tissue oxygenation.

In order to account for the decrease in cerebral blood flow caused by hypocapnia with our model of simulated hemorrhage (i.e., LBNP), we utilized end-tidal forcing as a technique to clamp $etCO_2$ and then assess the effect of forcing hemodynamic oscillations in arterial pressure on the cerebral blood flow and tissue oxygenation response. Surprisingly, clamping of $etCO_2$ prevented any decrease in cerebral blood flow or tissue oxygenation with either oscillatory or non-oscillatory LBNP. However, forcing hemodynamic oscillations during simulated hemorrhage protected stroke volume and mean arterial pressure, both of which may have contributed to the improvement in tolerance observed in this study. These results do highlight a potential use of hypercapnia as a therapy for preserving cerebral blood flow and tissue oxygenation under conditions of reduced cerebral perfusion.

In exploring the role of biological sex on the responses to forced hemodynamic oscillations during simulated hemorrhage, systemic cardiovascular and cerebral tissue

oxygenation responses were similar between the sexes. There was, however, a difference in the muscle tissue oxygenation response, with males exhibiting a decrease in oxygenation without forced oscillations, and a subsequent attenuation in this decrease with forced hemodynamic oscillations. Females did not exhibit a decrease in muscle tissue oxygenation in either condition. This finding highlights the potential protective effects of female sex hormones, such as estrogen, to such stresses as hemorrhage. It also highlights that certain benefits of forced hemodynamic oscillations may be sex specific.

Lastly, in the final study of this dissertation, I sought to assess the impact of high altitude on the response of forcing hemodynamic oscillations at 0.1 Hz. High altitude exposure resulted in baseline increases in arterial pressure oscillations, and arterial pressure oscillations increased during LBNP at both low and high altitude. Increases in MCAv oscillations only occurred at high altitude, but cerebral tissue oxygenation responded similarly at both altitudes. We also assessed the timing of generating oscillations in arterial pressure and cerebral blood flow between altitude conditions. Increases in arterial pressure oscillations occurred at similar times during LBNP for both altitudes, while cerebral blood flow oscillations only increased at high altitude, and only towards the end of the protocol. Together, these data indicate that forcing arterial pressure oscillations is equally protective at low or high altitude despite the increased physiological stress associated with hypobaric hypoxia.

Limitations

Several limitations for these studies should be addressed. For Specific Aim 1, participants were removed from the analysis if they were consistently breathing below 10 breaths per minute. This was necessary to remove the influence of breathing from the generation of low frequency

oscillations in arterial pressure and blood flow. Participants were also removed from the analysis if single large breaths were detected, as these breaths created noise in the analysis due to the breath setting off a small chain of oscillations. While this approach limited the number of participants in the analysis, it allowed for isolating endogenous oscillations created by sympathetic activity. Future work in this area should seek to pace participants' breathing frequency outside of the low frequency range, but also to examine the role of slow breathing within the low frequency range.

For our studies conducted at high altitude, testing occurred on days 5-7 of altitude exposure, which does allow for some physiological compensation to occur. It is likely that if we had tested participants earlier in their altitude exposure, the effect of hypoxia could have greater. Despite this, however, cerebral tissue oxygenation was largely attenuated during LBNP with forced oscillations. Another component to these studies was a lack of control in regard to experimental timing. Experiments occurred through the course of the day, and female participants were not tested at a controlled stage of their menstrual cycle. Each participant did, however, undergo testing at the same time of day for each experiment. Also, all female participants were on hormonal medication (3 intrauterine devices, and 1 oral hormonal medication) and 3 of the 4 reported either complete cessation of menstruation, or lengthy time intervals between menstruation.

The number of participants was limited in the high-altitude studies (N=8) and the isocapnic study (N=11). However, both study designs consisted of repeated measures which allows for greater confidence in our interpretations. Also, multiple measures of cerebral blood flow responded with consistent patterns within a given condition. For the isocapnia study, the number of female participants was low (N=2). While the cardiovascular responses of the females

in these studies were consistent with the male participants, more research is needed to understand whether the cardiovascular response in females is similar to males with forced hemodynamic oscillations during simulated hemorrhage with eucapnia.

As another consideration, the data used to compare sex differences in the response to LBNP with or without forced oscillations was drawn from two different study designs. While both studies utilized oscillatory and non-oscillatory LBNP with similar measurements, in one study all three profiles were completed within the same experimental session, while in the other study, each profile was completed in separate sessions. Sympathetic activity did not decrease back to resting levels when all three profiles were conducted in the same session, which could have produced a priming effect for subsequent experiments.

Lastly, our studies have been largely focused on the role hemodynamic oscillations play in the protection of tissue oxygenation. Our measurement of tissue oxygenation is made using near-infrared spectroscopy (NIRS) which has important assumptions to explain. Because the NIRS technique is measuring oxygenated and deoxygenated hemoglobin, oxygenation of a tissue is calculated from its vascular space. The vasculature that it is measured from is also a mixed sample of oxygenated and deoxygenated hemoglobin comprised of 75% venous, 20% arterial and 5% capillary blood (1). This means that the measurement of tissue oxygenation will be affected by delivery of oxygen and tissue metabolism (i.e., oxygen extraction). For our studies, indices of cerebral blood flow did not differ between LBNP profiles which would indicate that any changes in oxygenation would be related to oxygen extraction. More invasive techniques to (such as electrode sensors) could be used in animal models to better elucidate the effects of hemodynamic oscillations on more direct measures tissue oxygenation.

Applications and Future Directions

Our studies demonstrate the potential effects of hemodynamic oscillations and lay a groundwork for further research. Particularly important is the ability to translate our findings into clinical scenarios. Much of the future research should be focused on this issue.

Potential Therapeutic Approaches & Clinical Applications

Many of the studies assessing the effects of hemodynamic oscillations have been observational and focused on the endogenous generation of these oscillations either at rest, or under various stimuli (e.g., blood removal, simulated hemorrhage, limb occlusion, orthostatic challenge). For those studies where oscillations have been experimentally induced, various methods have been employed, including oscillatory LBNP (see Chapters III-VI), paced breathing, or sympathetic nerve stimulation. However, to be employed clinically, simpler methods need to be developed for ease of use in patient populations (who may or may not be conscious).

A relatively simple mechanical method for entraining oscillations in arterial pressure and blood flow is rhythmic leg compression via pneumatic cuffs. Katsogridakis et al. used cyclically inflating and deflating pneumatic thigh cuffs (between 0-150 mmHg) to drive oscillations in arterial pressure and cerebral blood flow at 0.1 Hz in humans at rest (2). When compared to baseline resting conditions, this maneuver effectively doubled the amplitude of oscillations in arterial pressure and also increased cerebral blood velocity oscillations. Hockin et al. recently employed rhythmic leg compression via pneumatic cuffs in humans undergoing a head-up tilt protocol, which induces caudal shifts in blood volume and subsequent central hypovolemia (3).

Pneumatic cuffs placed around the calves were cyclically inflated and deflated at various pressures and frequencies. Using this technique, stroke volume was protected when the leg cuffs were repeatedly inflated for 4-s and deflated for 11-s (~0.07 Hz cycle) with cuff pressures between 0 and 60-100 mmHg. In a follow up study, this group also tested the efficacy of cyclically inflating and deflating leg cuffs during a combined head-up tilt and LBNP protocol to presyncope (4). Tolerance to this hypovolemic stress was increased with cyclical leg compressions (at the same rate of inflation and deflation as their prior study, and a cuff compression pressure of 60 mmHg), and was associated with an attenuated decrease in arterial pressure, stroke volume, and cerebral blood velocity. These results indicate that cyclical leg compression can be an effective measure to protect central blood volume during conditions of reduced tissue perfusion. Rhythmic leg compression at varying frequencies is also commonly employed in other therapeutic scenarios, such as cardiac rehabilitation (i.e., external counter pulsation) (5), and for treatment of tissue damage in athletes (6), making this approach a prime candidate as a mode of delivering hemodynamic oscillations to patients.

When considering these therapeutic approaches, their utility may be affected by the underlying physiology of the patient. Much of the research on hemodynamic oscillations in humans (both endogenous and forced) has been performed in young and healthy participants. While this demographic is certainly represented in some clinical conditions of hypoperfusion, such as traumatic hemorrhage (7), other conditions such as stroke, myocardial infarction, and Alzheimer's Disease, are more prevalent in older populations (8). Accordingly, understanding endogenous and forced hemodynamic oscillations in the aging individual is essential. Xing et al. measured hemodynamic variability at rest and during sit-stand maneuvers at 0.05 Hz in young (21-44 y), middle aged (45-64 y), and older (65-80 y) participants (9). When comparing systolic

and diastolic arterial pressure and cerebral blood velocity across these three groups, low frequency oscillations (0.05-0.15 Hz) were lower with increasing age for all measures at rest. In the very low frequency range (0.02-0.07 Hz), mean arterial pressure oscillations showed an effect with age (P=0.057); however, directionality of this response was less clear across the three age groups, and no effect of age was observed for cerebral blood velocity oscillations. When oscillations in arterial pressure and cerebral blood flow were driven with the repeated sit-stand maneuver at 0.05 Hz, oscillatory power was greater with increasing age. As discussed, current models show that vasomotion at this same frequency may play a crucial role in the drainage of excess interstitial fluid from the brain via the glymphatic system. These data demonstrate that forced oscillations may provide an effective therapeutic approach in older populations, despite suppression of endogenous oscillations at rest.

Cognitive disorders, such as Alzheimer's disease, may be accompanied by an underlying or contributing vascular component (10). Cerebral blood flow oscillations around 0.1 Hz in individuals with Alzheimer's disease are elevated in comparison with healthy controls at rest (11), which may represent impaired cerebral autoregulation and/or baroreflex control, but could also be a compensatory response to improve oxygenation and tissue fluid clearance as previously described. However, there is little research in this area specifically, with many contending that the vascular dysfunction associated with Alzheimer's disease impairs vasomotion (12). Our understanding of the role of oscillatory blood flow on ß-amyloid clearance (see Xie et al., (13)), paired with known vascular changes in Alzheimer's disease, suggest that vasomotion (and oscillations in blood flow) in cerebral arterioles could be an important factor in aiding clearance of ß-amyloid from the interstitial fluid (12). Designing therapeutics to increase hemodynamic

oscillations further may provide an effective method for clearance of ß-amyloid as a potential treatment for Alzheimer's disease.

Other potential applications for clinical use of forced hemodynamic oscillations could include many conditions where tissue perfusion and oxygenation are impaired, including hemorrhage (especially during the transportation phase), recovery from stroke and cardiac arrest, and sepsis. Oscillatory hemodynamic therapies may improve tissue perfusion and oxygenation and improve clinical outcomes. An important consideration for these clinical populations, is the state of the microvasculature, and the subsequent effect of hemodynamic oscillations of tissue perfusion and oxygenation. For example, if inflammation and oxidative stress damages the microvascular endothelium, this could increase vascular permeability (14), impairing solute exchange (including oxygen), and the proposed beneficial effects of forced hemodynamic oscillations. Theoretically, however, forcing blood flow oscillations may be an approach to overcome impaired diffusion and solute exchange, with cyclical increases in pressure and concentration gradients from the vasculature to the tissue. Further studies in this field are essential to understand the implications of microvascular damage on solute exchange in these conditions.

Conclusion

Oscillations in arterial pressure and blood flow occur spontaneously at frequencies below the rate of respiration. Accumulating evidence points to a role of these oscillations in both the protection of tissue perfusion and oxygenation. The protection of cerebral tissue oxygenation can be aided by the forcing of arterial pressure and blood flow during simulated hemorrhage. As such, potential therapeutics can be developed for conditions such as traumatic hemorrhage,

sepsis, and recovery from stroke or cardiac arrest or even for the treatment of Alzheimer's disease. Continued research into the physiological mechanisms and benefits of inducing hemodynamic oscillations will facilitate an understanding of their therapeutic potential.

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