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FOREHEAD LASER DOPPLER AND TRANSCRANIAL DOPPLER DURING SIMULATED HYPOVOLEMIA

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Medicine

by

Kathleen Samuels

2009

FOREHEAD LASER DOPPLER AND TRANSCRANIAL DOPPLER DURING SIMULATED HYPOVOLEMIA.

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The present study employed lower body negative pressure (LBNP), a rapidly titratable, safe and reversible means of inducing simulated hypovolemia, for a comparison of transcranial Doppler (TCD) ultrasound of the middle cerebral artery and laser Doppler (LD) flowmetry of the forehead microvasculature.

With IRB approval, 9 healthy volunteers (26.3±2.7 years) were monitored continuously with EKG, noninvasive finger arterial blood pressure (BP), and TCD positioned at the transtemporal window. After a baseline (Base) period, subjects underwent rapid onset of LBNP to -70 mmHg over the course of 1 minute, followed by progressive declines of ~10 mmHg until lightheadedness or had a BP decline >20% of baseline BP. Changes in the peak (systolic) and trough (diastolic) values with each heart beat were analyzed at Base, at approx. 30 seconds prior to the onset of lightheadedness (Presympt) and at onset of symptoms (Sympt).

In the 6 subjects who subsequently became lightheaded, forehead LD flow decreased by 10.9±11.7% at Presympt (p=NS for interphase difference). It then decreased by an additional 20.4±18.7% with the onset of lightheadedness (p=0.035 for Presympt vs. Sympt). Peak TCD readings decreased by 29.3±9.7% from Base to the time of the Presympt measurement (p=0.001); they then increased by 4.1±12.9% with the onset of Sympt (p=NS). In the 2 subjects who remained asymptomatic, LD did not change significantly in the Presympt and Sympt phases where Sympt was the time when the study was terminated because the BP cutoff was reached. In these asymptomatic subjects, the TCD flow velocity declined progressively.

The present findings suggest that monitoring of the microvasculature in the distribution of the carotid arteries provides a better indication of changes in perfusion associated with lightheadedness than measurement of velocity at the middle cerebral artery. The discordance between LD and TCD is consistent with autoregulatory mechanisms at the level of the forehead microvasculature that have previously been reported in the context of systemic administration of phenylephrine.

Acknowledgements

Thank you, Dr. Keith Ruskin for all of the time and energy you put into the completion of this thesis project. Your red ink made it a thousand times better. Thank you, Dr. David Silverman for including me in your project and working through the data completely and thoroughly. Thanks, Nick Galante, for showing me how to run the experiments. Dr. Awad El-Ashry, I would not have been able to do the data analysis without your help – thank you. Simon, thank you for supporting me throughout this long process.

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Introduction

The goal of the present study is to determine whether there is preservation of blood flow in the middle cerebral artery and in the microcirculation of the forehead during hypoperfusion produced by inducing simulated hypovolemia. Up to a point, the body is able to compensate for blood loss and maintain a normal mean arterial blood pressure and heart rate via local and systemic autoregulation of the vasculature. However, the presence of a normal systemic blood pressure during hemorrhage implies that vasoconstriction is depriving some organ or organ system of its blood supply.[1] Hypotension and tachycardia are late findings in hypovolemic shock and "do not mark the beginning of circulatory failure, but rather represent the beginning of decompensation."[1] Hypovolemic shock occurs due to a decrease in intravascular volume, which causes a decrease in preload. Decreased preload leads to diminished stroke volume (SV), and thus decreased cardiac output (CO). Systemic vascular resistance (SVR) is typically increased in the setting of hypovolemic shock, as the vasoconstriction acts to maintain blood flow to vital organs (brain, heart, etc.).

Central microcirculation is preserved during vasoconstrictive challenges using phenylephrine,[2-4] the cold pressor test[5] and nicotine. High-frequency oscillations were noted in blood flow through the forehead microvasculature during these vasoconstrictive challenges,[2-5] although the significance of these oscillations has yet to be determined. For comparison of the forehead microcirculation laser Doppler (LD) flow to transcranial Doppler (TCD) velocity in the middle cerebral artery, progressive lower body negative pressure (LBNP) is a good model to use for impending circulatory collapse. LBNP

induces physiological changes comparable to 70-degree head-up tilt,[6] passive standing[6] and controlled hemorrhage,[6] but has advantages over other experimental methods. Previous studies have shown that lower body negative pressure accurately simulates blood loss and produces hemodynamic and autonomic responses consistent with central hypovolemia.[6-17] Depending upon the speed of the change in lower body negative pressure, compensation for the central volume loss may be observed at varying levels of the circulation.[6, 15] Our focus was on a fast LBNP protocol and the cardiovascular and neural reflex responses seen as the body quickly compensates for an acute loss of central volume. Our group postulates that the oscillations previously seen in the forehead microcirculation[2-5] could be related to the autoregulation of cerebral blood flow, given the common neural and vascular innervations of the forehead and cerebral hemispheres via the internal and external carotid arteries.[18]

Background

Cerebral Circulation:

The Circle of Willis represents the communicating system of arterial blood flow to the brain. The two internal carotid arteries and two vertebral arteries deliver the main blood supply to the brain. The vertebral arteries converge to form the basilar artery. The six major vessels supplying blood to the cerebral cortex are the left and right anterior, middle and posterior cerebral arteries (See Figure 1).

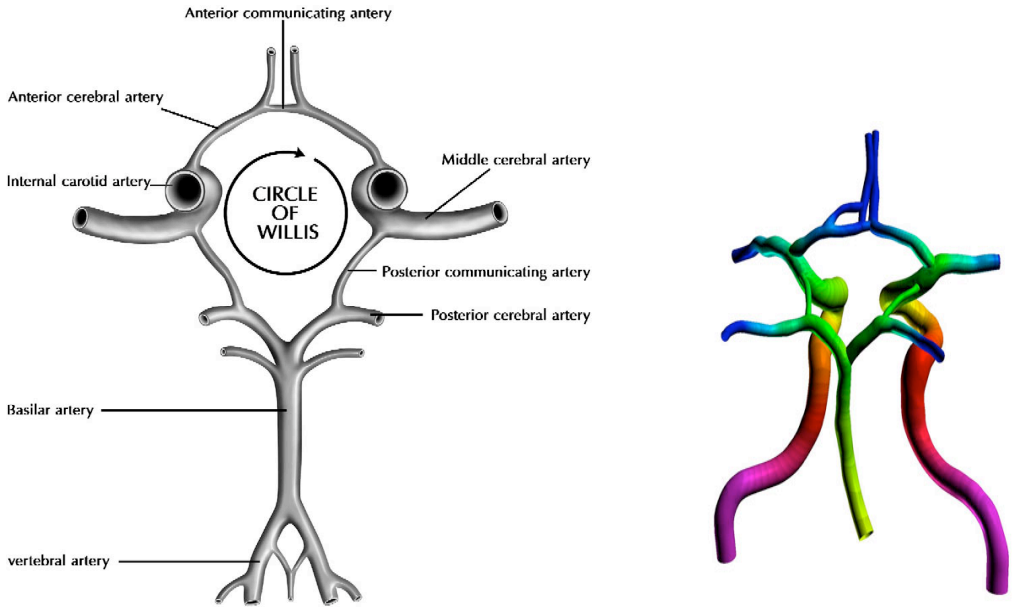

Figure 1. Circle of Willis. Left, two-dimensional representation of the communicating system of cerebral blood flow. Right, three-dimensional representation of the solution to the incompressible Navier-Stokes equations (INS3D) for flow through the Circle of Willis (Left, from Franco Folino, 2007; Right, from http://www.nas.nasa.gov/Resources/Applications/applications.html)

As intracranial arteries penetrate into the gray matter, they become progressively smaller and undergo characteristic changes (i.e. loss of smooth muscle) becoming arterioles and finally resulting in the formation of capillaries.[19] These capillaries have a tight endothelial layer (forming the blood-brain barrier) and are surrounded by pericytes and astrocytes.[19] Pericytes can directly induce changes in capillary diameter.[20]

Endothelium of both the systemic and cerebral vessels is said to have a complex and fundamental role in regulation of circulation and homeostasis of the local vessel.[21] Endothelium in cerebral vessels forms the blood-brain barrier via tight junctions, thus selectively prohibiting certain substances from entering the cerebral tissue. Cerebral vascular resistance is regulated on a local level via many substances produced by the endothelium or that act on the endothelium.[22, 23] Acetylcholine, adenosine diphosphate, adenosine triphosphate, and bradykinin are known to induce dilation of the cerebral vasculature via actions on the endothelium.[22, 23] These substances can cause dilation when they activate G-coupled protein receptors present on endothelium.[22, 23] They also lead to the production of prostanoids, endothelium-derived hyperpolarization factor, or nitric oxide, which induce vasodilation.[22, 23] Alternatively, vasoconstriction can be induced via other factors with effects on endothelium, including endothelin-1, thromboxane-A_2 and prostaglandin F2α.[19]

Additionally, the cerebral vascular bed is innervated by the autonomic nervous system (See Figure 2).[19, 24] Extracerebral arteries, located in the pia mater, are innervated by sympathetics originating in the superior cervical ganglion (SCG), parasympathetics

originating in the sphenopalatine (SPG) and otic ganglia (OG), and send sensory information via neurons directed toward the trigeminal ganglion (TG).[19, 25] When the extracerebral vessels enter the brain parenchyma, it is said that they lose this innervation.[19] The intracerebral vessels encounter neurons that are specific to the brain: subcortical neurons of the basal forebrain, raphe nucleus, thalamus, and locus coeruleus, and interneurons (See Figure 2).[19] Most of the perivascular nerves interact with the vessels via astrocytes that typically surround the vessels, rather than by direct contact.[19] These neurons can regulate the diameter of the microvessels by releasing neurotransmitters (acetylcholine, serotonin, norepinephrine, etc.) that interact directly with the smooth muscle or endothelium of the vessels, or with specific receptors on the astrocytes, and lead to vasoconstriction or vasodilation.[25]

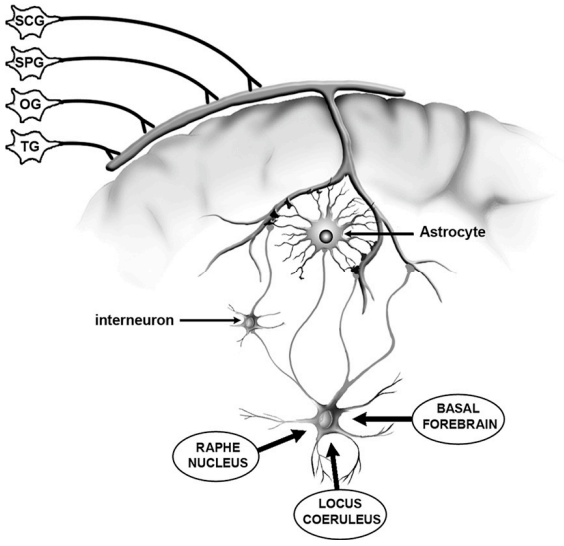

Figure 2. Innervation of extracerebral and intracerebral arteries. Arteries in the pia mater receive sympathetic innervation from the superior cervical ganglion (SCG), parasympathetic innervation from the sphenopalatine (SPG) and otic ganglion (OG), and sends sensory information to the trigeminal ganglion (TG).[19] Intracerebral arteries are innervated by subcortical neurons originating from the locus coeruleus, raphe nucleus, basal forebrain, and thalamus.[19] Nerve endings can directly contact a vessel or can contact interneurons or astrocyte bodies to affect the vessel.[19] (From Franco Folino, 2007)

Cerebral Autoregulation:

Cerebral Autoregulation (CA) is the ability of the cerebral vasculature to independently regulate and maintain cerebral blood flow despite changes in systemic arterial blood pressure, within a limited range of mean arterial pressures (MAP) – for example, 60-140mmHg.[19, 26] Cerebral arteries of all sizes can participate in CA, but principally arterioles less than 100 to 250 μm are involved.[19] Static autoregulation refers to steady-state changes in cerebral blood flow in response to progressive, gradual changes in blood pressure.[27] Dynamic autoregulation refers to the rapid modifications in cerebral blood flow in response to abrupt changes in arterial blood pressure, over the course of seconds.[27] Dynamic cerebral autoregulation may have different mechanisms of action than static autoregulation.[27]

Four pathways potentially mediate cerebral autoregulation: myogenic, metabolic, neurogenic,[28] and endothelial function.[21] Smooth muscle cells of the arterioles have an intrinsic ability to induce vasoconstriction in response to elevated cerebral blood pressure,[29] increasing the resistance and limiting the amount of pressure to which the cerebral tissue is exposed. Changes in arteriolar resistance occur in the setting of modifications of local or systemic metabolism,[30] including changes in concentration of oxygen and adenosine. The concentration of CO_2 also affects vessel diameter, with hypocapnia leading to vasoconstriction and hypercapnia leading to vasodilation.[31] Cerebral vessels are innervated by both sympathetic and parasympathetic nerves.[32] Sympathetic nerves are thought to be the principal autonomic neurogenic modifiers of cerebral blood flow.[33] The sympathetic nervous system acts via tonically active[34]

norepinephrine and neuropeptide Y secretion,[33] which leads to vasoconstriction. Despite potential vasodilator effects of brain vessels by the parasympathetic nervous system, via mediators such as vasoactive intestinal peptide, acetylcholine and nitric oxide, current literature states that the parasympathetic nervous system does not play a relevant role in cerebral autoregulation.[24] Neurally-mediated control of autoregulation may be more effective during dynamic than static autoregulation.[35] Endothelium influences vessel diameter as it releases mediators, such as nitric oxide, and as it modulates the three mechanisms just listed, inducing constriction or dilation.[21]

In response to changes in systemic blood pressure, the diameter of cerebral arteries change in order to maintain a constant cerebral blood flow.[19] Cerebral blood flow is directly proportional to the blood pressure and inversely proportional to the cerebral vascular resistance.[19] Total cerebral blood flow at rest is approximately 15 to 20% of total cardiac output, or about 800 mL/min.[27] Efficient autoregulation requires synchronized vasoactive mechanisms in the pial and intracerebral arteries.[27] Pial vasoconstriction limits the ability of the downstream intracerebral arteries to vasodilate.[27] If the intracerebral arteries vasodilate because of the direct effect of surrounding neurons, then this vasodilatory stimulus is propagated retrogradely to the pial arteries.[36]

Cerebral blood flow is dependent upon the pressure gradient across the cerebral vasculature[19]; the pressure gradient decreases as the arterial blood pressure is rapidly reduced,[37] thus the cerebral blood flow is also reduced. During acute changes in systemic blood pressure (BP), the cerebral blood flow should remain stable if dynamic CA is intact

(See Figure 3).[37] If there is a precipitous fall in BP, however, there can be a transient, acute drop in cerebral blood flow.[38] In this setting, when dynamic CA is intact the recovery of the cerebral blood flow occurs despite continually depressed BP[37] because of the reflex modulation of cerebrovascular resistance.[19] Impairment of CA, alternatively, leads to a continued fall in cerebral blood flow that follows the trend of MAP.[37] Prodromic symptoms occur when cerebral blood flow falls below 40 - 50% of baseline levels; loss of consciousness typically occurs after 8 to 10 seconds of interrupted cerebral blood flow.[27, 39]

Regardless of the underlying disease process in syncope, the ultimate cause of loss of consciousness is "insufficient cerebral perfusion, with a critical reduction in blood flow to the reticular activating system, the neuronal network of the brainstem responsible for maintaining consciousness."[19] Symptomatic orthostatic hypotension that is associated with decreased cerebral blood flow velocity (CBFV) may occur because of a transient reduction in perfusion pressure,[37] a paradoxical increase in cerebral vascular resistance elicited by sympathoexcitation or hypocapnia,[38, 40] or a combination of factors.[37] Occasionally, there is a paradoxical increase in cerebral vascular resistance in response to a decrease in systemic blood pressure, which leads to a critical reduction in cerebral blood flow and neurally mediated syncope.[19] Lower CBFV during orthostatic hypotension may be the physiological reason for symptoms, but the symptoms can occur despite the maintenance of cerebral autoregulation.[37]

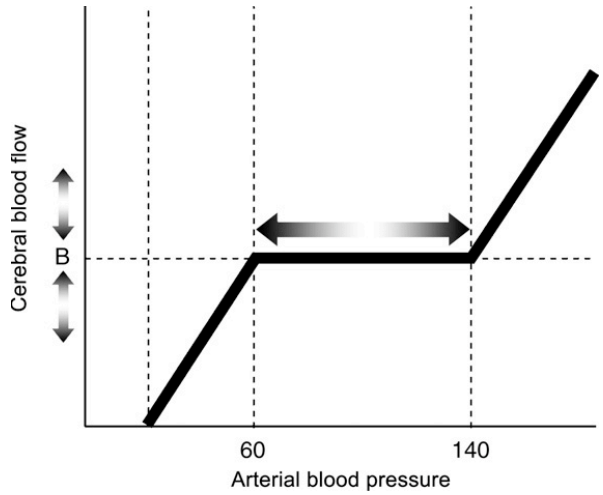

Figure 3. Flow-pressure curve of the cerebral circulation. Cerebral autoregulation maintains a constant cerebral blood flow through a range of normal mean arterial pressures (60-140 mmHg), despite changes in systemic blood pressure. When the MAP falls below 60 mmHg, cerebral perfusion decreases proportionally to MAP. When MAP exceeds 140 mmHg, cerebral perfusion increases proportionally to MAP. (From Franco Folino, 2007)

The flow-pressure curve of cerebral circulation is not fixed.[19] An individual's baseline arterial blood pressure and chronic diseases can induce a shift of the curve to the left or right in order to protect the cerebral tissue.[41] For example, hypertensive patients have a curve that is shifted to the right[41] (See Figure 4) because of static autoregulation. This right shift allows for constant cerebral blood flow, and avoidance of hyperperfusion and excessive pressures, at higher systemic blood pressures.[19] A right shift of the curve leaves a patient more susceptible to declines in cerebral blood flow (and potentially an ischemic event) if the systemic blood pressure falls below the lower limit of the range in which autoregulation can occur.[19] In patients with hypotension, static autoregulation leads to a left shift of the flow-pressure curve.[41] Hypotensive patients maintain a constant cerebral blood flow, despite lower than normal systemic blood pressures.[41] These hypotensive patients are at risk of damage to the cerebrovascular endothelium and disruption of the blood-brain barrier in the setting of hypertension.[42]

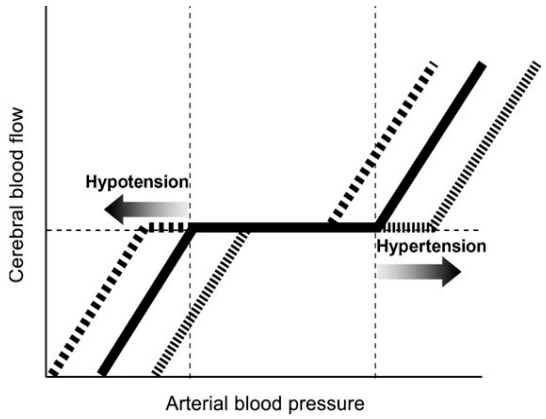

Figure 4. Flow-pressure curve of the cerebral circulation shifts in hypertensive and hypotensive patients. There is a right shift of the curve in patients with hypertension, because the static cerebral autoregulation acts to maintain cerebral blood flow at a constant level, despite higher systemic blood pressures. In hypotensive patients, the curve shifts to the left and static autoregulation acts to keep cerebral blood flow constant despite lower systemic blood pressures. (From Franco Folino, 2007)

In addition to hypertension and hypotension, there are many pathologic conditions that cause changes in CA.[19] In ischemic stroke, dynamic and static autoregulation are impaired.[43] Carotid artery stenosis causes impaired CA that is a marker for patients at higher risk for stroke,[44] and which is reversible upon endarterectomy or stenting.[45] Head injury,[46] focal ischemia[47] and subarachnoid hemorrhage[48] also affect CA. Aging, on the other hand, is associated with lower cerebral blood flow and velocity,[49] but is not associated with a decline in CA.[50]

Discovery of Oscillations in the Microvasculature

Heart Rate Variability:

Heart rate variability (HRV) was first noted in the 18th century when Stephen Hales carried out experiments that led to quantitative measurements of arterial blood pressure, which could then be compared to the respiratory cycle and beat-to-beat intervals.[51] Oscillations in signals (flow, velocity, electrical conduction, etc.) are also known to exist

within the cardiovascular system. The variation noticed in the beat-to-beat interval has been considered clinically normal but its importance was first emphasized in fetal monitoring.[52] Loss of fetal HRV is a sign of distress and is an indication for prompt delivery.[52]

Beat-to-beat variations represent the dynamic response that is occurring in the homeostatic mechanism when there are perturbations in the cardiovascular system.[52] The variations in arterial and venous blood pressure stem from changes in intrathoracic volume and pressure during the respiratory cycle and also from changes in the peripheral vascular resistance when local autoregulation of blood flow occurs at the tissue level.[52] These changes are sensed by a variety of chemoreceptors and baroreceptors[52] that in turn modify the heart rate, electrical conduction and contractility of the heart, and arterial and venous tone in order to maintain homeostasis.[52]

Spectral analysis breaks a signal into its component waveforms. The sinusoidal components of an electrocardiogram (ECG) can be described as very-low-frequency (VLF; <0.04 Hz), low-frequency (LF; 0.04-0.10 Hz) and high-frequency (HF; >0.10 Hz) spectral components. The heart rate power spectrum is regulated at each frequency by one or more control mechanisms that either induces or amplifies the oscillation. VLF oscillations are attributed to the sympathetic nervous system, thermoregulation and regulation by the renin-angiotensin system.[52-54] LF oscillations are mediated by both sympathetic and parasympathetic inputs, but also by baroreceptor reflexes.[52, 53] HF oscillations are attributed to the parasympathetic nervous system, but are also related to

respiratory rate.[52-54] The parasympathetic response time is much shorter than that of the renin-angiotensin or sympathetic nervous systems.[53] Cholinergic activity is known to be a predominant component of heart rate fluctuations. [55, 56]

The reason for the lack of sympathetic control at higher frequencies has been elucidated by Stauss, HM et al., with a series of experiments on rat vascular smooth muscle cells[53]. These experiments demonstrated that periodic depolarization was possible at up to 0.5 Hz when K+ was used, but that when phenylephrine was the depolarizing agent, the cells could not contract periodically any faster than 0.1 Hz; tonic contraction occurred at frequencies higher than 0.5 Hz and 0.1 Hz, respectively[53]. This implies that smooth muscle is physically capable of contracting more rapidly than 0.1 Hz, but the rate-limiting step is the sympathetic activation of the smooth muscle[53]. The slow adrenergic signal transduction system accounts for the sympathetic component being limited to VLF and LF oscillations[53].

VLF, LF, and HF oscillations have been discovered in the heart rate[52], blood pressure[55], and microvascular flow.[57] There is sympathetic involvement in the oscillatory control of the blood pressure and microcirculation[55, 57]. During times of cardiovascular stress, sympathetic control of HRV increases and the parasympathetic contribution is diminished[56]. As a person's ability to continue to mount the autonomic response diminishes, there is sympathetic withdrawal and parasympathetic tone predominates[56]. In the setting of trauma or hypovolemia, a high parasympathetic to sympathetic ratio in the HRV power spectral analysis is a predictor of impending death[56]. The sympathovagal

balance in the HRV has been used as an indicator to try to predict mortality in many clinical conditions.[56, 58]

Cold Pressor Test:

During the cold pressor test, the subject places his or her hand in ice water (4 degrees Celsius), producing reflex systemic vasoconstriction. In a study by Awad et al. in 2001, plethysmographic signals at the ear was not significantly changed while a significant decrease was seen in the finger signal.[5] The plethysmographic amplitude depends on systemic intravascular pulse pressure (ΔP) and the distensibility (D) of the vascular wall[5, 59]. This relationship is similar to that of blood volume pulsations (ΔV) seen during flowmetry, where $\Delta V = \Delta P \times D$. [5, 59] The finger plethysmographic amplitude is determined by the autonomic nervous system,[5, 60] because of the high density of adrenergic receptors at this site.

In the finger, the large sympathetic response to the cold pressor test is mediated by the a-adrenergic receptors that line the cutaneous vessel walls.[5] It is therefore postulated that the finger can provide valuable information about changes in sympathetic tone.[5] Whereas changes in the amplitude of the ear plethysmograph may more closely reflect changes in pulse pressure.[5] Awad et al., in 2001, noted that the changes in the plethysmographic signal and the blood pressure are correlated and postulated that there is a generalized synchronization of oscillations in the low frequency range in different areas of the body, i.e. the finger and the ear.[5, 61]

Laser Doppler:

Cutaneous LD flowmetry uses a noninvasive fiber optic probe to quantify the phase shift of laser light induced by moving red blood cells in the 10-60 capillaries under a 1-mm^2 area of skin.[2, 3] The laser Doppler probe must be affixed to a single site throughout a given study because movement and positioning of the probe will affect the results.[2] The probe can detect the concentration of moving red blood cells (CMBC) and red cell flux (CMBC times red blood cell velocity) in the arteriolar-capillary network at a depth of 500 to 700 nm below the surface of the skin.[2] CMBC is primarily sensitive to local changes in vessel caliber and hematocrit.[2] Red cell flux is more sensitive than CMBC to RBC velocity.[2] LD can identify a microsvascular response to a vasoconstrictive stimulus and, thus, provides monitoring of changes in cutaneous perfusion.[2]

Changes in cutaneous microvascular flow may be a surrogate marker for changes in perfusion of vital organs.[2] Importantly, atherosclerotic coronary arteries are sensitive to α-adrenoreceptor agonists, cold pressor testing and mental stress.[2] Toda et al. examined the patterns of blood flow and sympathetic nerve activity of vital organs during nonpulsatile cardiopulmonary bypass and noted a decrease in renal blood flow (with a concurrent increase in sympathetic nerve activity), but no change in blood flow to the heart or brain.[54, 62] The Toda group proposed a possible vagally-mediated mechanism by which certain critical organs maintain perfusion during increased sympathetic nervous system activity.[54, 62]

Prior to the availability of LD flowmetry, it was postulated that there is a process at the

microcirculatory level that maintains perfusion during arterial vasoconstriction.[3, 63] Discovery of nonadrenergic neural elements [64] and cholinergic receptor activity at the precapillary level,[65] acetylcholine modulation of perivascular adrenergic receptors[66] and of acetylcholine-induced release of endothelium-dependent vasodilatory factors,[67] led to the development of a theory that the parasympathetic nervous system was involved in the regulation of microvascular blood flow.[3]

The Effects of Phenylephrine on Forehead Laser Doppler:[2]

Microvascular smooth muscle cells oscillate out of synchrony with neighboring cell populations when not under extrinsic (autonomic) regulation.[3] Out of phase oscillations damp the laser Doppler signal, which is sensitive to the phase relationships of underlying capillaries.[3] Under neural control, oscillation of the capillaries of the same branching order become synchronized, resulting in a stronger LD signal.[3] Resistance of the vascular bed declines as more capillaries are recruited to synchronous oscillation, because the resistance of a vessel (or vascular bed) whose diameter varies sinusoidally is lower than that of a constant-caliber vessel with the same average diameter.[3, 68]

Almost 70 years ago, Hertzman and Dillon reported that there is a specific distribution of vasoconstriction within the body in response to a vasoconstrictive stimulus. [69] Regions innervated more densely by adrenergic fibers (i.e. finger) respond with decreased perfusion while more central regions (i.e. forearm, forehead) seem to have maintained blood flow during a vasoconstrictive challenge. [3, 69] While the parasympathetic nervous system has been shown in blood pressure and heart rate oscillations, the peripheral

vasculature has been labeled as devoid of parasympathetic input.[4]

In a study by Silverman et al., LD probes were attached to the palmar surface of the fourth finger and the volar surface of the forearm.[2] These two sites have distinct vascular anatomy, innervation, function and responsiveness to autonomically active substances.[2] Phenylephrine was infused at a rate of 0.4, 0.8 and 1.6 ug/kg/min for 10-minute intervals.[2] The finger LD showed a decrease in flux and CMBC during all of the infusions, while the forearm LD revealed a variable response to the infusions and achieved a statistically significant increase in flux and CMBC only during the 0.4 ug/kg/min infusion.[2] The decreased LD flow in the finger is consistent with the predicted results, given the rich concentration of α_1-adrenoreceptors and a vasoconstrictive stimulus (phenylephrine).[2] However, the apparent vasodilatory response at the forearm during the lowest rate of infusion of the α_1-receptor agonist, and variable flow during the higher rates of infusion, leads one to believe that a homeostatic response may be occurring, which allows for both vasoconstriction and vasodilation.[2] This vasodilation in the forearm, at the time of a decrease in heart rate, potentially represents vagally mediated baroreceptor activity and occurs even in the absence of a change in blood pressure. [2] This theory is supported by data from head-down tilt studies that show an increase in forearm blood flow with concurrent decrease in heart rate.[70] Additionally, there is evidence that phenylephrine can directly affect baroreceptors, even in the absence of a pressure change.[71, 72]

Cholinergic Oscillatory Control in the Forehead Microvasculature:

In an unpublished study by Silverman et al.•, the disparity in forehead and finger blood flow was monitored using laser Doppler flowmetry, during which subjects consumed nicotine lozenges to produce vasoconstriction. During this study, nine subjects were monitored and two of these subjects developed symptoms of lightheadedness and nausea. All nine of the subjects had signs of vasoconstriction and each one developed oscillations of the forehead laser Doppler signal in the high frequency range with exposure to the nicotine. In the two subjects who became lightheaded, the oscillations at 0.13 Hz +/-0.3 Hz disappeared immediately prior to the onset of the symptoms. These results provided supportive evidence for the idea that the oscillations in the microvasculature function in a protective or homeostatic manner.

In a subsequent study, Silverman et al. investigated the microvascular blood flow at the finger and forehead during systemic phenylephrine and atropine infusions.[3] During phenylephrine infusion, there was a decrease in blood flow to the finger and maintenance of flow at the forehead site of laser Doppler.[3] The autonomic activity noted in the spectral analysis of the finger during phenylephrine infusion was primarily in the low-frequency (sympathetic) range.[3] Alternatively, the primary peak in spectral power at the forehead was in the high-frequency (parasympathetic) range at 0.12-0.18 Hz,[3] consistent with cholinergic oscillatory control (termed COC_{VASC} by Silverman et al., 2001).[3] At the point in the study during which atropine was added to the phenylephrine infusion, the high-frequency oscillations in the forehead disappeared.[3] Because phenylephrine is known to

• Personal communication with investigators

activate a parasympathetic homeostatic response at the level of the heart, the investigators concluded that it could induce a parasympathetic homeostatic response at the level of the microcirculation.[3] Additionally, because atropine is an anti-cholinergic medication and it inhibited the oscillations in the microcirculation, a parasympathetic process may mediate these oscillations.[3]

Prior to the 2001 Silverman study, there was no discussion of parasympathetic involvement in the microvascular oscillations. The HF oscillations that have been documented to occur in the peripheral microvasculature had previously been attributed to mechanical transduction of respiration-related changes in heart rate and stroke volume.[55, 73] Acetylcholine played a role in thermal reflexes and "local functional hyperemia," [4, 65] but acetylcholine was not considered to be an important vasomotor neurotransmitter. [65] The prevailing theory since that time has been that the parasympathetic nervous system is not involved in regulating peripheral vascular resistance.[74] The autonomic innervation of the peripheral vasculature is believed to be almost entirely sympathetic,[55, 73, 75] which would lead to low frequency oscillations on spectral-domain analysis.

The Silverman et al. 2002 investigation[4] was designed to determine whether the oscillations in the microvasculature originate in the microcirculation or if they are the result of transmitted changes in the diameter of more proximal vessels. Previously, investigators have postulated that the high frequency oscillations in the peripheral microvasculature are due to mechanical transmission of oscillations from the heart rate, cardiac stroke volume and systemic blood pressure.[55, 73] Additionally, high frequency

microvascular oscillations in the have been associated with respiration in the same way that HRV is associated with respiration.[55, 73] There are direct drug effects on microvascular oscillatory activity and indirect baroreceptor-mediated reflexes at the level of the microvasculature.[75] The microvasculature has been considered "relatively passive," as it does not have a significant amount of adrenergic innervation.[55, 73, 75]

To further explore the nature of the oscillations in the forehead laser Doppler signal, Silverman et al. infused phenylephrine, followed by atropine, into 15 healthy volunteers[4]. This study showed that the most prominent oscillatory peak in the forehead LD signal was at 0.14 +/- 0.2 Hz and that this peak is atropine sensitive,[4] consistent with earlier work[3]. Spectral-domain analysis of the R-wave to R-wave interval (from the ECG) revealed an oscillatory peak at 0.2 Hz and this peak was also atropine sensitive[4] (consistent with previous HRV studies that show predominant cholinergic control of heart rate).[52] The respiratory frequency, systemic pressure and systemic flow also had the most power at 0.2 Hz and they did not have a peak at 0.14 Hz.[4] As expected, phenylephrine did not cause atropine-sensitive oscillations in the finger laser Doppler signal.[4] The forehead LD signal and R-wave to R-wave interval signal had distinct and non-overlapping predominant peaks at 0.14 and 0.20 Hz, respectively.[4] The changes in amplitude of the signals were not proportional and the signals oscillated out of synchrony from one another.[4] Additionally, the forehead LD signal did not have overlapping peaks with systemic blood flow or pressure in the power spectral analysis.[4] These discrepancies lead one to believe that there is no physical, down-stream relationship between the heart rate oscillations/blood pressure oscillations and the forehead microvascular oscillations.[4]

Furthermore, these data support the theory that the oscillations are parasympathetic in nature.[4]

Additionally, forehead LD probes placed at different sites on the forehead transmit signals from microvessels that were oscillating out of synchrony from neighboring vessels.[4] In a small study, a topical local anesthetic was applied to the forehead of four subjects 45 minutes prior to LD probe placement. Forehead oscillations in the microvasculature were eliminated by the local anesthetic.[4] These two additional investigations support the proposal that the oscillations in the microvasculature that occur during phenylephrine infusion are a local, peripheral phenomenon and are not the result of transmission from proximal sites.[4] The cholinergic oscillatory control of the microvasculature (COC_{VASC})[3] may function to maintain microvascular homeostasis.[4]

Synchronous Rhythmical Vasomotion in the Microvasculature During Nonpulsatile Cardiopulmonary Bypass:

Traditional understanding of microvascular oscillation has been that only low-frequency oscillations (0.04 – 0.10 Hz) originate from the microcirculation and that high-frequency oscillations (>0.12 Hz) are transmitted from changes in stroke volume caused by respiration or through mechanical transmission from more proximal vessels.[57] In 2002, Silverman *et al* used approximate entropy (ApEn) to determine whether oscillations arise in the absence of cardiac and respiratory activity, in the setting of nonpulsatile cardiopulmonary bypass (NP-CPB).[54] Approximate entropy is a tool that evaluates the overall complexity of a signal; as the oscillations become less chaotic and more regular,

the approximate entropy decreases.[54] NP-CPB was associated with the development of high-frequency forehead oscillations (0.13 +/- 0.03 Hz) and low-frequency finger oscillations (0.07 +/- 0.02 Hz) in the LD flow.[54] In addition to increased power in the high-frequency spectral domain analysis, as the NP-CPB proceeded, there was a decrease in approximate entropy.[54] The decrease in entropy was because of an increase in synchronization of oscillations in the microvasculature.[54]

The emergence of organized, high-frequency oscillations in the microvasculature during NP-CPB may have implications about the local, and even systemic, vasoregulatory mechanisms.[54] This potentially homeostatic mechanism occurs at the level of the microcirculation in the setting of a vasoconstrictive challenge and also during systemic depulsation and may protect the body's vital organs from ischemia.[54] HF oscillations have been demonstrated in numerous cholinergically-rich locations within the body, including the brain[76], viscera[77], and centrally located skin (head, trunk, proximal arm).[3, 4, 78, 79] HF oscillations could be induced with hyperventilation[78], arousal[79], cerebral vasoconstriction[76] and transient arterial occlusion.[80] Absence of HF oscillations is associated with poor outcome in diabetic wound healing.[81] It has therefore been proposed that HF oscillations could represent a cholinergically-mediated homeostatic mechanism that preserves blood flow during vasoconstrictive challenges.[54]

Study Design

Lower Body Negative Pressure (LBNP):

Studies involving the local application of reduced atmospheric pressure were first conducted in 1841 by Junod,[6] who used reduced pressure to draw blood away from diseased organs, cause a severe, localized hyperemia, and also to induce syncope.[82] This induced syncope was used to create a "satisfactory state for the conduct of surgery."[6] By the mid-1960's, LBNP was introduced to medical research by Stevens and Lamb.[15] LBNP is used to study orthostatic stress,[83] cardiovascular response to hypovolemia[11] and the ability of baroreceptor reflexes to change peripheral vascular resistance during simulated hemorrhage.[16] Blood pressure regulation[84] and the effects of physiologic and pharmacologic interventions on cardiovascular reflexes[12] can also be examined. LBNP has been used clinically to create dry operating fields during surgery[6, 15] and also to promote a foot-ward shift of blood after hip replacement.[85] LBNP has been used to reverse the orthostatic intolerance caused by spaceflight, bed rest and head-down tilt.[86]

LBNP can be used to simulate gravity during exercise in sub-gravity conditions and has also been employed to simulate partial gravity environments, like the Moon or Mars.[87] LBNP does not rely on the earth's gravitational field, and may therefore be useful for preventing complications of spaceflight.[6] Astronauts in spaceflight can use LBNP not only to reverse orthostatic intolerance, but also to prevent musculoskeletal deconditioning, neuromotor dysfunction and to maintain bone health.[88] LBNP is also used experimentally during vertical acceleration on high-performance aircraft.[8] There are a

multitude of other uses for LBNP that have been reviewed thoroughly in the articles by Goswami et al, 2008[15] and Wolthuis et al, 1974.[6] LBNP is useful for testing autonomic function[89] during simulated hypovolemia.

LBNP is used as a model for central hypovolemia because of its noninvasive, reproducible, and easily reversible nature.[6, 11, 15] During supine LBNP, a subject lies horizontally with his/her legs and pelvis inside of the LBNP chamber. A seal is created after which a vacuum pump decreases pressure inside the chamber while a barometer measures the internal pressure. The hemodynamic effects of LBNP are similar to those following $+G_z$ loading,[90] passive stand,[6] controlled hemorrhage[6] and 70 degree head-up tilt.[6] LBNP is less expensive than a human centrifuge, but remains an accurate way to study the effects of G loads on the cardiovascular system.[7, 91] Compared to passive standing, LBNP provides a better model for central hypovolemia because it allows for quantification of approximate blood "loss" or pooling, and there are progressive stages of severity. Large volume controlled hemorrhage is no longer performed in unanaesthetized humans for ethical reasons, but there are sentinel papers that review outcomes from these types of experiments.[92] 70-degree head-up tilt and LBNP both induce central hypovolemia, blood pooling and decreased cardiac output. However, 70-degree head-up tilt and LBNP produce different physiological responses to the challenges and LBNP provides a more controllable, progressive environment.

The body responds to LBNP with similar cardiovascular and neurohumoral reflexes as during acute hemorrhage because the mechanism by which LBNP acts leads to a similar

decrease in venous blood return, preload, stroke volume and cardiac output.[11] LBNP causes reproducible physiologic changes, including specific hemodynamic and neurohormonal reflexes[93] in response to induced hypotension.[6, 11, 15] Changing the level of negative pressure adjusts the degree of hypotension.[6, 11, 15] LBNP reduces thoracic blood volume, decreases central venous pressure, diminishes stroke volume and cardiac output, and increases peripheral vascular resistance while causing blood to pool.[11, 94] LBNP decreases preload, resulting in decreased stroke volume via the Frank-Sterling mechanism.[11] Depending on the magnitude of the pressure used and the speed of the progression of application of the LBNP, the physiologic responses to LBNP will be different.[6, 15, 95] During –30 to –50 mmHg of LBNP 0.5-1.0 liter of blood is pooled in the lower body.[6] With higher pressures more blood tends to pool, with an estimate of 1.5L pooling with the use of –80 mmHg LBNP.[96]

There are three distinct phases of the hemodynamic response to acute central hypovolemia.[11] Phase I involves the maintenance of arterial blood pressure despite falling cardiac output, mediated by baroreceptor-reflex generated vasoconstriction and sustained tachycardia.[92] Phase II occurs when the cardiac output falls below a critical value (50-60% of resting cardiac output or 30% of blood volume) and vasoconstriction fails and is marked by bradycardia and hypotension.[92] Phase II is associated with fainting. During phase II there is increased parasympathetic and decreased sympathetic activity.[11] In severe hemorrhage, a third phase can occur, during which tachycardia reappears and is accompanied by inability to maintain microvascular circulation.[97-99] This third phase is associated with ischemia, cellular dysfunction and death.[97-99] These three stages of

hemorrhagic shock are also described in subjects undergoing LBNP.[100]

The greater the amount of LBNP applied to a subject, the greater the amount of pooling of blood in the lower extremities.[11] Also, with a larger amount of LBNP, there will be a more substantial decline in a subject's central venous pressure.[11] The CVP generally declines by 3 mmHg at -10 mmHg LBNP and by 7 mmHg at -60 mmHg LBNP, with a 1 mmHg decline in CVP per -10mmHg progression in LBNP.[11] However, the degree of hypotension and lower extremity blood pooling experienced by each subject will vary at a given LBNP based on the subject's individual response to the challenge.[101]

The duration and speed of onset of negative pressure, as well as the size of the decrease, affect the physiological response of a given subject to LBNP.[102] LBNP that is applied for a short amount of time and is advanced quickly will facilitate study of the sympathetic and parasympathetic circulatory reflexes in response to acute central hypovolemia.[103] Brief exposure to LBNP of less than 15 minutes total[104] will not cause hormonal compensation to the hypovolemia[105] and will allow for a study that manipulates only the neural and baroreflex systems.[15] Most of the cardiovascular effects due to changes of the LBNP occur in the first 3 minutes after the change.[106] Longer exposures to LBNP, of 20 minutes or more, lead to more complex neurohormonal responses.[95] Low pressure (10 mmHg) LBNP for 60 minutes leads to a decrease in central, splanchnic and forearm blood flow,[107] while the mean arterial pressure and pulse pressure remain unchanged. The change in blood flow without a concurrent change in blood pressure is thought to be secondary to sympathetic[108] and renin-aldosterone system[90] activation.

When LBNP is adjusted in increments – steps of a varying pressure after a set amount of time – there is a pressure-dependent change in the neural and hormonal (specifically, renal) systems,[109] cardiac preload reduction,[110] vasoconstriction and tachycardia.[111] Graded LBNP which is gradually and continuously increased, rather than in steps, can reduce CVP in a stepwise fashion, which enhances baroreceptor sensitivity.[112] Graded LBNP has been used to study the integrity of vasomotor reflexes.[90] Using a moderate to strong level of LBNP with a "ramp" of the pressure (rapid, graded increase), allows for the study of cardiovascular and autonomic responses to moderate to severe stress.[15]

Using the LBNP model for impending cardiovascular collapse, it is possible to safely induce progressive decreases in cerebral perfusion to the point of presyncope during which subjects feel lightheaded, dizzy, sweaty, or nauseous. The cardiovascular response to this physiologic challenge consists of reflexes that attempt to maintain arterial pressure and cerebral perfusion.[113] When these compensatory reflexes are overwhelmed, circulatory collapse occurs leading to decreased perfusion of the brain with associated loss of consciousness.[6] At the end of the study, the blood that has pooled in the lower body will be returned rapidly as the suction is decreased. This restored blood volume leads to resolution of symptoms and normal circulatory hemostasis.[6]

Transcranial Doppler (TCD) Ultrasound:

When a wave front strikes a moving object, it is reflected back towards its source, but its frequency is shifted. The change, or shift, in the frequency between the incident and

reflected waves is known as the Doppler effect. The Doppler frequency is within the audible range. Mathematically, this can be represented: $F_d = f_e - f_o$, where F_d is the Doppler shift, f_e is the echo (reflected wave) and f_o is the incident wave (with a known frequency). According to the Doppler equation for a reflector, the velocity of the moving target can be calculated with the equation:

$$v = \frac{c * f_o}{2 * f_o * \cos \Theta}$$

where *v* is the velocity of the moving target, and c is the speed of the incident wave. The target moves in the direction of the probe at an angle of Θ, the Doppler angle.

In 1982, the first transcranial Doppler (TCD) was introduced and allowed non-invasive assessment of intracranial hemodynamics.[114] The low-frequency (2-4 Mhz) Doppler probe measures blood flow velocity in cerebral arteries through the thinnest portions of the intact skull.[115] The TCD signal is displayed as a velocity-time waveform. The peak systolic (PSV) and end-diastolic (EDV) blood flow velocities can be ascertained directly from the TCD waveform.[116] The mean blood flow velocity (MV) can be calculated:

$$MV = \frac{PSV + (EDV * 2)}{3}$$

According to the Hagen-Poiseuille law, flow in a rigid tube is:

$$F = \frac{P * \pi * r^4}{8 * \eta * l}$$

where F is flow, r is the radius of the tube, l is the length of the tube, P is the change in the pressure over the length of the tube, and η is the viscosity of the fluid.

The relationship between flow and velocity through a tube is defined:

$$F = v * \pi * r^2$$

Combining the previous formula for flow with the Hagen-Poiseuille law[117]:

$$v = \frac{P * r^2}{8 * \eta * l}$$

TCD is used for interrogation of the cerebral circulation through four "windows" in the intact skull.[114] The four windows are in the temporal, orbital, submandibular, and suboccipital areas.[114] The temporal window is used to measure blood velocity in middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA) and communicating arteries (Figure 5).[114] The transorbital approach allows assessment of blood velocity in the ophthalmic artery (OA) and internal carotid artery (ICA) siphon.[114] The submandibular window is used to measure blood velocity in the ICA as it enters the

skull.[114] The suboccipital approach allows assessment of blood velocity in the terminal vertebral arteries (VA) and basilar artery (BA) through the foramen magnum.[114]

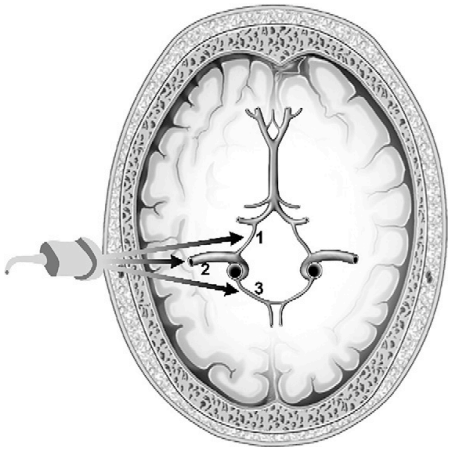

Figure 5. Schematic drawing of insonation through the temporal window using transcranial Doppler. Transcranial Doppler measures the cerebral blood flow velocity in the cerebral arteries. The transtemporal approach allows for measurement of the (1) posterior (2) middle and (3) anterior cerebral arteries. (From Franco Folino, 2007)

In 1982, Aaslid determined a range of normal blood flow velocities and depths of insonation for each of the major intracranial arteries[116, 118]. The blood flow velocity depends on the angle of insonation, vessel diameter, cerebral blood flow and collateral flow.[116] The range that was established for MCA blood flow velocity was 46-86 cm/s, toward the ultrasound probe through the temporal window, at a depth of 35-60 mm.[116, 118] The mean velocity obtained in the MCA by Aaslid et al. in 1982 was 62 +/- 12 cm/s.[118] Cerebral blood flow is dependent on the radius of the vessel and the blood flow velocity through the vessel.[117] The blood flow velocity is dependent on the perfusion pressure across the vessel, the radius of the vessel and the hematocrit of the blood.[117] Physiological factors that influence blood flow velocity are similar to those that change cerebral blood flow: mean arterial pressure, partial pressure of CO_2, and hematocrit.[116]

The TCD waveform is determined by a number of factors including cardiac output and blood pressure, as well as autoregulatory or vasomotor responses and focal arterial lesions within the brain.[114] Hypertension (both chronic and acute) increases blood flow pulsatility and may increase the mean flow velocity.[114] Hyperventilation decreases the mean flow velocity and increases the pulsatility of flow.[114] Hypercapnia increases the mean flow velocity and decreases pulsatility of flow.[114] A waveform pattern can also be affected by downstream circulatory conditions, such as loss of autoregulation or elevated intracranial pressure.[114] When homologous arterial segments are compared, normal variations of up to 30% in flow velocities and pulsatility indices can be expected.[114] Variations in the angle of insonation can account for 15% of a normal velocity/pulsatility difference and the resistance of downstream vasculature during breathing cycles for another 15%.[114]

The importance of assessing cerebral blood flow as indicated by cerebral blood flow velocity is based on the knowledge that cerebral blood circulation appears to directly influence cerebral function.[119] Cerebral blood flow and mean flow velocities decrease with age in adults.[117] TCD is used to evaluate traumatic brain injuries, acute and chronic cerbrovascular disease, and unexplained syncope.[19] Cerebral blood flow monitoring with TCD during circulatory arrest and cardiopulmonary bypass[116] leads to improved neurological outcomes in neonates and children undergoing congenital heart repair.[117] TCD has been useful for detecting emboli,[116, 117] improper cannulation or cross clamping of aortic arch vessels during cardiac surgery.[117] TCD is utilized for the clinical evaluation

of cerebral vasospasm following subarachnoid hemorrhage and monitoring of cerebral hemodynamics following head trauma.[116] Less frequently, TCD is used to diagnose brain death.[116]

Transcranial Doppler is the most popular way to assess cerebral autoregulation;[115, 116] it can be used to measure both dynamic and static autoregulation.[19] Specifically, it provides real-time information about changes in cerebral blood flow velocity and allows for the study of dynamic autoregulation.[19] An index of autoregulation can be calculated as a percentage change in cerebrovascular resistance (MAP/CBFV) per 1% change in MAP (CBFV is measured using transcranial Doppler).[116] A value lower than 0.4 indicates impaired autoregulation.[116] Using spectral-domain analysis, one can determine the contribution of parasympathetic, sympathetic and other influences on the regulation of the middle cerebral artery.

Calculation of the Pulsatility Index (PI) and Resistance Index (RI) from the measured blood flow velocities gives information about the resistance of more distal vasculature (PI) and increased intracranial pressure (PI and RI).[116]

$$PI = \frac{PSV - EDV}{MV}$$

$$RI = \frac{PSV - EDV}{PSV}$$

PSV is peak systolic blood flow velocity, EDV is end diastolic blood flow velocity, and MV is mean velocity (calculation of MV shown above).

Using TCD to evaluate absolute blood flow is not possible because the precise diameter of the blood vessel is not known.[117] Changes in cerebral blood flow can, however, can be accurately estimated by the change in cerebral blood flow velocity measured by TCD in the MCA.[117] The caveat to the relationship is that the CBF and cerebral blood flow velocities are correlated in a linear fashion only if the angle of insonation and vessel caliber remains constant throughout the exam.[116] There are some studies that suggest that the diameter of the MCA does not change significantly during cardiovascular challenges such as pediatric cardiac surgery, with the vascular changes occurring only in resistance arteries and arterioles.[117, 120]

Use of TCD is limited by multiple factors. It is operator dependent,[19] with a steep learning curve before technical proficiency is gained. It can only measure velocity in large arteries.[115] Regional cerebral autoregulation defects could be missed because of averaging with areas that have better perfusion.[115] Collateral circulation can weaken the correlation between cerebral blood flow velocities and cerebral blood flow.[121] Significant moment to moment variation means that intermittent TCD could miss important peaks or troughs in blood flow velocities.[116] Up to 8% of subjects do not have an adequate acoustic window for insonation.[116, 122]

Research Applications for TCD:

Using TCD during experimental models of hypoperfusion is convenient because it provides a non-invasive, objective measure of the amount of blood perfusing the brain.[117] The mean cerebral blood flow velocity (CBFV) seems to be the most important value for describing cerebral perfusion, rather than systolic cerebral blood flow velocity.[7, 117] During one study by Balldin et al., with maximal LBNP prior to syncope, a decline in mean CBFV of 60% from baseline was been noted.[7] This diminished flow velocity continues at the third heartbeat after the release of LBNP, but begins to recover by 30 seconds after release (84% of baseline).[7] Mean CBFV is not completely restored to baseline levels up to 30 seconds after the release of LBNP.[7]

Specific Aims of the Present Study

The present study was undertaken to determine if the Cholinergic Oscillatory Control of the microvasculature (COC_{VASC})[3] is present in the setting of hypoperfusion, as it was in the setting of vasoconstrictive challenges.[2-5] The change in blood flow at the level of the microcirculation is compared to the change in blood flow velocity in the middle cerebral artery to determine if either monitor better predicts symptom onset during hypoperfusion. An evaluation of the power in the autonomic spectra of the signals generated by the TCD and the forehead LD allows for the quantification of the relative contributions of the sympathetic and parasympathetic nervous systems.

Methods

With Institutional Review Board approval, 11 healthy non-smoking volunteers were given informed consent in the protocols and procedures involved in the experiment. Subjects were instructed to refrain from caffeine and other known vasoactive compounds for at least 8 h and to eat only a light meal prior to their scheduled experiment time.

Instrumentation:

Subjects were positioned within the LBNP chamber such that their anterior superior iliac crests were aligned with the opening and then a piece of wood with a cutout for their abdomen was placed at the edge of the chamber. The kayak skirt was stretched over the lip of the chamber entrance and the top of the skirt was secured tightly with a Velcro strap. A leg roll was placed under the knees and a footrest was positioned at the end of the chamber. The climbing harness was connected to eye-loops in the chamber via nylon rope and the subject was fastened into place. The Plexiglas lid was then closed and locked. The negative pressure is turned on and can be adjusted using a manual dial. A manometer is present to monitor the pressure inside of the chamber.

Protocol:

Subjects were placed in the recumbent position on a table with their legs and pelvis inside of a horizontal, wooden lower body negative pressure chamber, in a temperature-regulated room (25°C). They were fitted with a mountain-climbing harness and kayak skirt prior to lying down. Surface electrodes were applied for monitoring the

electrocardiogram (ECG), a respiration belt was placed around the thorax, and a noninvasive blood pressure cuff was placed on one arm. Blood pressure, mean arterial pressure and stroke volume were registered by a noninvasive pneumatic finger cuff (Finapres, Ohmeda model 2300, Madison, WI). Laser Doppler flowmetry probes (Periflux 2B; Perimed, Sweden) were applied to the skin via double-stick tape on the forehead and on the ventral finger, contralateral to the arm blood pressure cuff. A transcranial Doppler transducer in a manually adjustable headset was positioned over the temporal acoustic window until a mean blood flow velocity of at least 50 cm/s was obtained. The depth and gain was adjusted to optimize the signal for each subject.

Data Collection:

The ECG, respiration, blood pressure, stroke volume, TCD blood flow velocity and laser Doppler flux were recorded through a PowerLab data acquisition unit, at 250 Hz, with commercially available data acquisition software (LabChart software; ADInstruments). For forehead LD, TCD, and Finapres arterial blood pressure, systolic (peak) and diastolic (trough) values were recorded. Data from Chart was analyzed using peak analysis and the average, minimum and maximum values during specific 30-second intervals were transferred to Microsoft Excel. In Microsoft Excel, mean and standard deviation calculations, and two-tailed paired t-tests were performed on each interval. These intervals were labeled “Baseline,” “Presympt,” “Sympt” and “Recovery.”

There was a baseline period of data collection that lasted approximately 3 minutes in each subject, during which LBNP was not applied to the subject. The “Baseline” interval used

in data analysis was described as a 30-second interval during the baseline period of data collection. The volunteers were subjected to a rapidly progressive LBNP protocol (-70 mmHg in 1 min followed by progressive declines of ~10 mmHg until they reported lightheadedness or had a BP decline >20% baseline BP). Presympt is the pre-symptomatic period. All subjects have a Presympt period at approximately 30 seconds before Sympt, even if they did not become symptomatic. Presympt is a marker of the time of the maximum LBNP and occurs just prior to discontinuation of LBNP. Sympt is a 30-second interval that begins with the subject reporting symptoms of nausea or lightheadedness. However, subjects who did not report symptoms during LBNP still have a Sympt period, which is the time of discontinuation of LBNP for safety reasons. LBNP was discontinued for safety if a subject had a decline in SBP >20% baseline BP or a doubling of their heart rate. LBNP was also discontinued for discomfort in one subject who had groin pain.

Data Analysis:

Customized modifications of the spectral-domain analysis program in commercially available software (Chart; Igor Pro, Wavemetrics, Inc., Lake Oswego, OR) were used to enable analysis of continuous flow. The frequency and power of the oscillatory activity of the R-R tachogram, transcranial Doppler flow velocity, and laser Doppler flux signals were characterized with the autopower spectral density (APSD):

$$G_{aa} = [\text{ave}(S_a\, S_a^*)]/df,$$

wherein G_{aa} is the instantaneous amplitude spectral density of channel "a" [e.g., R-R intervals (in msec) or forehead flow (in volts) or transcranial Doppler flow velocity (in cm/s)], S_a is instantaneous amplitude spectrum of channel "a", S_a* is complex conjugate of S_a, and df is frequency resolution [0.01 Hz for a 40 sec window (e.g., $APSD_{0-40}$)]. These were displayed with frequency (from 0.05 – 0.30 Hz) on the x-axis and power for each frequency (in $msec^2$ /Hz or $volt^2$ /Hz or $(cm/s)^2$/Hz) on the y-axis. Joint time-frequency analysis (JTFA) was performed on the "moving," overlapping windows. After the $APSD_{0-40}$ window was generated, subsequent windows were automatically shifted every 20 sec, such that each successive window incorporated the next 20 sec block of data and excluded the prior window's earliest 20 sec block of data (20-60 sec, 40-80 sec …). The data for the successive windows were converted to a spreadsheet (Excel, Microsoft, Redmond, WA) with successive 0.025 Hz frequency bins in each column and successive 20 sec increments of the $APSD_{40}$ in each row. Initially, $APSD_{40}$ were generated for the entire TCD and the continuous forehead flow waveform. These were converted to the aforementioned spreadsheet with successive 0.025 Hz frequency bins in each column. The eleven 0.025 Hz-wide bins between 0.05 and 0.30 Hz were grouped into the following frequency bands: low (0.05-0.11 Hz), intermediate (0.12-0.18 Hz), respiratory (0.19-0.21 Hz) and remainder of the high (0.21-0.30).

Finally, the results of spectral-domain analysis for each individual was correlated to the timing in Chart and labeled with "Baseline," "Presympt," "Sympt," and "Recovery" intervals. The entire baseline period of the study was averaged for each spectral-domain frequency interval (e.g. 0.05, 0.075, 0.1, etc.) for each individual. In order to show a

difference from baseline, the 85th percentile of baseline was calculated and was then compared to the "Presympt," "Sympt," and "Recovery" 30-second intervals. The absolute difference of the Presympt, Sympt or Recovery value minus the upper limit of the 85th percentile of the baseline interval was then divided by the average of the baseline and multiplied by 100 to give percent change from baseline. If the Presympt, Sympt or Recovery value was lower than the 85th percentile baseline range, then the Presympt, Sympt or Recovery value was subtracted from the minimum value of the baseline range and divided by the average of the baseline and multiplied by –100 to give percent change from baseline (with a negative value indicating that it is below the baseline range).

Results

Of the eleven healthy volunteers enrolled in the study, two were excluded based on poor quality of data collection. Nine subjects were able to tolerate the lower body negative pressure challenge. Seven of the nine subjects became symptomatic – six with lightheadedness, plus or minus nausea, and one subject with nausea only. Two subjects did not become symptomatic with progressive LBNP – one subject had significant groin pain and the study was terminated because of pain, and one subject had no symptoms until after the LBNP was discontinued, at which point he experienced nausea. Subjects have been grouped, therefore, into the following categories: All (all nine subjects); symptomatic (seven symptomatic); lightheaded (six symptomatic subjects with lightheadedness); nauseous (two subjects with nausea – one during LBNP and one immediately after its discontinuation); asymptomatic (two subjects who did not have symptoms prior to discontinuation of LBNP). Maximum LBNP tolerated by each subject group is listed in Table A. The average of maximum LBNP tolerated did not vary significantly between symptomatic and asymptomatic subject groups, though there was a trend toward further progression of LBNP in asymptomatic subjects (-110 mmHg in asymptomatic vs. –94.3 mmHg in symptomatic subjects, p= NS)

Subject Group (Individual Subjects)	Maximum LBNP
All Subjects (A-I)	-97.8 mmHg
Symptomatic (A-G)	-94.3 mmHg
Lightheaded (B-G)	-95 mmHg
Nauseous (A, I)	-97.5 mmHg
Asymptomatic (H, I)	-110 mmHg

Table A. Maximum Lower Body Negative Pressure (LBNP) tolerated in each subject group

Blood Pressure:

Lower body negative pressure induced predictable changes in systemic blood pressure and heart rate (See Tables B and C; Figures 1 and 2).

Average Systemic Pressure	Baseline	Presympt	Sympt	Recovery
All:				
Systolic	126.211	115.14*	101.73*	119.644
Diastolic	72.464	80.96*	72.342	76.451
Pulse Pressure	53.738	34.20*	29.40*	43.20*
Symptomatic:				
Systolic	128.200	117.41*	105.58*	120.457
Diastolic	72.261	81.73*	72.886	76.491
Pulse Pressure	55.930	35.70*	32.71*	43.97*
Lightheaded:				
Systolic	131.800	119.22*	103.50*	120.47*
Diastolic	75.420	83.49*	72.248	78.533
Pulse Pressure	56.375	35.74*	31.26*	41.95*
Nauseous:				
Systolic	110.950	104.800	99.785	117.350
Diastolic	63.155	73.130	70.575	68.020
Pulse Pressure	47.770	31.690	29.205	49.315
Asymptomatic:				
Systolic	119.250	107.2*	88.265	116.800
Diastolic	73.175	78.275	70.440	76.310
Pulse Pressure^	46.065	28.950	17.820	40.475

Table B. Average Systemic Blood Pressure. The average systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure for each subject group are shown, during Baseline, Presympt, Sympt and Recovery. Significant changes from Baseline are represented with "*" and signify $p \leq 0.05$. Significant changes between Presympt and Sympt are represented with "^" and signify $p \leq 0.05$.

Heart Rate				
	Baseline	Presympt	Sympt	Recovery
All Subjects	67.9	108.7*	101.2*	59.6*
Symptomatic	64.8	104.3*	101.4*	57.7*
Lightheaded	66	103.3*	99.7*	57.2*
Nauseous	72.5	113.1	112.4	67.4
Asymptomatic	78.8	124.2	100.7	66.3*

Table C. Average Heart Rate (HR). The average heart rate during Baseline, Presympt, Sympt and Recovery for each subject group is shown. Significant changes from Baseline are represented with "*" and signify $p \leq 0.05$. There were no significant changes between Presympt and Sympt in any subject group.

In all subjects, the average systolic blood pressure (SBP) declined from Baseline at 126.2 mmHg to Presympt at 115.1 mmHg ($p<0.001$) and to Sympt at 101.7 mmHg ($p = 0.008$); diastolic blood pressure (DBP) increased significantly from Baseline at 72.5 mmHg to Presympt at 81.0 mmHg ($p = 0.005$); pulse pressure declined from Baseline at 53.7 mmHg to Presympt at 34.2 mmHg ($p<0.001$) and to Sympt at 29.4 mmHg ($p<0.001$) and to Recovery at 43.2 mmHg ($p = 0.017$). There were no significant changes in SBP, DBP or pulse pressure between Presympt and Sympt (p = NS). In symptomatic subjects, average SBP declined from Baseline at 128.2 mmHg to Presympt at 117.4 mmHg ($p = 0.003$) and to Sympt at 105.6 mmHg ($p = 0.044$); DBP increased significantly from Baseline at 72.2 mmHg to Presympt at 81.7 mmHg ($p = 0.012$); pulse pressure declined from Baseline at 55.9 mmHg to Presympt at 35.7 mmHg ($p<0.001$) and to Sympt at 32.7 mmHg ($p = 0.002$) and to Recovery at 44.0 mmHg ($p = 0.030$). There were no significant changes in SBP, DBP or pulse pressure between Presympt and Sympt (p = NS). In lightheaded subjects, average SBP declined from Baseline at 131.8 mmHg to Presympt at 119.2 mmHg ($p<0.001$) and to Sympt at 103.5 mmHg ($p = 0.017$) and to Recovery at 120.5 ($p = 0.045$); DBP increased significantly from Baseline at 75.4 mmHg to Presympt at 83.5 mmHg ($p = 0.030$); pulse pressure declined from Baseline at 56.4 mmHg to Presympt at 35.7 mmHg ($p<0.001$) and to Sympt at 31.3 mmHg ($p = 0.003$) and to Recovery at 42.0 mmHg ($p = 0.017$). There were no significant changes in SBP, DBP or pulse pressure between Presympt and Sympt (p = NS). In nauseous subjects, average SBP, DBP and pulse pressure did not change significantly between any of the analyzed stages. In asymptomatic subjects, average SBP declined from Baseline at 119.3 mmHg to Presympt at 107.2 mmHg ($p = 0.013$), however there were no other significant changes in

SBP, DBP. Pulse pressure changed significantly from Presympt at 29.0 mmHg to Sympt at 17.8 mmHg (p = 0.011). (See Table B)

Heart Rate:

In all subjects, the heart rate (HR) increased from Baseline at 67.9 bpm to Presympt at 108.7 (p = 0.0008 for interphase difference by two-tailed paired t-test) and from Baseline to Sympt at 101.2 bpm (p = 0.003) and HR declined from Baseline to Recovery at 59.6 bpm (p = 0.0022). In symptomatic subjects, HR increased from Baseline at 64.8 bpm to Presympt at 104.3 (p = 0.006) and to Sympt at 101.4 bpm (p = 0.010); HR declined from Baseline to Recovery at 57.7 bpm (p = 0.019). In lightheaded subjects, HR increased from Baseline at 66 bpm to Presympt 103.3 bpm (p = 0.019) and to Sympt at 99.7 bpm (p = 0.031); HR declined from Baseline to Recovery at 57.2 (p = 0.005). There was no significant change in HR in nauseous subjects (p = NS). In asymptomatic subjects, the only significant change in HR was between Baseline at 78.8 bpm and Recovery at 66.3 bpm (p = 0.012). There was no difference in HR between Presympt and Sympt (p = NS) in any group. (See Table C)

Forehead Laser Doppler Flow:

Forehead LD Flow changed significantly only from $Baseline_{SYS}$ to $Sympt_{SYS}$ (*p=0.048) in all subjects. In symptomatic subjects, the average Forehead LD Flow changed significantly only from $Presympt_{SYS}$ to $Sympt_{SYS}$ (p=0.040). In lightheaded subjects, the Forehead LD Flow changed significantly only from $Presympt_{SYS}$ to $Sympt_{SYS}$ (p=0.040). In nauseous subjects, the Forehead LD Flow changed significantly only from

$Baseline_{HEIGHT}$ to $Presympt_{HEIGHT}$ (0.004). In asymptomatic subjects, the Forehead LD Flow did not change significantly between any measured intervals. Any p-value not shown is p= not significant (NS). (See Figures 1 and 2)

Transcranial Doppler Flow:

Transcranial Doppler Flow Velocity changed significantly in all subjects from $Baseline_{SYS}$ to $Presympt_{SYS}$ (*p<0.001), $Baseline_{SYS}$ to $Sympt_{SYS}$ (*p<0.001), $Baseline_{DIAS}$ to $Presympt_{DIAS}$ (*p=0.018), $Baseline_{DIAS}$ to $Sympt_{DIAS}$ (*p=0.003), $Presympt_{DIAS}$ to $Sympt_{DIAS}$ (^p=0.012), $Baseline_{HEIGHT}$ to $Presympt_{HEIGHT}$ (p<0.001) $Baseline_{HEIGHT}$ to $Sympt_{HEIGHT}$ (p=0.008). In symptomatic subjects, the TCD Flow Velocity changed significantly from $Baseline_{SYS}$ to $Presympt_{SYS}$ (p<0.001), $Baseline_{SYS}$ to $Sympt_{SYS}$ (p=0.002), $Baseline_{DIAS}$ to $Presympt_{DIAS}$ (p=0.022), $Baseline_{DIAS}$ to $Sympt_{DIAS}$ (p=0.014), $Presympt_{DIAS}$ to $Sympt_{DIAS}$ (p=0.045), and $Baseline_{HEIGHT}$ to $Presympt_{HEIGHT}$ (p<0.001). In lightheaded subjects, the TCD Flow Velocity changed significantly from $Baseline_{SYS}$ to $Presympt_{SYS}$ (p=0.001), $Baseline_{SYS}$ to $Sympt_{SYS}$ (p=0.005), $Baseline_{DIAS}$ to $Presympt_{DIAS}$ (p=0.026), $Baseline_{DIAS}$ to $Sympt_{DIAS}$ (p=0.009), $Presympt_{DIAS}$ to $Sympt_{DIAS}$ (p=0.016), and $Baseline_{HEIGHT}$ to $Presympt_{HEIGHT}$ (p=0.001). In nauseous subjects, TCD Flow Velocity changed only significantly from $Baseline_{HEIGHT}$ to $Sympt_{HEIGHT}$ (p=0.021). In asymptomatic subjects, the TCD Flow Velocity changed significantly from $Baseline_{SYS}$ to $Recovery_{SYS}$ (p=0.047), $Baseline_{HEIGHT}$ to $Presympt_{HEIGHT}$ (p=0.014), $Baseline_{HEIGHT}$ to $Sympt_{HEIGHT}$ (p=0.022). Any p-value not shown is p = NS. (See Figures 6 and 7)

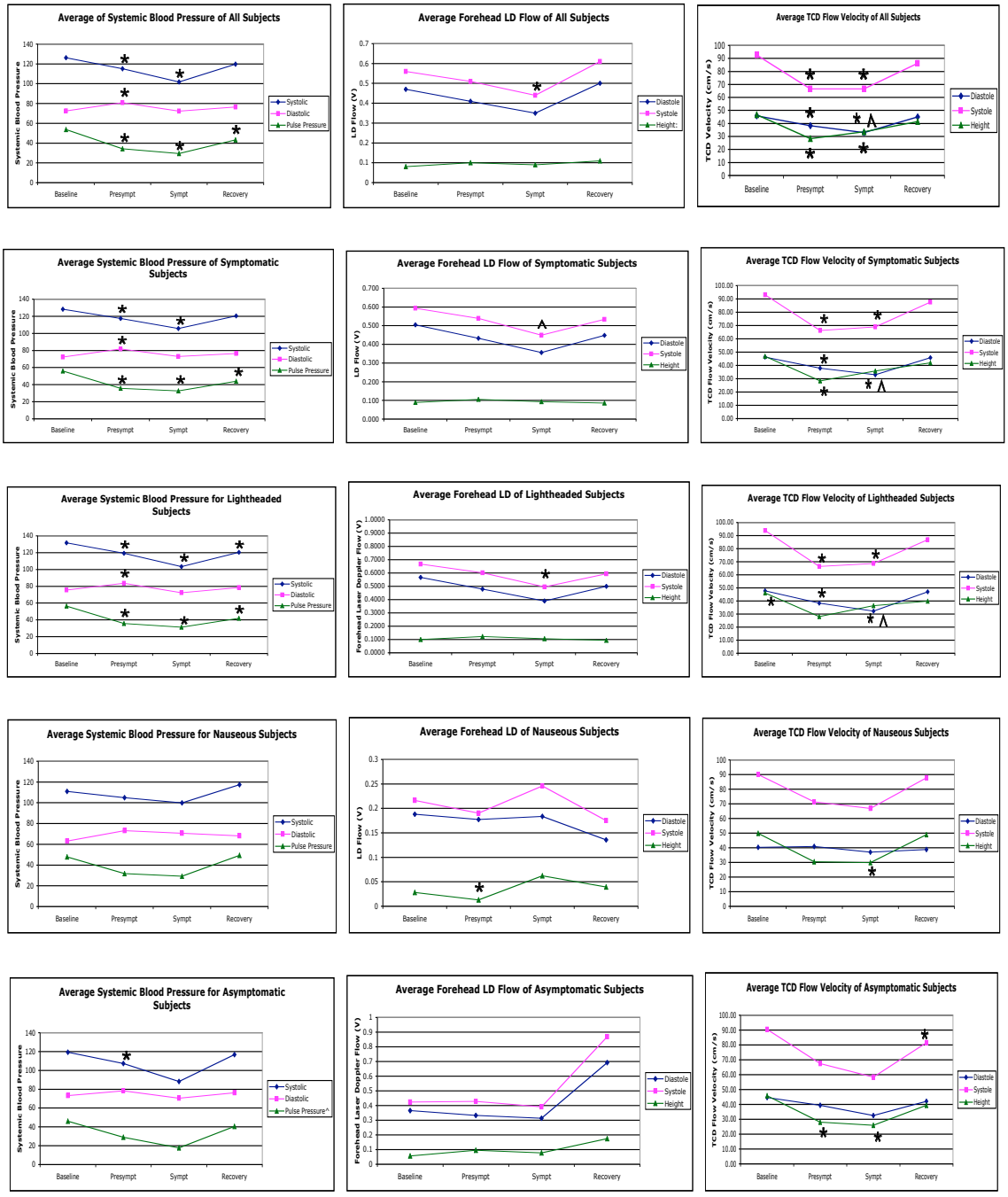

Figure 6. Comparison of Average Forehead Laser Doppler Flow to Transcranial Doppler Flow Velocity. The average Forehead LD Flow and TCD Flow Velocity during Baseline, Presympt, Sympt and Recovery are shown for each group of subjects. Significant changes from Baseline are represented with "*" and signify $p \leq 0.05$. Significant changes between Presympt and Sympt are represented with "^" and signify $p \leq 0.05$.

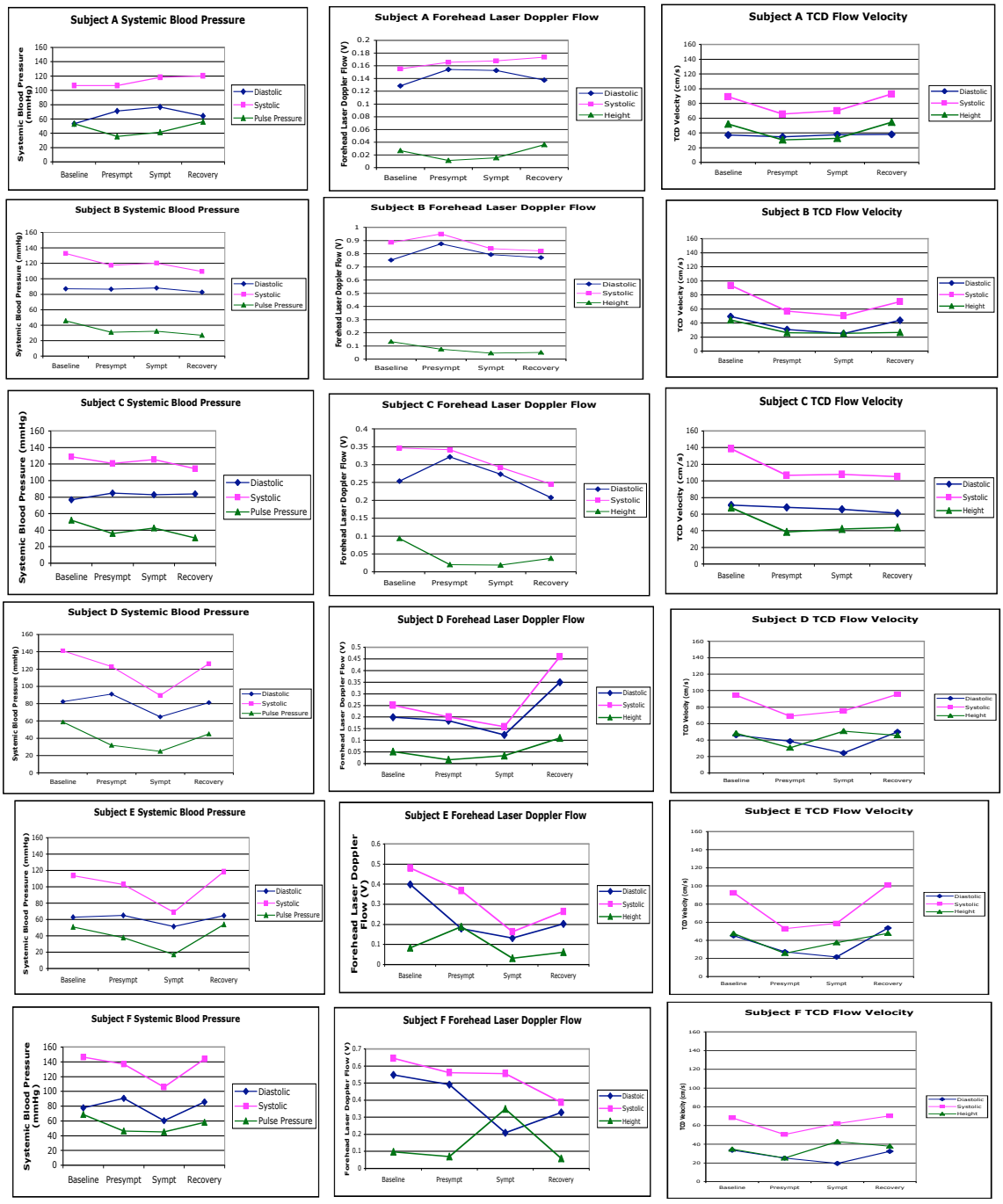

Figure 7. Systemic Blood Pressure, Forehead Laser Doppler Flow and Transcranial Doppler Flow Velocity. Individual patterns of systemic blood pressure, forehead LD and TCD are represented in the above graphs. Subjects A-G are symptomatic; Subject A became nauseous with LBNP and Subjects B-G became lightheaded and nauseous. Subject H was asymptomatic, but had groin pain at the end of the study. Subject I was asymptomatic and became nauseous after the LBNP was discontinued. Systolic: Peak value; Diastolic: Trough value; Pulse Pressure or Height: numerical difference between peak and trough.

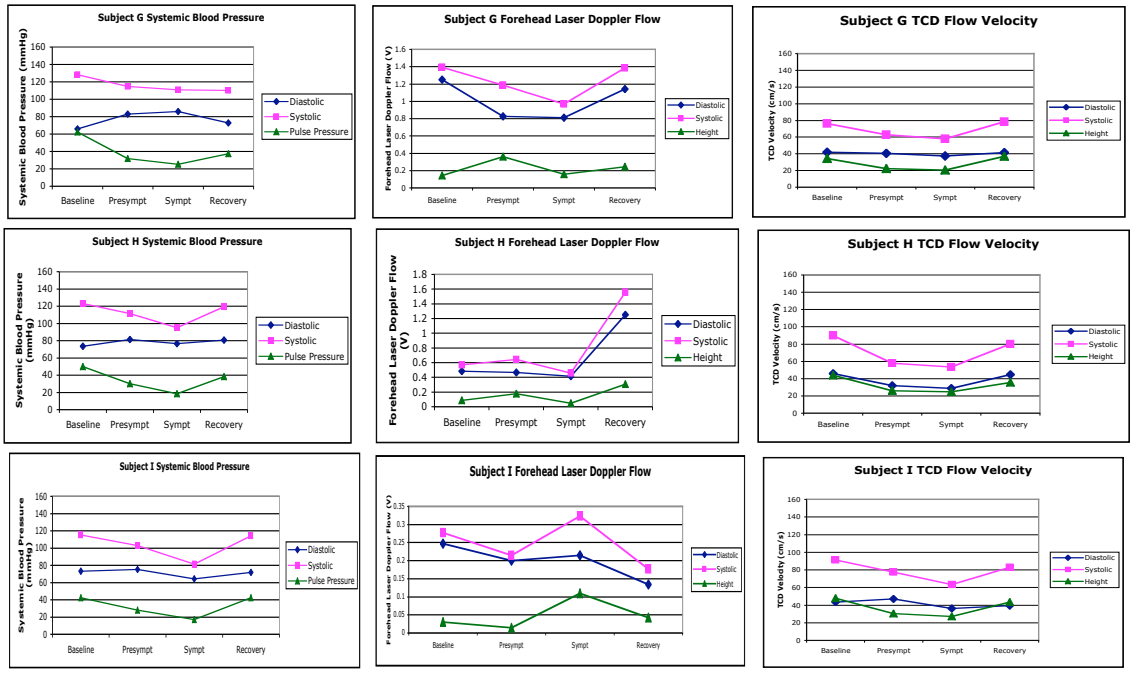

Figure 7 (cont). Systemic Blood Pressure, Forehead Laser Doppler Flow and Transcranial Doppler Flow Velocity. Individual patterns of systemic blood pressure, forehead LD and TCD are represented in the above graphs. Subjects A-G are symptomatic; Subject A became nauseous with LBNP and Subjects B-G became lightheaded and nauseous. Subject H was asymptomatic, but had groin pain at the end of the study. Subject I was asymptomatic and became nauseous after the LBNP was discontinued. Systolic: Peak value; Diastolic: Trough value; Pulse Pressure or Height: numerical difference between peak and trough.

Autonomic Power:

The overall changes in autonomics were analyzed via spectral-domain analysis of the Heart Rate Variability (HRV), Transcranial Doppler (TCD) Flow Velocity and Forehead Laser Doppler (LD) Flux. HRV varied significantly between Presympt and Sympt in the asymptomatic subjects ($p = 0.021$), but did not have a significant change in other subject groups during the course of progressive LBNP. (See Table D)

HRV: LF/HF				
	Baseline	Presympt	Sympt	Recovery
All	2.77	27.65	14.12	2.90
Symptomatic	3.06	35.04	16.51	3.08
Lightheaded	2.69	38.40	17.86	2.64
Nauseous	2.76	8.56	7.24	3.61
Asymptomatic^	1.77	1.79	5.78	2.28

Table D. Heart Rate Variability (HRV). The average HRV during Baseline, Presympt, Sympt and Recovery is shown for each subject group. Significant changes between Presympt and Sympt are represented with "^" and signify $p \leq 0.05$. There were no significant changes in HRV between Baseline and Presympt, Sympt or Recovery.

Changes in the autonomic power in the Forehead LD Flow and TCD Flow Velocity for each subject group are listed below. The data represents a percentage change from a "baseline range." The baseline range includes approximately 85% of numerical values you would expect to find during baseline. If a percentage change is equal to 200%, this indicates that the power at this frequency is 200% (or twice the power) of the maximum baseline range value. If the percentage is negative, this indicates that the change in power is below the minimum number in the baseline range. (See Figure 8; See Tables E-I and J-N)

All Subjects	Forehead LD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0
Presympt	6.8	131.5	154.6	182.1	262.6	203.5	243.0	270.8	286.7
Sympt	48.8	313.7	529.9	446.5	310.5	600.0	262.8	578.2	306.5
Recovery	2.2	-4.3	-11.6	-5.7	23.7	19.6	0.8	101.7	433.3

Table E. Percent change in Forehead LD flow in All Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Symptomatic Subjects		Forehead LD % Change from Baseline							
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0
Presympt	-1.3	29.3	95.7	210.7	271.0	261.6	311.9	333.9	299.2
Sympt	5.6	33.2	-4.4	-12.2	299.3	719.2	48.4	419.1	309.9
Recovery	4.1	-3.6	-6.8	-7.4	31.2	30.1	10.5	130.7	557.1

Table F. Percent change in Forehead LD flow in Symptomatic Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Lightheaded Subjects	Forehead LD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.0	0.0
Presympt	-1.6	34.2	70.7	44.2	75.7	116.1	321.3	389.6	231.6
Sympt	-0.2	38.8	-6.1	-14.2	127.5	18.5	53.9	443.7	349.6
Recovery	7.0	-1.4	-7.7	-8.6	33.7	9.8	10.8	152.5	653.9

Table G. Percent change in Forehead LD flow in Lightheaded Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Nauseous Subjects	Forehead LD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Presympt	46.5	150.9	128.5	684.6	846.3	567.4	138.3	10.4	341.2
Sympt	220.0	1248.6	2360.6	2052.2	1031.2	2661.5	1021.0	1184.3	208.9
Recovery	-11.2	-15.2	-29.1	0.0	8.0	75.8	-6.5	0.0	-12.1

Table H. Percent change in Forehead LD flow in Nauseous Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Asymptomatic Subjects	Forehead LD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Presympt	35.1	489.2	360.4	82.0	233.2	0.0	1.9	49.8	242.9
Sympt	199.8	1295.3	2400.1	2052.2	349.7	182.8	1013.1	1135.0	294.9
Recovery	-4.4	-6.8	-28.4	0.0	-2.4	-17.0	-32.9	0.0	0.0

Table I. Percent change in Forehead LD flow in Asymptomatic Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

All Subjects	TCD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.9	0.0	0.0	3.1	0.0	0.0
Presympt	68.6	70.8	216.6	101.2	56.2	77.3	189.6	172.9	249.4
Sympt	27.2	59.5	255.6	457.8	201.0	308.8	435.2	168.6	340.4
Recovery	-6.6	23.1	45.8	67.3	69.4	87.0	119.5	49.2	25.1

Table J. Percent change in TCD Flow Velocity in All Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Symptomatic Subjects	TCD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0
Presympt	83.1	31.6	171.6	120.2	50.2	78.8	241.8	217.5	320.6
Sympt	37.8	76.5	143.6	258.6	93.1	397.0	413.9	153.9	134.6
Recovery	-12.5	34.1	64.4	88.6	92.1	111.9	124.3	63.2	34.4

Table K. Percent change in TCD Flow Velocity in Symptomatic Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Lightheaded Subjects	TCD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0
Presympt	97.0	17.6	70.9	93.5	25.6	2.3	268.9	238.7	314.4
Sympt	-3.4	89.3	167.6	301.6	2.3	440.7	482.9	168.8	145.7
Recovery	-14.5	43.8	75.2	103.4	107.5	130.6	146.6	73.8	40.1

Table L. Percent change in TCD Flow Velocity in Lightheaded Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline

Nauseous Subjects	TCD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.00	0.00	0.00	0.00	0.00	0.00	14.0	0.00	0.00
Presympt	26.2	265.6	771.3	174.6	176.3	324.0	46.4	63.8	178.9
Sympt	142.5	0.00	648.9	1159.4	897.4	67.3	509.6	32.5	894.1
Recovery	-2.8	-27.6	-5.7	0.00	-10.00	0.00	97.7	0.00	-7.6

Table M. Percent change in TCD Flow Velocity in Nauseous Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

Asymptomatic Subjects	TCD % Change from Baseline								
	0.050	**0.075**	**0.100**	**0.125**	**0.150**	**0.175**	**0.200**	**0.225**	**0.250**
Baseline	0.0	0.0	0.0	0.0	0.0	0.0	14.0	0.0	0.0
Presympt	17.5	207.9	374.0	34.5	77.3	72.0	6.7	16.7	0.0
Sympt	-9.6	0.0	647.4	1155.0	578.6	0.0	509.6	219.8	1060.7
Recovery	14.0	-15.7	-19.6	-7.1	-10.0	0.0	102.4	0.0	-7.6

Table N. Percent change in TCD Flow Velocity in Asymptomatic Subjects. Numbers listed represent the difference between the maximum power in the baseline range (approximately the 85th percentile) and the power at each frequency during the specified interval, divided by the average of baseline and multiplied by 100%. If a number is negative, the power at that frequency was subtracted from the minimum power in the baseline range and was then divided by the average of baseline and multiplied by –100%. Negative numbers therefore represent a change that is below what would be expected at baseline.

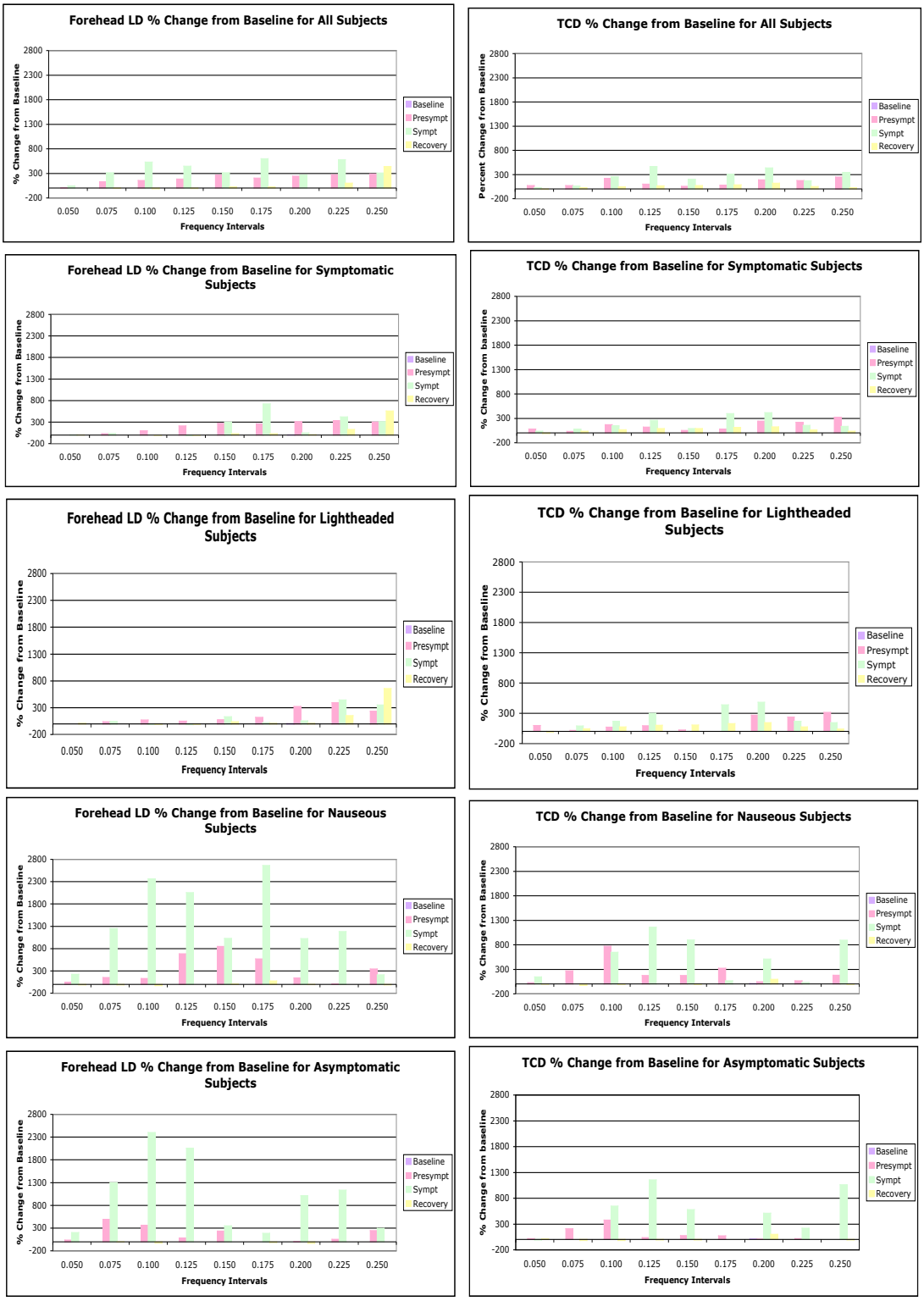

Figure 8. Comparison of Forehead LD Change in Power from Baseline and TCD Percent Change from Baseline. Graphical representation of the average percent change in power from Baseline at each frequency in the different subject groups.

Discussion

Rapid ramping of LBNP successfully simulated acute central hypovolemia during the present research protocol. Progressive LBNP resulted in strong evidence of systemic sympathetic compensation, including tachycardia, decreased heart rate variability, decreased flow measurements in the extremities (finger laser Doppler flow declined during progressive LBNP, data not shown), decreased stroke volume (data not shown) and decreased arterial pulse pressure. Of the nine subjects who tolerated LBNP, six became lightheaded, one became nauseous only, and two did not develop symptoms during the progression of LBNP. Upon analysis of the data and consideration of subject symptom characteristics, a decision was made to divide lightheaded subjects from nauseous subjects, because lightheadedness is a clear indicator of decreased cerebral perfusion, whereas nausea may be a vagal response or could be mediated by some other process unrelated to cerebral perfusion.

In the 6 subjects who became lightheaded, forehead LD decreased by 10.9±11.7% from Baseline at Presympt (p=NS for interphase difference). It then decreased by an additional 20.4±18.7% with the onset of lightheadedness (p=0.035 for Presympt vs. Sympt). Forehead LD flow was predictive of symptom onset. Forehead LD changed significantly in a manner that predicted the occurrence of lightheadedness in a subject. The forehead LD peak (systole) and trough (diastole) values did not change during the course of LBNP in the nauseous or asymptomatic groups (where Sympt was the time when the study was terminated because the BP cutoff was reached). In the symptomatic and lightheaded

groups, the only significant change in Forehead LD was between $Presympt_{SYS}$ and $Sympt_{SYS}$.

TCD flow velocity changed frequently throughout the progression of LBNP. Peak TCD readings in lightheaded subjects decreased by 29.3±9.7% from Baseline to the time of the Presympt measurement (p=0.001); they then increased by 4.1±12.9% with the onset of Sympt (p=NS). (The relatively high standard deviations reflect inter-subject, not intra-subject, variability.) There was no significant change in the TCD flow velocity in the nauseous or asymptomatic subjects, but the downward trends may have reached significance if there were enough subjects in those groups.

The specificity of the forehead LD is ideal as a predictor of impending circulatory collapse. Forehead LD flow could be a surrogate for microvasculature in the brain because the vascular supply is the same (the internal and external carotid arteries) and because the forehead microvascular flow is spared during vasoconstriction and hypovolemia until symptoms occur.

TCD, however, may not consistently predict symptoms in the context of acute hypovolemia. The lack of a continued decline in TCD velocity might be attributed to a stabilization of stroke volume; however, the data suggest that stroke volume continues to decline at the levels of LBNP used in the present study.[89] Vasoconstriction at the site of TCD measurement would increase velocity within the vessel segment; however, this would not account for divergent responses during systole and diastole. When the TCD

trend follows systemic MAP, cerebral autoregulation has been compromised. However, when systemic MAP continues to fall, but TCD levels off, this is evidence of dynamic cerebral autoregulation (an effort to maintain perfusion of the brain in the setting of decreased cardiac output). In the symptomatic and lightheaded groups, the TCD diastolic value continues to fall between Presympt and Sympt – along with MAP – which is an indication that the subjects have lost the ability to protect the brain's blood supply.

The discordance between LD and TCD may be consistent with autoregulatory mechanisms at the level of the forehead microvasculature that have been reported in the context of systemic administration of phenylephrine.[3, 4] Subjects in the phenylephrine studies were not pushed to the point of symptoms and the cerebral blood flow was not monitored, therefore our data gives more global information about the subjects and their ability to autoregulate during a hypoperfusion challenge.

Despite potential vasodilator effects of brain vessels by the parasympathetic nervous system, via mediators such as vasoactive intestinal peptide, acetylcholine and nitric oxide, current literature states that the parasympathetic nervous system does not play a relevant role in cerebral autoregulation.[24] However, our data presents an alternative theory. Post-hoc analysis of spectral domain patterns reveal that, despite evidence of sympathetic activity at the level of the heart and the periphery, the low-frequency to high frequency ratio (which correlates with relative sympathetic activity) at the level of the TCD declined from Base to Sympt. This decreased LF/HF ratio implies a sympathetic withdrawal and a potential parasympathetic increase in power. Our results may be

consistent with cholinergically-mediated oscillatory control (COC_{VASC}) previously described in the forehead microvasculature.[3, 4] This cholinergically-mediated homeostatic mechanism may increase compliance to allow a relatively greater percentage of the stroke volume into the cranial circulation and thereby maintain TCD systolic values.

The HRV LF/HF trended upward between Baseline and Presympt indicating an increase in sympathetic power in each subject group, except in the asymptomatic subjects, however the changes were not statistically significant. This trend is plausible physiologically because it represents compensation for perceived blood loss with sympathetic activation. Then, in each group except asymptomatic, the HRV LF/HF tended to decline at the time of Sympt, indicating a sympathetic withdrawal, or the autonomic system's inability to continue compensating for the cardiovascular insult, but this also did not reach statistical significance. Sympathetic withdrawal at the time of cardiovascular decompensation is well documented.[11] In the asymptomatic subjects, HRV LF/HF remained steady through Presympt and then increased significantly between Presympt and Sympt. The increase in HRV LF/HF at the point of Sympt in this group is logical because the individuals are not actually symptomatic. The subjects in the asymptomatic group continued to mount a sympathetic response to the induced hypovolemia at the point when a safety cutoff for blood pressure was reached. The HRV LF/HF returned to Baseline values during recovery in all groups.

In lightheaded subjects, Forehead LD average change from baseline to Presympt for LF power was +34.4%±81.3%; the change for HF power was +139.3±115.8% ($p=0.027$ by

two-tailed paired t-test). Oscillatory activity at the HF (parasympathetic frequency) appears to have been generated at the level of the forehead microvasculature, as the oscillatory activity in the middle cerebral artery (as measured with transcranial Doppler) showed a predominance of sympathetic activity at Base as well as Presympt. The TCD did not demonstrate an increase in parasympathetic activity until the onset of symptoms. We propose a homeostatic mechanism that occurs at all levels of the circulation from the parasympathetic control of the microcirculation to the parasympathetic oscillations in larger vessels (i.e. MCA) and vagal control systemically. In response to simulated hypovolemia, the microvasculature of the forehead has an increase in HF (parasympathetic) activity that is distinct from the predominantly LF activation throughout the body. This may contribute to autoregulation and cerebral protection.

The present data also confirmed that serial advancement of overlapping windows with JTFA significantly improves the temporal resolution of spectral-domain analysis in the presence of an acute challenge. JTFA more effectively delineated the intermediate frequency response of the forehead microvasculature, such that the LBNP-induced increase in power delineated by JTFA was significantly greater than that delineated by a traditional APSD. The improved temporal resolution of JTFA likewise identified significant changes in the R-R interval power spectrum (HRV). These findings suggest that JTFA should prove to be especially helpful when one is unsure as to when a challenge will occur and as to the time course of brief homeostatic and therapeutic responses.

Limitations:

Statistical analysis of the subgroup data is challenging because of the limited number of subjects enrolled in the study. It is difficult to prove differences between groups when only two subjects remained asymptomatic. It was determined during the analysis that Subject H (asymptomatic) could actually be skewing the data because of his groin pain. Perhaps his sympathetic activation because of groin pain increased the LF/HF ratio in the HRV analysis and also increased the LF power in all of the spectral analyses. It is possible that he would have become symptomatic during the same progression of LBNP had he been more comfortable. Future analyses of this data of may exclude Subject H because of his pain.

We make the assumption that autonomic activity at the level of the microcirculation, middle cerebral artery, or finger has implications for the autonomic status of the entire body.[53] It is possible that baroreceptor activity, or some other factors not related to the autonomic nervous system, might affect the vasculature in the frequency ranges studied.[53]

The respiratory rate was not controlled in this study, leading to potential complications in data interpretation. The respiratory frequency should be analyzed using JTFA and compared to the TCD, Forehead LD and HRV power spectra to evaluate overlap of the respiratory power with our frequency bands of interest (HF = 0.125 – 0.200). Forehead LD and TCD require complex signal interpretation. TCD also has many limitations (as mentioned previously), including a steep learning curve before technical proficiency is gained. Forehead LD and TCD are both affected significantly by movement during the

study. Both tools are costly and fragile, limiting the general utilization of the techniques in the clinical setting.

Summary:

This investigation into the significance of the high-frequency oscillations in the forehead microvasculature during hypoperfusion induced by LBNP demonstrated a potential homeostatic mechanism. The preservation of blood flow in the forehead microvasculature at the Presympt phase was accompanied by an increase in HF oscillations that was four times the increase in LF power. The body exhibits primarily sympathetic tone (greater LF than HF), at the time when HF increased at the forehead LD. An increase in parasympathetic (HF) activity would lead to vasodilation and decreased resistance. This decreased downstream resistance provides a mechanism for increased cerebral perfusion gradient and therefore increased cerebral blood flow. When the oscillations cease, the HF power diminishes, and then the resistance increases and the cerebral blood flow velocity decreases and symptoms occur.

These findings have important clinical ramifications. Forehead LD has potential use in intensive care units and emergency departments for patients whose mental status is compromised (i.e. intubation, head injury), and for whom there is concern for hemodynamic decompensation. A monitor that could predict cardiovascular collapse and allow for earlier intervention would be invaluable. Conversely, in patients known to be undergoing a vasoconstrictive challenge, forehead LD could provide information about the integrity of their autonomic nervous function[4] (i.e. in diabetic, hypertensive, or

atherosclerotic patients). Additionally, HRV functions as a tool by which mortality can be predicted after a myocardial infarction or during progressive heart failure.[53] If forehead LD is a similar marker of autonomic function, its predictive value may be equal to that of HRV. Finally, because of the common vascular supply and neural innervation, the forehead microvasculature may act as a surrogate for the cerebral microcirculation and forehead LD could be a useful tool for monitoring cerebral perfusion during stroke, subarachnoid hemorrhage, head injury, surgery (including carotid endarterectomy) and many more clinical settings.

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