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PHOTOPLETHYSMOGRAPHIC WAVEFORM ANALYSIS DURING LOWER BODY NEGATIVE PRESSURE SIMULATED HYPOVOLEMIA AS A TOOL TO DISTINGUISH REGIONAL DIFFERENCES IN MICROVASCULAR BLOOD FLOW REGULATION.

A Thesis Submitted to the
Yale University School of Medicine
In Partial Requirement for the Degree of
Doctor of Medicine

By
Nicholas Joseph Galante
2009

ABSTRACT

PHOTOPLETHYSMOGRAPHIC WAVEFORM ANALYSIS DURING LOWER BODY NEGATIVE PRESSURE SIMULATED HYPOVOLEMIA AS A TOOL TO DISTINGUISH REGIONAL DIFFERENCES IN MICROVASCULAR BLOOD FLOW REGULATION.

Nicholas J. Galante, Lars J. Grimm, Aymen A. Alian, Nina S. Stachenfeld, Kirk H. Shelley, and David G. Silverman, Department of Anesthesiology, Yale University, School of Medicine, New Haven, CT

The purpose of this investigation was to explore modulation of the photoplethsymographic (PPG) waveform in the setting of simulated hypovolemia as a tool to distinguish regional differences in regulation of the microvasculature. The primary goal was to glean useful physiological and clinical information as it pertains to these regional differences in regulation of microvascular blood flow. This entailed examining the cardiovascular, autonomic nervous, and respiratory systems' interplay in the functional hemodynamics of regulation of microvascular blood flow to both central (ear, forehead) and peripheral (finger) sites.

We monitored ten healthy volunteers (both men and women age 24-37) non-invasively with central and peripheral photoplethysmographs and laser Doppler flowmeters during Lower Body Negative Pressure (LBNP). Waveform amplitude, width, and oscillatory changes were characterized using waveform analysis software (Chart, ADInstruments). Data were analyzed with the Wilcoxon Signed Ranks Test, paired t-tests, and linear regression.

Finger PPG amplitude decreased by 34.6 ± 17.6% ($p = 0.009$) between baseline and the highest tolerated LBNP. In contrast, forehead amplitude changed by only 2.4 ± 16.0% (p=NS). Forehead and finger PPG width decreased by 48.4% and 32.7%, respectively. Linear regression analysis of the forehead and finger PPG

waveform widths as functions of time generated slopes of -1.113 (R = -0.727) and -0.591 (R = -0.666), respectively. A 150% increase in amplitude density of the ear PPG waveform was noted within the range encompassing the respiratory frequency (0.19-0.3Hz) (p=0.021) attributable to changes in stroke volume. We also noted autonomic modulation of the ear PPG signal in a different frequency band (0.12 – 0.18 Hz).

The data indicate that during a hypovolemic challenge, healthy volunteers had a relative sparing of central cutaneous blood flow when compared to a peripheral site as indicated by observable and quantifiable changes in the PPG waveform. These results are the first documentation of a local vasodilatation at the level of the terminal arterioles of the forehead that may be attributable to recently documented cholinergic mechanisms on the microvasculature.

ACKNOWLEDGEMENTS

To my advisors, David Silverman and Kirk Shelley, I express my most sincere and overwhelming appreciation for your tremendously gracious devotion of time, energy, support, guidance, and mentoring. To my fellow collaborators, Aymen Alian, Nina Stachenfeld, and Lars Grimm, I am most grateful for the aid, suggestions, and commentary that have gone into this project and thesis. You made things fun and worthwhile. To Donna Corranzo and Mae Geter, I am most thankful for your patience and assistance. To the Yale Office of Student Research, I am indebted to their generous funding and support of this project through the James G. Hirsch, M.D., Endowed Medical Student Research Fellowship. Most importantly, the utmost respect and appreciation goes to my friends and family, without which little could have been accomplished.

TABLE OF CONTENTS

INTRODUCTION

The photoelectric plethysmograph (PPG) was first described over seventy years ago[1-3]. Since that time, the PPG waveform has been studied and used clinically for a multitude of purposes. The discovery of its utility in the calculation of arterial oxygen saturation in the mid 1970's, however, had such a profound impact on clinical monitoring that the other potential uses of the waveform quickly faded from the attention of clinicians. Further neglect came from the waveform's absence from early stand-alone pulse oximeters, where the pulse was indicated by either a bouncing bar or flashing heart symbol.[4]

The pulse oximeter, as a photoelectric plethysmograph, non-invasively measures minute changes in blood volume of a vascular bed (e.g finger, ear, or forehead) over time. In this capacity, it has the ability to monitor differences between those microvascular beds with respect to blood flow. Regional differences exist in the regulation of blood flow in the microvasculature. Specifically, sympathetic activation, such as cold pressor testing and use of pharmacological agents (i.e. phenylephrine), cause disproportionate vasoconstriction of an adrenergically rich region such as the finger, as opposed to the ear or forehead, which have less adrenergic innervation [5-7].

Where previous investigation of the PPG has focused on its potential as an anesthesia monitoring device[8], over the course of its history, remarkably little has been done in the way of studying the device's ability to measure the aforementioned regional terminal arteriolar and microvascular differences[4]. This thesis will explore modulation of the PPG waveform in the setting of simulated hypovolemia. The primary aim of this investigation was to glean useful physiological and clinical information as it pertains to regional differences in regulation of microvascular blood flow. To this end, we examined the cardiovascular, autonomic nervous, and respiratory systems' interplay in the functional hemodynamics of regulation of microvascular blood flow to both

central (ear, forehead) and peripheral (finger) sites. The underlying hypothesis is that the PPG waveform can be used to detect, elucidate, and characterize these differences, as well as offer plausible explanations for these differing vascular responses to hypovolemia.

SOURCE OF THE WAVEFORM

The term "plethysmograph" is derived from the Greek root "plethysmos" meaning "to increase." Photoplethysmography, in simplest terms, is an optical measurement technique that can be used to detect blood volume changes in a microvascular bed in the tissue. There is a close correlation (r = 0.9) between the PPG and the more traditional strain gauge plethysmograph[9].

The elements that contribute to the pulse oximeter waveform are described by Beer's law of light:

$$A_{total} = E_1C_1L_1 + E_2C_2L_2 + \ldots E_nC_nL_n$$

Where,
A_{total} = absorption at a given wavelength
E_n = extinction coefficient (absorbency)
C_n = concentration
L_n = path length

The PPG itself is a simple device. It consists of a light source (most commonly an LED) and light detector (photo diode). The detector can be placed either directly across from the light source for transmission plethysmography or next to the light source for reflective plethysmography (Figure 1).

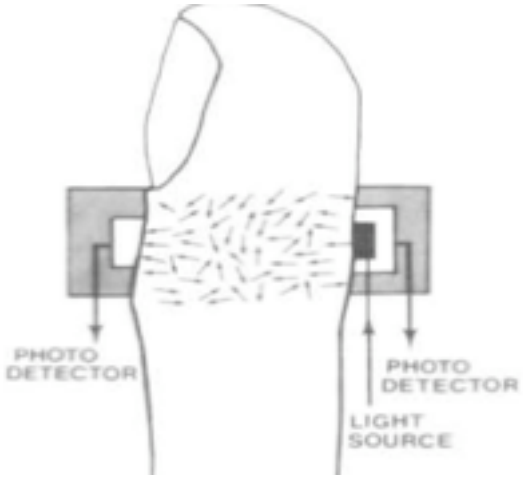

Figure 1. A transmission PPG consisting of a light source (most commonly an LED), light detector (photo diode), and tissue to be studied (path).

Conceptually, the pulse oximeter waveform is most easily viewed as measuring the change in blood volume (more specifically path length), during a cardiac cycle, in the region being studied (typically the fingertip or earlobe). The general consensus is that the waveform comes from the site of maximum pulsation within the arteriolar vessels where pulsatile energy is converted to smooth flow just before the level of the capillaries[9, 10].

The pulsatile component of the PPG waveform is often called the AC component and has a fundamental frequency typically around 1 Hz, depending on heart rate (Figure 2). This AC component is superimposed onto a large DC component that relates to the average tissue blood volume.[11] The DC component varies slowly due to respiration, vasomotor activity and vasoconstrictor waves, Traube Hering Mayer (THM) waves, and thermoregulation.[6, 12-22]

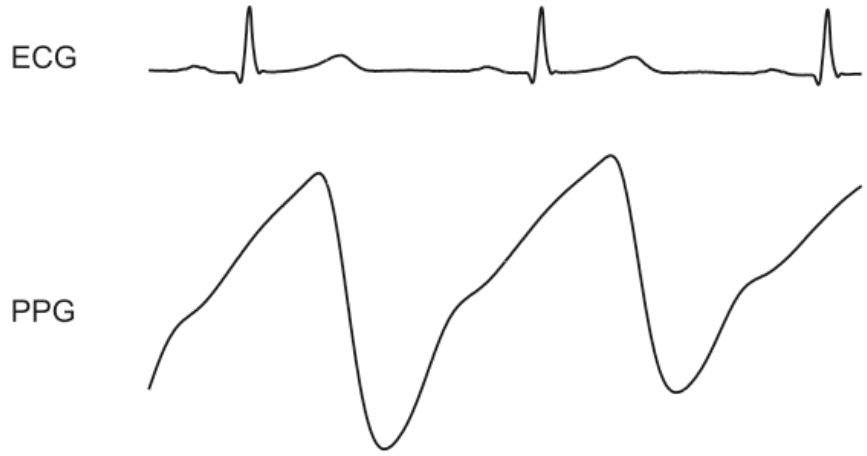

Figure 2. The pulsatile (AC) component of the PPG signal and corresponding electrocardiogram (ECG). The AC component is actually superimposed on a much larger DC component that relates to the tissues and to the average blood volume within the sample. It represents the increased light attenuation associated with the increase in terminal arteriolar blood volume with each heartbeat.[11]

In researching the PPG waveform, it is paramount to appreciate the fact that the waveform displayed on commercial pulse oximeters is a highly processed and filtered signal. Normally, equipment manufacturers use both auto-centering and auto-gain routines on the displayed PPG waveforms, as well as implement both high and low pass filtering systems (Figure 3).[4]

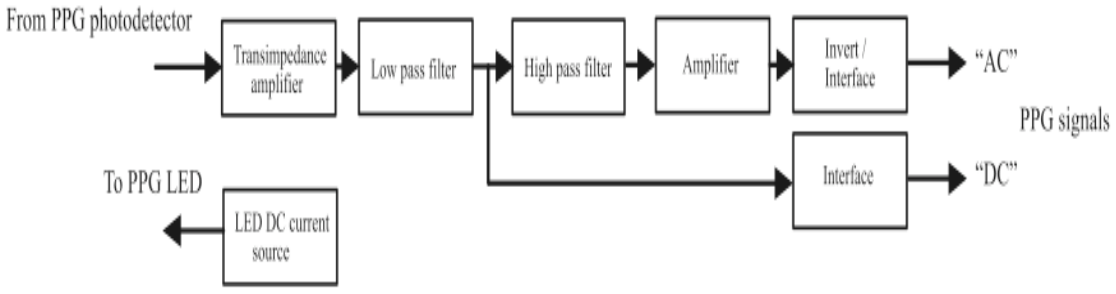

Figure 3. The signal conditioning stages surrounding the transimpedance amplifier which include low pass filtering, high pass filtering and further amplification, inversion and signal interfaces.[11]

Typically, there are two wavelengths measured by the pulse oximeter: 940 nm and 660 nm. Traditionally only the infrared signal (940 nm) is presented, because it is more stable over time, especially when compared to the red signal (660 nm), which is more susceptible to changes in the oxygen saturation.[4] In addition, only the pulsatile component or AC portion is displayed. The DC component is eliminated by an auto centering routine used to ensure the waveform remains on the display screen.[4] With changes in the degree of venous congestion, the waveform can be noted to drift partly off the screen and then return via the auto-centering algorithm. The auto-gain function is designed to maximize the size of the waveform displayed.[23] However, with its use, it becomes impossible to analyze parameters such as the amplitude of the PPG waveform.

WAVEFORM ANALYSIS

Despite the esthetic processing, the PPG waveform is still rich in information regarding the physiology of the patient. It contains a complex mixture of the influences of arterial, venous, autonomic, and respiratory systems on the central and peripheral circulation. When using clinical monitoring devices as research devices, one must learn how to cope with proprietary filters and algorithms, so as to extract underlying signals that contribute to the complex waveform and to study how these underlying signals vary over time[24]. Key to the successful interpretation of this waveform is the ability to separate it into fundamental components through both time and frequency domain analysis.

MONOTORING SITES

When examining the PPG waveform, taking note of the region of the body being measured is important. By their nature, pulse oximeters are pulse-dependent, and a site with adequate perfusion is required. Thus the performance of sensors placed on the extremities can be impaired by multiple conditions such as low perfusion[25,

[26] and motion artifact[27]. One solution to this problem is the placement of sensors on the head (e.g. ear, forehead, lip, and nose)[28]. This, of course, creates other issues such as challenges with method of attachment and potential reduction of signal strength.

In the finger, where the walls of the cutaneous vessels are richly innervated by alpha-adrenoceptors, the sensitivity to changes in the sympathetic nervous system are greater than when compared to other areas of the body such as the earlobe[23]. Thus, a technique such as plethysmography may be more influenced by changes in adrenergic tone at the finger than at other sites. This has prompted clinicians to explore the usefulness of plethysmographic signals at other sites as a means of minimizing the effect of local vasoconstriction on the measurements of oxygen saturation and assessments of waveform morphology[7,28].

During a cold immersion test, which is a classic method of eliciting a vasoconstrictive reflex by immersing a hand in ice water (4 ◦ C), it has been shown that the amplitude of the ear PPG was relatively immune to vasoconstrictive effect while the contralateral finger PPG waveform showed marked reduction in amplitude. This underscores an important idea in site specific PPG waveform analysis: the ear tracing may reflect central hemodynamic changes while the finger pulse oximeter waveform might be used as a monitor of sympathetic tone and peripheral vascular events[23].

Additional support for differing regulation of the central and peripheral microcirculation comes from an investigation showing contrasting responses, as measured by laser Doppler flowmetry, in forehead and finger microvascular flow in response to systemic administration of phenylephrine, a vasoconstrictive drug. This was accompanied by an induced oscillatory response that was detectable in the forehead microvasculature by measuring red cell flux with laser Doppler flowmetry. The oscillations occurred at a “high” frequency (0.18 Hz), which was consistent with mediation via parasympathetic pathways.[29-33] The cholinergic

etiology of these high frequency oscillations was confirmed by their elimination by systemic administration of atropine.[34]

Prior analysis of the PPG waveform during hypovolemia has shown that it can be modulated by positive pressure ventilation similar to ventilation induced variation of systolic blood pressure[35-37]. Furthermore, following the withdrawal of 450 ml of blood in healthy volunteers, there was a significant increase in the respiratory induced oscillation of the ear PPG tracing during spontaneous and continuous positive airway pressure ventilation despite the absence of changes in heart rate or blood pressure[38] In addition, prior work by members of our group has demonstrated the respiratory signal when measured in the ear PPG waveform is 10 times stronger compared to the finger PPG [39].

AMPLITUDE ANALYSIS

Plethysmographic analysis has typically focused on the amplitude of the waveform (Figure 5). Amplitude changes can be concealed by the auto-gain function found on most pulse oximeters. By turning off the auto-gain, certain observations can be made. For example, over a remarkably wide range of cardiac output, the amplitude of the PPG signal is directly proportional to stroke volume and local vascular distensibility[8]. With a decrease in stroke volume, a smaller volume of blood is delivered to the circulation with each cardiac cycle. This will result in decreased waveform amplitude, and vice versa. Additionally, if the vascular compliance is low, as in episodes of increased sympathetic tone, the pulse oximeter waveform amplitude is also low; in contrast, during vasodilatation, the pulse oximeter waveform amplitude is increased. It is important to realize that a large pulse amplitude does not imply the presence of high arterial blood pressure nor vice versa.[4] The pulse oximeter waveform amplitude decreases during significant increases in arterial blood pressure due to increased sympathetic tone. For example, during surgical incision there is normally a profound decrease in the amplitude of the finger PPG when compared to the ear

pinna (Figure 4). This observation has been interpreted as being directly related to the response of the peripheral vascular bed to sympathetic stimuli[7].

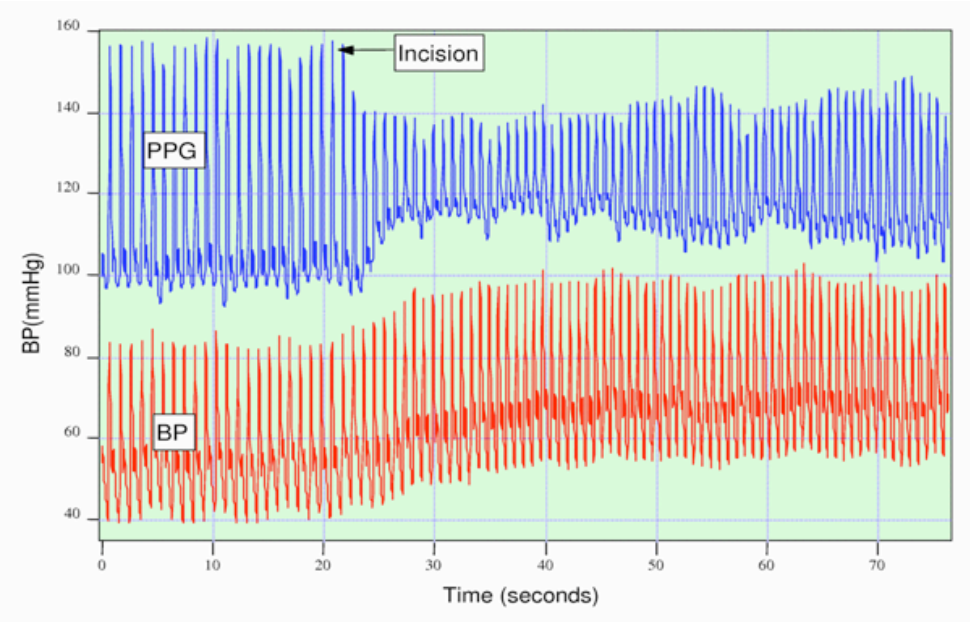

Figure 4. Effect of surgical incision on a subject under general anesthesia on the photoplethysmograph (PPG) and blood pressure (BP). With an increase in sympathetic tone, SVR increases as does BP, where as PPG amplitude decreases as a result of decreased vascular compliance seen with vasoconstriction.[40]

Once a baseline measurement has been established, the pulse oximeter amplitude can be followed as a gauge of sympathetic tone and systemic vascular resistance[41-43]. A potential use for such monitoring is in changes in arterial blood pressure, particularly hypotension, in critically ill patients. Hypotension is a late feature of circulatory collapse because compensatory measures, including changes in systemic vascular resistance (SVR), often mask hemorrhage and other forms of volume loss. The ability to continuously measure SVR and flow allows for early detection of active compensatory mechanisms, thereby enabling early detection of volume loss and characterization of the nature of the shock.[4] However, continuous monitoring of SVR requires use of invasive techniques, whereas with the PPG, this same information can potentially be obtained non-invasively.

WIDTH ANALYSIS

An appreciation of the changes in PPG pulse width (Figure 5) may provide valuable information with respect to changes in peripheral vascular tone, vessel caliber, blood pressure, and stroke volume. The PPG waveform width is measured in seconds and represents the time through which a volume of blood moves through a given vascular bed. When a fluid is in motion, it must move in such a way that mass (energy) is conserved. This principle is described by the continuity equation:

$A_1V_1=A_2V_2$

Where

A= cross sectional area

V= velocity

This equation, when applied to the microvasculature, is not an ideal representation of hemodynamics. First, the equation assumes a rigid container, and does not account for the distensibility and variable compliance of the vascular system. Second, the equation assumes that there is only one pathway by which fluid can move, whereas there are millions of vascular beds in series and parallel in the terminal arterioles and microcirculation. Still, it is a useful tool for describing what happens to blood velocity when vessel caliber is changed.

With a change in vessel diameter, blood will either change velocity, be redistributed to another region (parallel vascular bed), or a combination of these possibilities, in order to conserve energy. It is difficult to appreciate which of these factors predominates with vasomotor changes, due to the previously mentioned factors.

Thus, the PPG width is a complex measurement, especially in regions such as the

finger, which are highly susceptible to vasomotor activity. Width is also dependent on heart rate, stroke volume, and PPG amplitude. A decrease in stroke volume whether due to hypovolemia or an increase in heart rate will cause the width to decrease regardless of what is happening to vessel diameter. Also, as width is conventionally taken at the point of half the amplitude, as amplitude changes so does the point at which width is measured.

Despite these confounding factors, the usefulness of PPG width as a measure of vasculature tone and thus autonomic activity is intriguing and will be further investigated in this thesis. Prior research by our team has shown that the ear PPG pulse width correlates highly with changes in systolic blood pressure, mean blood pressure, and pulse pressure during open heart surgery[44]. Specifically, the ear pulse oximeter beat width was a strong predictor ($r = 0.80$) of blood pressure measured invasively from the radial artery[44]. Further, the ear PPG width was inversely related to CO and HR with correlation coefficients of ($r = –0.71$) and ($r = –0.824$) respectively during open-heart surgery. These findings suggest that with the increase in CO (either with increase HR and/or increase in contractility), the entire body receives more blood flow and therefore the duration of the PPG waveforms become shorter (reduction in the width of the pulse wave).[45]

Additional investigation by our group with a cold pressor test, a classic method of eliciting a vasoconstrictive reflex by immersing a hand in ice water (4° C), demonstrated that upon hand immersion there was an increase in both systolic and diastolic pressures and reflexive decrease in heart rate. As a result of the increase in sympathetic tone, there was also a reduction of the average finger PPG width and a concurrent increase in ear PPG width. In proposing an explanation, we assumed that blood moved from an area of vasoconstriction to other sites that are less vasoconstricted, for example the ear or face. Thus, as a result of an increased blood volume load, paired with the low vascular tone of the ear blood vessels, the width of the ear pulse oximeter waveform increased.[45] In summary, with regard to insight into vascular response, the PPG width can be seen as a measure of blood

volume transit time.

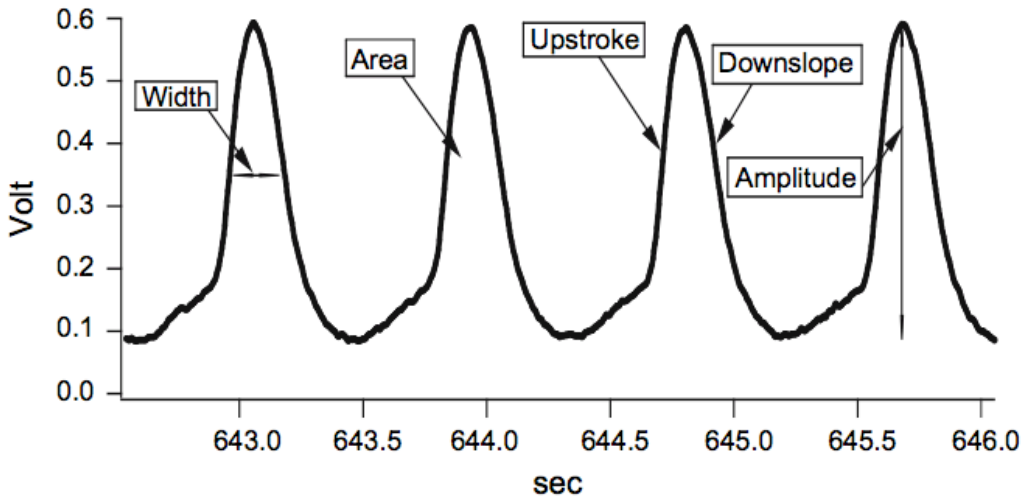

Figure 5. Parameters studied when analyzing the photoplethsymographic waveform.

RESPIRATORY VARIABILITY ANALYSIS

Adolf Kussmaul initially described inspiration induced pulsus-paradoxus in 1873[46]. Since that time the effect of respiration on the peripheral pulse has been described in a number of contexts.

Positive pressure ventilation of an intubated patient can have a significant impact on both cardiac preload and afterload and, hence, stroke volume[47-50]. Perel and associates used the term ‘‘systolic pressure variation’’ to describe the impact of positive pressure ventilation on the arterial pressure waveform. These investigators concluded that the degree of systolic pressure variation as a consequence of the positive pressure is a sensitive indicator of hypovolemia[51-53]. Moreover, this variation is significantly more sensitive than heart rate (HR), systemic blood pressure (BP) and central venous pressure for identifying blood loss[52, 53]. In addition, changes in systolic pressure variation as well as pulse

pressure variation correspond closely to changes in pulmonary artery wedge pressure, left ventricular end-diastolic area, and stroke volume[35, 37, 54-58].

Consistent with Kussmaul's initial report[46], systolic BP variation of the arterial waveform also has been noted in spontaneously ventilating non-intubated patients. However, the effect has been less pronounced and less consistent than during mechanical ventilation[57]. .It has been hypothesized that with each positive pressure breath, venous return to the heart is impeded resulting in a temporary reduction in cardiac output [49]. As a patient becomes volume depleted, with a resulting decrease in venous pressure, positive pressure ventilation has an exaggerated impact on the arterial blood pressure. Positive pressure-induced changes have also been detected in the PPG tracing of pulse oximeters (figure 6)[8, 36, 59-62]. The ability to detect the influence of the respiratory system on the cardiovascular system opens many possibilities. For example, respiratory rate may be reliably determined using the plethysmographic waveform[63-67]. Addionally, several groups have demonstrated that ventilation-induced waveform variability of the PPG signal could be used as an indicator of hypovolemia[36, 69].

Both the IR and red PPG traces show considerable low-frequency modulation of the waveform amplitude and baseline, occurring at the respiratory frequency (10/min [almost equal to] 0.17 Hz, Fig 6). Ventilation has two distinct effects on the PPG waveform: fluctuation of both the baseline (DC) and pulsatile (AC) components of the PPG waveform with both spontaneous and positive pressure ventilation.[38] The most common modulation is a shift in baseline with each breath, which is recorded as a modulation in the DC component of the waveform (Figure 7). Shifts in the baseline are associated with changes in the venous (i.e. nonpulsating) volume in the vasculature. The other phenomenon, less commonly seen, is variation in amplitude of the pulse beats. This AC modulation is caused by changes in arterial pressure induced by positive pressure ventilation and is only significant in hypovolemic states[38].

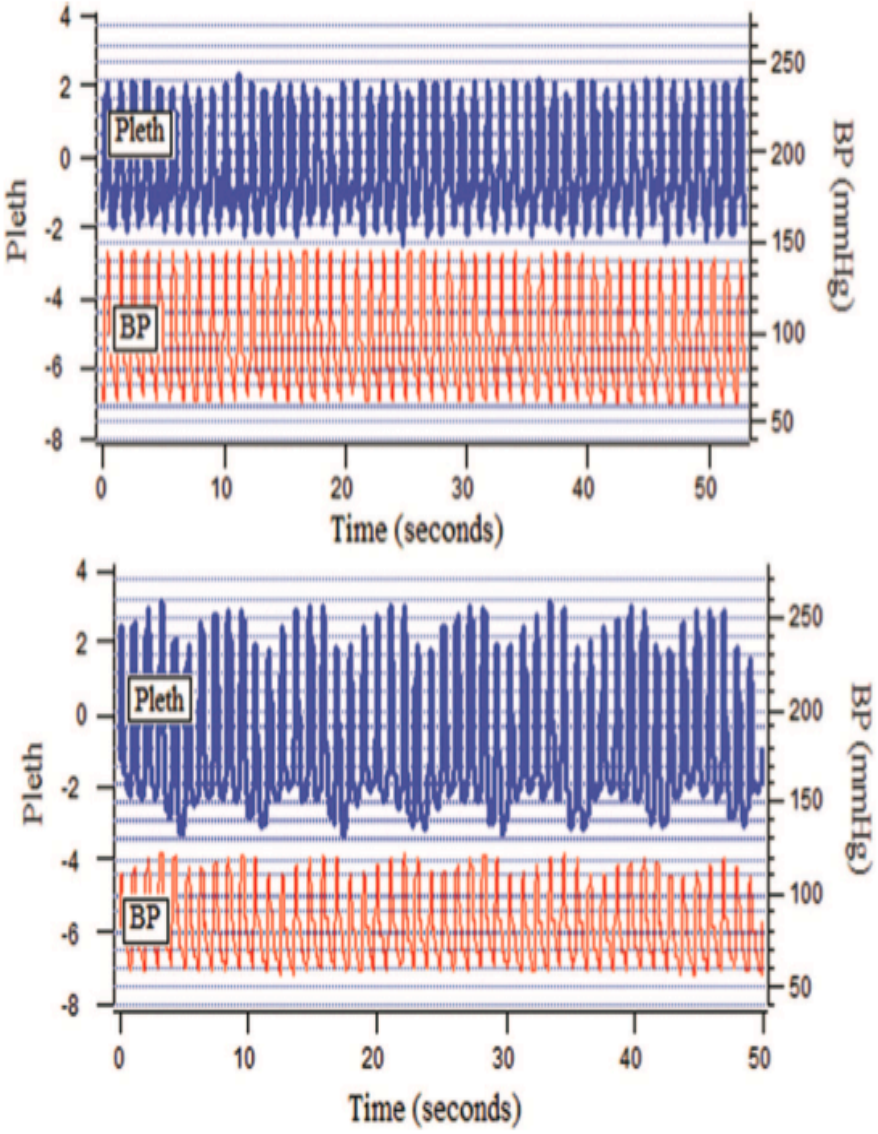

Figure 6. The effect of blood loss on the pulse oximeter waveform (Pleth) and arterial pressure waveform (BP). The upper diagram shows the baseline waveforms of the patient under general anesthesia with positive pressure ventilation. The lower diagram is after a 1000 mL blood loss. The effect of positive pressure ventilation is apparent.[38]

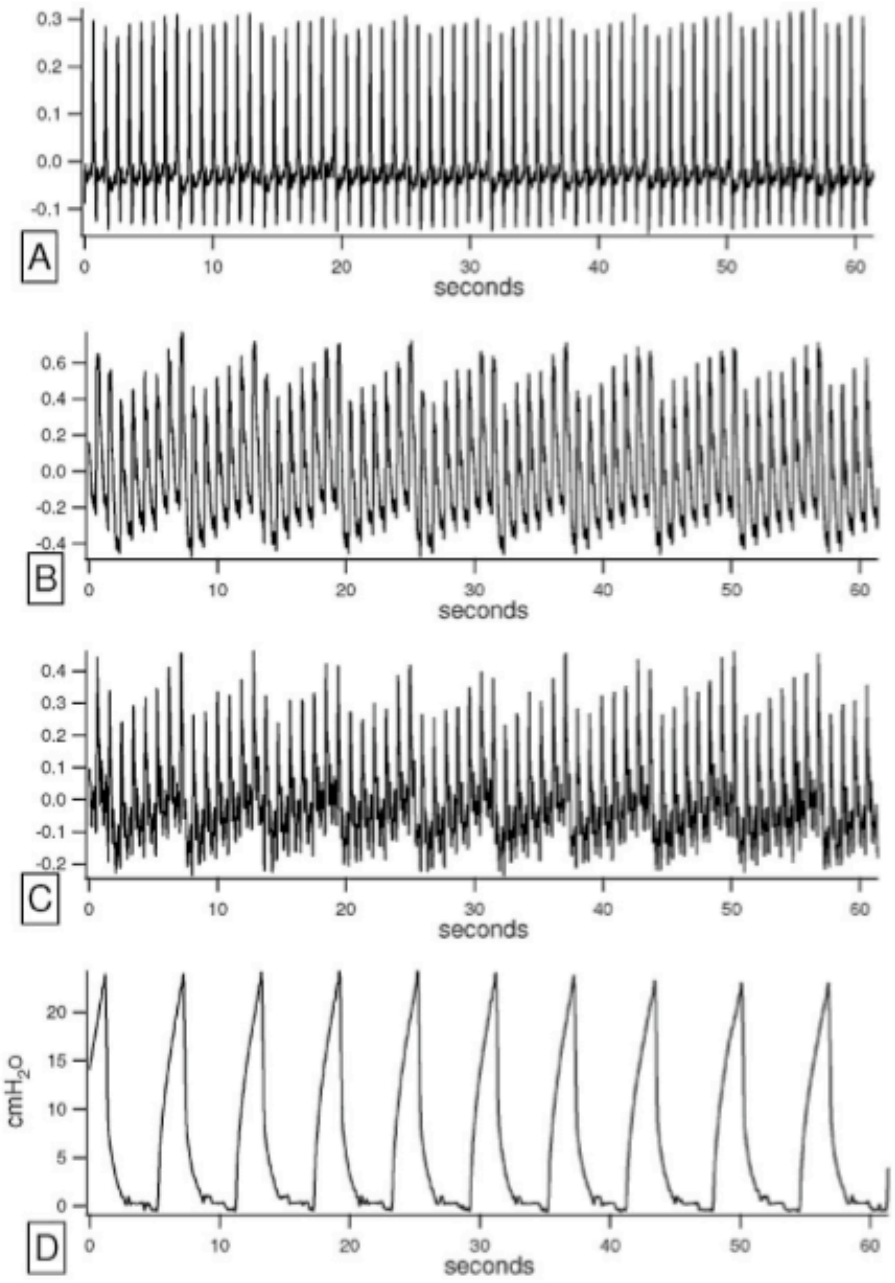

Figure 7. Tracings of the plethysmographs of the finger (A), ear (B), forehead (C), and peak airway pressure (D) from a patient undergoing radical prostatectomy (60-s segment).[39]

Monitoring the respiratory variability seen in the pulse oximeter waveform may be a useful method of detecting occult hemorrhage, with its resulting hypovolemia[70, 71]. There are ongoing research efforts designed to find the best site and method of analysis for quantifying the effects of ventilation on the PPG waveform[39, 72].

However, since the main purpose of these devices is determination of arterial oxygen saturation, most oximeters have an auto-centering algorithm and a high-pass filter, which excludes frequencies below those of cardiac pulsations – the goal being to facilitate visualization of the pulsatile signal rather than facilitate assessment of respiration-induced changes. In addition, oximeters most commonly are used on the finger, a region rich in sympathetic innervation that often reflects local (as opposed to systemic) alterations in vascular tone and volume status[5, 8, 23, 37].

In contrast to positive pressure mechanical ventilation[49], spontaneous ventilation generates negative inspiratory pressure with transient shifting of blood from the peripheral vasculature to the lungs and pooling therein. This blood volume shift causes a decrease in peripheral venous volume as a well as a transient decrease (followed by an increase) in left ventricular filling. These constant shifts in central volume cause different effects (magnitude as well as relative timing) than positive pressure ventilation on venous volume in the periphery, venous flow from the peripheral to the pulmonary vasculature, and emptying of pulmonary beds (left ventricular preload) as well as on left ventricular afterload[4, 38].

Our colleagues have previously examined the theory that blood loss could be monitored through the PPG. A study was undertaken to determine the effect of the removal of 450 ml of blood on respiration- induced oscillations of the ear PPG waveform during spontaneous ventilation in healthy volunteers.[38] That study indicated that assessment and quantification of the respiration-induced changes in

the ear PPG waveform has the potential of detecting volume changes in the non-intubated patient, through quantification of respiration-induced cyclical variation of the ear PPG.

AUTOMONIC MODULATION

To date little has been studied or documented regarding autonomic influences modulating the PPG signal.

LOWER BODY NEGATIVE PRESSURE

Lower body negative pressure (LBNP) will serve as our experimental model of hypovolemia. Application of negative pressure to the lower body redistributes fluid from the upper body to the lower extremities, allowing for the study of hemodynamic responses to central hypovolemia[73-75]. Since the first description of this research tool[76], LBNP has been used by aerospace investigators to study such physiological phenomena as post space flight orthostatic intolerance and exposure to vertical acceleration in high-performance aircraft[73, 75]. Convertino[73] investigated the utility and reproducibility of LBNP as a technique to study cardiovascular adjustments to stressors such as hemorrhage and shock and suggested that LBNP is a useful surrogate to study these factors in humans. Thus LBNP is an established model for the study of central hypovolemia[73].

Based on linear relationships between either hemorrhage or LBNP and central venous pressure or stroke volume, it appears that moderate LBNP on the order of 10–20 mmHg is equivalent to blood loss of 400–550 ml[77]. Data outlining relationships between more severe hemorrhage and levels of LBNP are less clear, but suggest that LBNP between 20 and 40 mmHg corresponds to blood loss of between 500 and 1,000 ml, and LBNP of 40–60 mmHg corresponds to 1,000-ml blood loss (Table 1).

LBNP	Hemorrhage
10–20 mmHg 400–550 ml fluid displaced	Mild 400–550 ml ≅10% of total blood volume
20–40 mmHg 500–1,000 ml fluid displaced	Moderate 550–1,000 ml ≅10–20% of total blood volume
≥40 mmHg ≥1,000 ml fluid displaced	Severe >1,000 ml >20% of total blood volume

Table 1. Classification of hemorrhage severity in humans and magnitudes of lower body negative pressure.[77]

A summary of physiological response comparisons between hemorrhage and LBNP based on an outstanding review on the subject by Convertino and Cooke is shown in Table 2 and is as follows: Heart rate progressively increases with hemorrhage or LBNP until shock or cardiovascular collapse occurs. Collapse is associated with relative bradycardia at high-level LBNP and during hemorrhagic shock, although 60– 70% of severely bleeding patients respond with tachycardia. Arterial pressures are either maintained or slightly increased with progressive reductions of stroke volume, cardiac output, and central venous pressure, until the onset of shock or collapse associated with abrupt hypotension. Under both conditions of hemorrhage and LBNP, sympathetic neural activation is fundamental to the maintenance of arterial pressure, and either blunted or exaggerated sympathetic activation occurs before shock or collapse. However, the onset of hypotension occurs in conjunction with sympathetic neural withdrawal. Also shown in Table 2 are differential vasoactive and volume regulatory hormonal responses at various stages of hemorrhage and levels of LBNP. [77]

Classification	Stage I	Stage II	Stage II	Stage III
Hemorrhage	Mild	Moderate	Severe	Shock
LBNP	10–20 mmHg	20–40 mmHg	>40 mmHg	Collapse
Variable				
HR	↑ ↔	↑ ↑	↑ ↑	↑ ↓ ↓
MAP	↔ ↔	↑ ↔	↑ ↑	↓ ↓
SV	↓ ↓	↓ ↓	↓ ↓	↓ ↓
Q̇c	↓ ↓	↓ ↓	↓ ↓	↓ ↓
CVP	↓ ↓	↓ ↓	↓ ↓	↓ ↔ ↓↔
SNA	↑ ↑	↑ ↑	↑ ↑	↓ ↓
NE	↑ ↑	↑ ↑	↑ ↑↔*	↓ ↓
PVR	↑ ↑	↑ ↑	↑ ↑	↓ ↓
AVP	↔ ↔	↔ ↔	↔ ↔↑*	**NA** ↑
PR	↔ ↔	↔ ↔	↔ ↔↑*	**NA** ↑
ANG II	**NA** ↔	**NA** ↔	**NA** ↑	**NA** ↑
PPH	↔ ↔	↔ ↔	↑ ↑	↑ ↑

Under each condition, variables either increase (↑), decrease (↓), do not change (↔), or show differential responses (↓↔; ↔↑). Responses to hemorrhage are shown in bold font, and responses to LBNP are shown in regular font. NA, data not available or too limited to present; HR, heart rate; MAP, mean arterial pressure; SV, stroke volume; Q̇c, cardiac output; CVP, central venous pressure; SNA, sympathetic nerve activity; NE, norepinephrine; PVR, peripheral vascular resistance; AVP, arginine vasopressin; PR, plasma renin; ANG II, angiotensin II; PPH, pancreatic polypeptide hormone. *Directional changes only in subjects susceptible to hemodynamic collapse or at the onset of hypotension.

Table 2. Comparison of global physiological responses to hemorrhage and LBNP.[77]

Due to local autoregulation of various vascular beds, evaluation of catecholamines and other vasoactive hormones from plasma samples provides little insight beyond global responses into progression to cardiovascular collapse. The summary data presented in Table 2 show that, physiological responses to hemorrhage and LBNP are similar, suggesting that LBNP is a useful model to simulate acute hemorrhage in humans.[77]

THE LASER DOPPLER FLOWMETR AS AN ADJUNCT INVESTIGATION TOOL

Laser Doppler flowmetry is a method of non-invasive, continuous measurement of the microcirculation, thought to be at the level of the precapillaries and

capillaries. The technique is based on the values of the Doppler effect of low-power laser light scattered randomly by static structures and moving tissue particulates. Like the PPG, it too is useful for measuring regional difference in microvascular flow as well as both sympathetic and parasympathetic influences on the microvasculature.[34, 78]

Previous investigations showed a differing response in forehead and finger microvascular flow in response to systemic administration of phenylephrine, a vasoconstrictive drug. This was accompanied by an induced oscillatory response that was detectable in the forehead microvasculature by measuring red cell flux with laser Doppler flowmetry. The oscillations occurred at a "high" frequency (0.12 Hz), which was consistent with mediation via parasympathetic pathways.[29-33] The cholinergic etiology of these HF oscillations was confirmed by their elimination by systemic administration of atropine.[34]

In part because of its complex second-messenger system, sympathetic transmission to adrenergic effector sites does not elicit oscillatory responses at frequencies > 0.12 Hz.[79] Spontaneous (myogenic) activity also tends to occur at a slower frequency and is less coordinated.[34] Further, the elicitation of the oscillatory activity by phenylephrine, a known activator of a parasympathetic homeostatic response at the heart, and elimination of the oscillatory activity by atropine provided strong evidence of a parasympathetic microcirculatory process.[34]

Laser Doppler flowmetry can thus provide a useful corollary to the study of the PPG waveform. It provides direct information about microvascular flow at a site just distal to that measured by the PPG. Additionally, it contains information pertaining to autonomic activity in the microcirculation.

PURPOSE AND HYPOTHESIS

The purpose of this investigation was to explore modulation of the PPG waveform in the setting of simulated hypovolemia as a tool to distinguish regional differences in regulation of the microvasculature. To this end, we examined the interaction of the cardiovascular, autonomic nervous, and respiratory systems in regulating the functional hemodynamics of microvascular blood flow to both central (ear, forehead) and peripheral (finger) sites. This study can be broken down into two parts: time domain analysis and frequency domain analysis

We first utilized time domain analysis to characterize changes in the amplitude and width of the PPG waveform during LBNP simulated hypovolemia. The underlying hypothesis is that the PPG waveform can be used to detect, elucidate, and characterize these differences, as well as offer plausible explanations for these differing vascular responses to hypovolemia. We reasoned that monitoring site-specific morphological differences would provide insight into the differing regulatory vascular response to hypovolemia: amplitude is more reflective of vessel compliance and caliber; width is more sensitive to blood flow transit time.

In addition to these regional measurements, we also monitored capillary and pre capillary flow of the finger and forehead (via laser Doppler flowmetry), heart rate, heart rate variability, systolic, diastolic, and mean blood pressure, and an indirect measure of stroke volume.

We also studied, through frequency domain analysis, the effect of hypovolemia on respiration-induced oscillations of the ear PPG waveform during spontaneous ventilation in healthy volunteers. We hypothesized that quantification of these oscillations can be used as monitor of volume status that is more sensitive than the classically used changes in heart rate and blood pressure. The presence of steadily increasing oscillations occurring at the respiratory frequency would support our hypothesis as well as underscore the potential of PPG as a non-invasive monitor

of volume status.

METHODS

Subjects

A total of 10 nonsmoking subjects (8 men and 2 women) volunteered to participate in this study (age, 24-37; height, 152.4 – 190.5 cm; weight, 46.4 – 90.4 kg). Before admission into the study, all subjects underwent a brief medical history to ensure they had no previous or current medical conditions that might preclude their participation (such as cardiovascular or pulmonary disease). Subjects received a verbal briefing and written descriptions of all procedures and risks associated with the study and were made familiar with the laboratory, the protocol, and procedures. Subjects were encouraged to ask questions of the investigators, and then they signed an informed consent form approved by the Institutional Review Board for the protection of human subjects.

Experimental Protocol

Central hypovolemia was induced by application of LBNP to simulate, as closely as possible in healthy human volunteers, the hemodynamic challenges associated with severe hemorrhage. Subjects were positioned supine within an airtight chamber that was sealed at the level of the iliac crest by way of a neoprene skirt. Breathing frequency was regulated, with subjects breathing to a metronome set at a rate of 12 breathes per minute (0.2Hz). Each subject underwent exposure to progressive LBNP until the point of impending cardiovascular collapse. The full LBNP protocol consisted of 3-minute windows at baseline, -30, -60, -75, and -90 mm Hg of chamber decompression, followed by a variable recovery period. The end point of the study was completion of the protocol or cardiovascular collapse as defined by one or a combination of the following criteria:

- Onset of hypovolemia associated symptoms (light headedness, nausea, diaphoresis).
- Predefined change in the subject's vital signs (a doubling of baseline heart rate, systolic blood pressure less than 90 mmHg with the onset of any symptoms, or systolic blood pressure less than 80 mmHg regardless of symptoms).
- Voluntary subject termination due to discomfort.

This study was performed in a climate-controlled chamber with temperature set at 25ºC and humidity set at 20%.

To account for differences in LBNP tolerance between subjects, the highest level of negative pressure that a subject was able to tolerate, as indicated by a stability of vital signs and absence of symptoms, was titled "pre-symptomatic". Additionally, the level of negative pressure during which a subject experienced symptoms was titled "symptomatic". All other common intervals were recorded as barometric readings of the negative pressure chamber.

Clinical Monitoring

All subjects were monitored non-invasively. A standard 3 lead ECG was used to monitor heart rate and rhythm. A finger blood pressure cuff recorded continuous beat-by-beat finger arterial pressure (Finometer Blood Pressure Monitor, TNO-TPD Biomedical Instrumentation, Amsterdam, The Netherlands). Plethysmographic monitoring was obtained with fixed-gain pulse oximeters (Oxypleth; Novametrix Medical Systems, Inc., Wallingford, CT) placed on the forehead, ear, and finger contralateral to the blood pressure cuff. 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 blood pressure cuff. Application of a force transduction belt around the chest enabled breath-by-breath detection of respiratory rate and depth. Figure 8 shows

an image of the experimental setup.

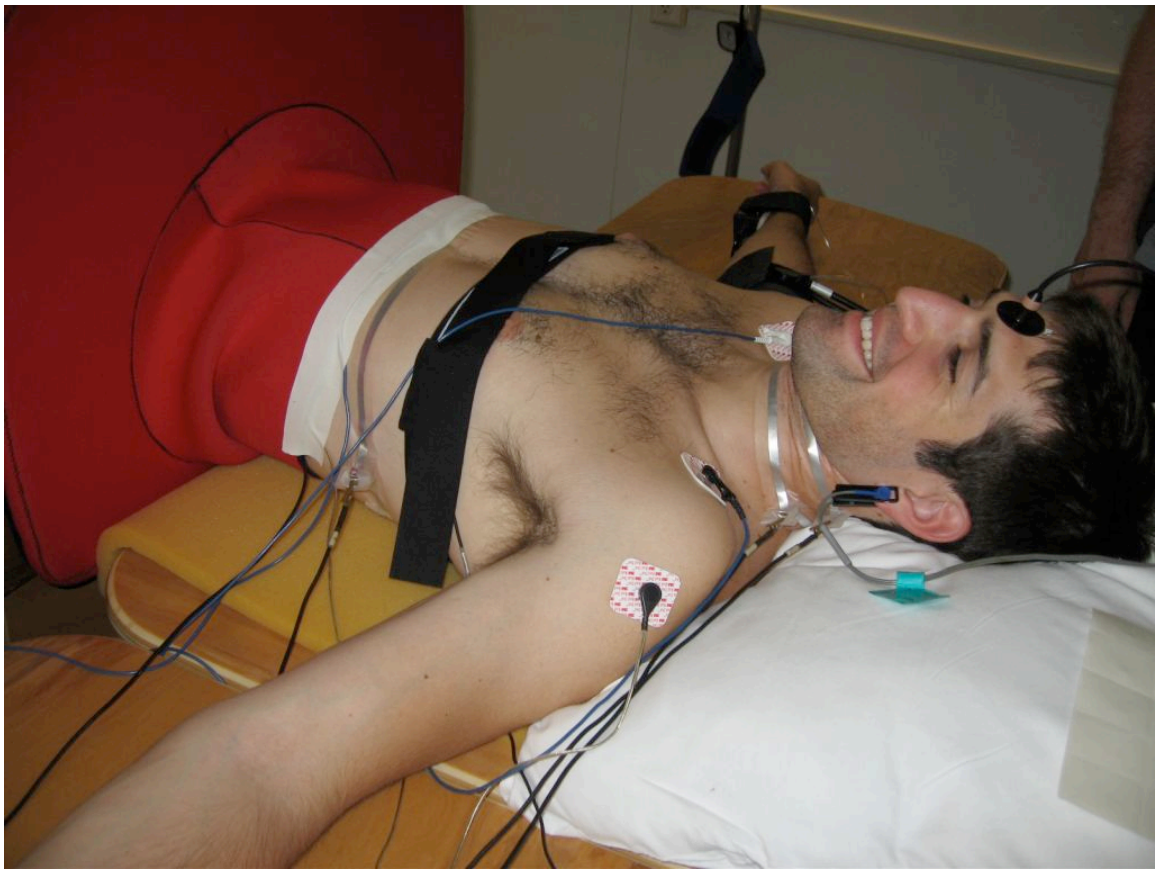

Figure 8. The experimental setup displaying the monitoring and lower body negative pressure chamber.

Data Analysis

All waveform tracings were sampled at 200 Hz and digitized to computer with a microprocessor-based data acquisition system, using commercially available data acquisition software (PowerLab, ADInstruments). Sixty second samples of data from each LBNP stage were extracted for analysis.

The PPG tracing was analyzed in the frequency domain using a fast Fourier transform (FFT) algorithm for detection and quantification of respiratory induced oscillations. The resulting discrete Fourier transform shows the spectrum of

frequencies contributing to the PPG signal, plotted as amplitude against frequency. The amplitude spectrum was produced using a 90 s 8192-point Hamming window and the zero-frequency component was removed.

The PPG data from each method during each phase were analyzed with the spectral-domain analysis module of the Chart software (ADInstruments) using a Hanning window with 50% overlap based upon the formula:

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

Where in G_{aa} is the instantaneous amplitude spectral density of channel ''a'' (i.e., of the PPG waveform), S_a is instantaneous amplitude spectrum of channel ''a'', $S_a^*$is complex conjugate of Sa, and df is frequency resolution. These were displayed with oscillatory frequency (Hz) on the x-axis and oscillatory power at each frequency (volt2/Hz) on the y-axis. The respiration-induced variation of the ear plethysmogram was quantified at the set respiratory frequency (0.2Hz).

Further, time domain analysis of height and width was accomplished with the peak and cyclic variable analysis module of the Chart software (ADInstruments), respectively. Waveform amplitude was taken at the maximum height from baseline and width was taken as the pulse width at the 50% peak amplitude height (see figure 5).

Statistical Analysis

All statistical analysis was done using SPSS. For the time domain analysis, data were compared and analyzed with paired t-tests (for vital signs and PPG amplitude), and linear regression analysis (for PPG width analysis). For the frequency domain analysis, data were compared and analyzed using the Wilcoxon Signed Ranks Test (For respiratory and autonomic modulation). P-values less than 0.05 were considered statistically significant. P-values greater than 0.05 are

reported as NS. Power analysis indicated that six subjects would be required to detect a 50% intra-subject change in respiratory induced oscillations of the plethysmographic signal, with an alpha of 0.05 and a power of 0.90.[38]

RESULTS

The level of negative pressure tolerated by each subjects was variable, and ranged from -40mmHg to -110mmHg of chamber decompression. Two subjects, one male one female, did not experience symptoms and thus were not included in the final analysis as the presence of symptoms (SYMPT) was taken as an indicator of initial cardiovascular decompensation. This brought the total number of subjects included in the study to 8. The reason for study termination for all other subjects was a combination of vital sign changes and symptomatology.

VITAL SIGNS

Mean finger arterial blood pressure declined slowly with progressive increases in LBNP. The overall change in mean arterial pressure from the baseline period to onset of symptoms was 7.4% ± 3.8% ($p < 0.05$), from 99.7mmHg ± 10.3mmHg to 90.7mmHg ± 11.8 mmHg. There was a 52.4% ± 13% ($p < 0.05$) decrease in mean pulse pressure, from 56.5mmHg ± 8.6mmHg to 25.8mmHg ± 9.8mmHg. This was attributable to a 17.9% ± 4.4% ($p < 0.05$) decrease in mean systolic pressure and a 4.7% ± 2.5% ($p < 0.05$) increase in mean diastolic pressure. This change was accompanied by a 65.7% ± 26.5% ($p < 0.05$) progressive decrease in area of the pressure waveform and a progressive decrease in stroke volume based upon contour of the Finometer waveform. These results can be seen in table 3.

Table 3

	Baseline	**Presympt**	**Sympt**	**Recovery**
SBP	138.7(9.0)	121.3(98)	113.3(10.0)	129.4(7.4)
DBP	82.2(12.0)	84.1(11.9)	87.5(11.6)	83.4(11.1)
PP	56.5(8.6)	37.3(9.3)	25.8(6.6)	46.0(5.6)
MBP	99.7(10.3)	91.1(11.6)	90.7(11.8)	97.9(9.8)

Table 3. Changes in MAP, systolic, and diastolic blood pressure during LBNP.

Over the course of the lower body negative pressure protocol, the heart rate (mean ± SD) increased 62.0% from 66.4 ± 12.4 beats/min at baseline to 107.6 ± 29.2 beats/min at the symptomatic phase, and decreased to 57.81 ± 9.0 beats/min during recovery ($p < 0.05$ for recovery vs. baseline and for recovery vs. symptomatic (Figure 9)).

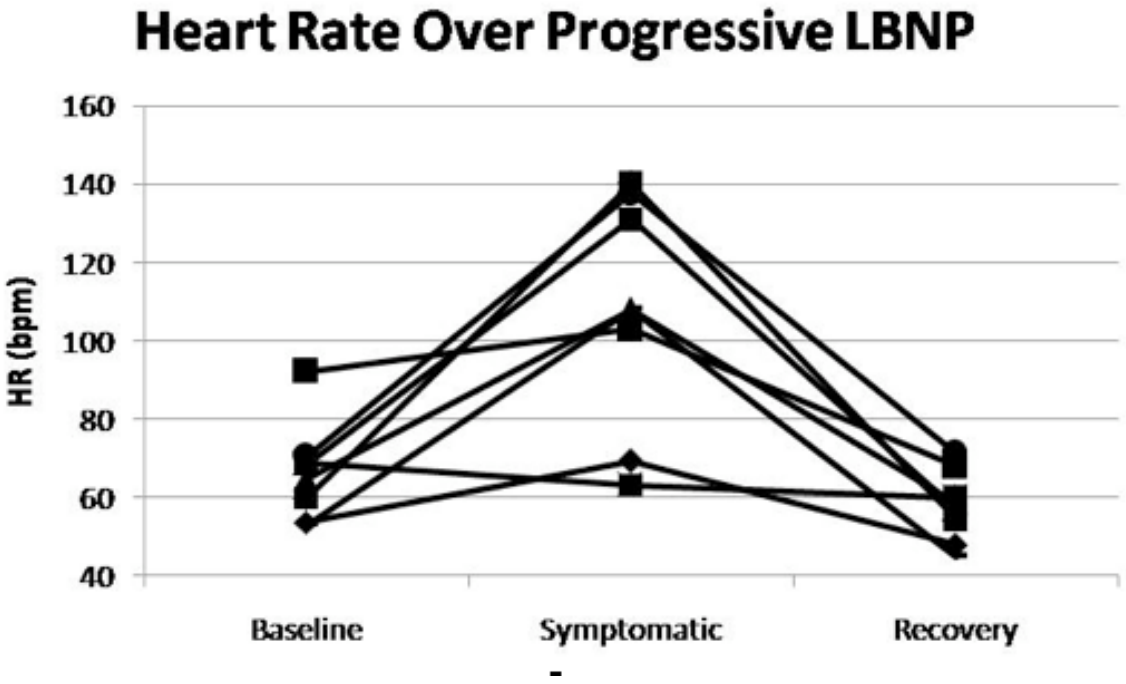

Figure 9. Mean Heart rate increased by substantially over the course of the LBNP protocol and decreased to sub baseline levels during the recovery period.

Figure 10 summarizes the changes in HR and systolic BP.

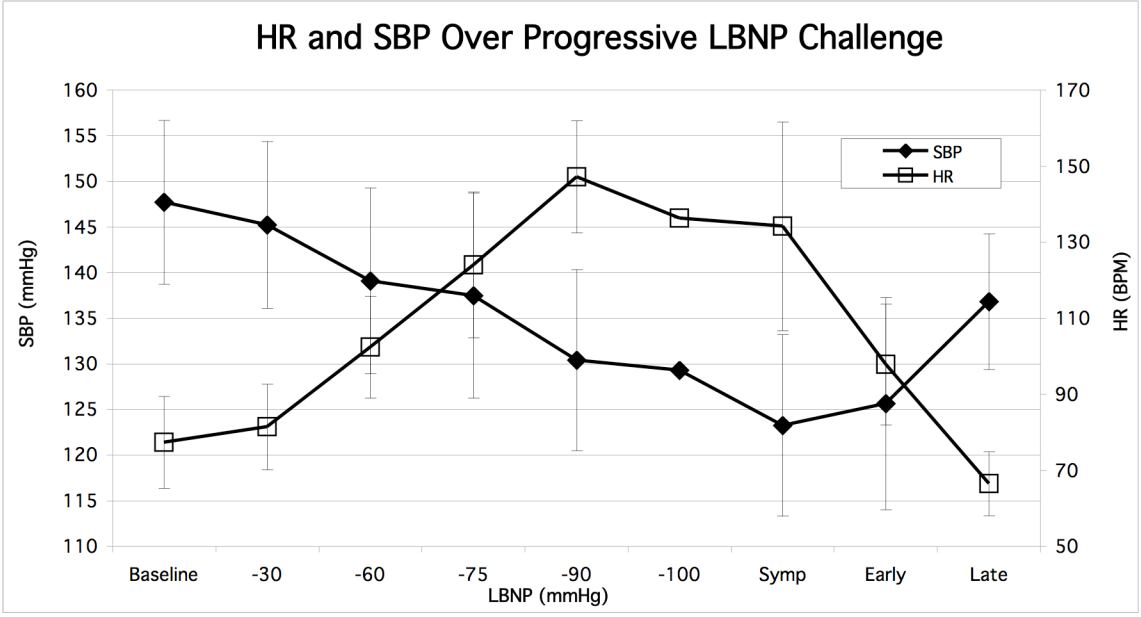

Figure 10. The classically described and expected observation, hypotension and tachycardia, were the responses to LNP induced hypovolemic challenge.

AMPLITUDE ANALYSIS

Finger PPG amplitude (height) decreased in all subjects during progressive lower body negative pressure challenge. Overall, the finger PPG amplitude decreased by 34.6 ± 17.6% ($p = 0.009$; Fig 11) between baseline and the highest tolerated LBNP. In contrast, forehead amplitude changed by only 2.4 ± 16.0% (p=NS; Fig 12). The intra-subject differential response between changes in finger and forehead was statistically significant ($p = 0.02$).

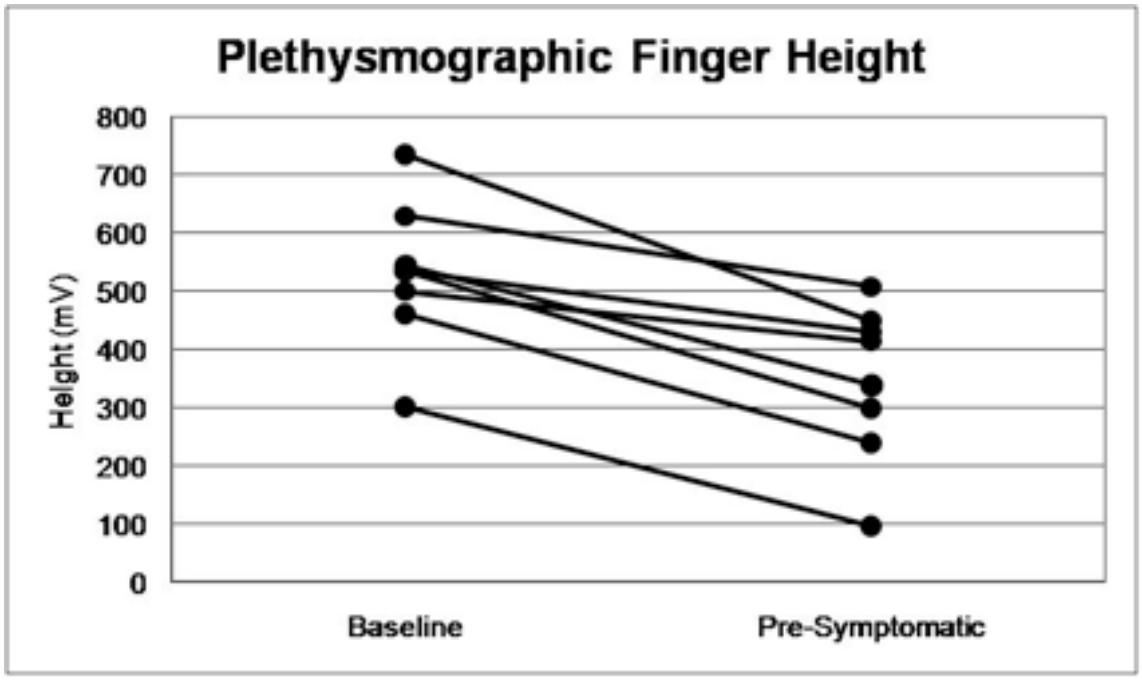

Figure 11. Overall, the finger PPG amplitude decreased by 34.6 from the start of the study to the highest level of tolerated lower body negative pressure.

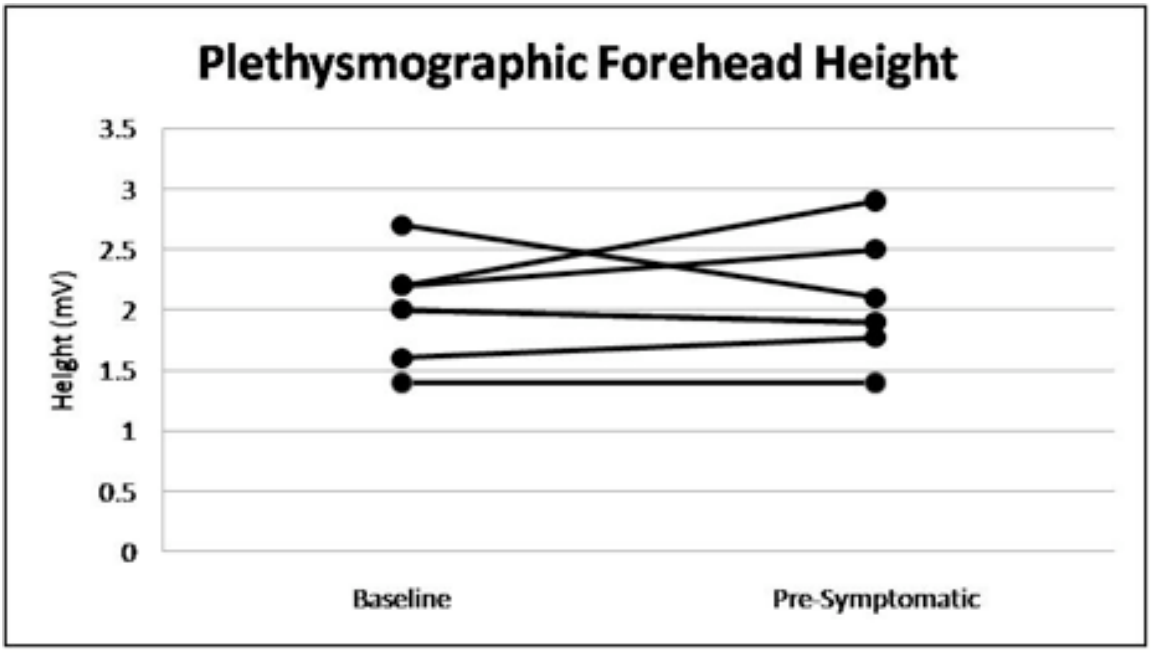

Figure 12. There was overall no change in the forehead PPG amplitude between the start of the study and the highest tolerated level of LBNP.

WIDTH ANALYSIS

Over the course of a 40-minute progressive LBNP challenge, the average decrease in MAP was only 7.4 mmHg. There nonetheless were declines in forehead and finger PPG width of 48.4% and 32.7%, respectively. Linear regression analysis of the forehead and finger plethysmographic waveform widths generated slopes of -1.113 (R = -0.727, Fig. 13) and -0.591 (R = -0.666, Fig. 14), respectively. Linear regression of forehead is included in Figure 14 for comparison (dashed line). Forehead width was also significantly greater than that of the finger at baseline, 210.4ms compared to 163.2ms. However, by the end of the study, both widths had converged to similar durations ~120ms.

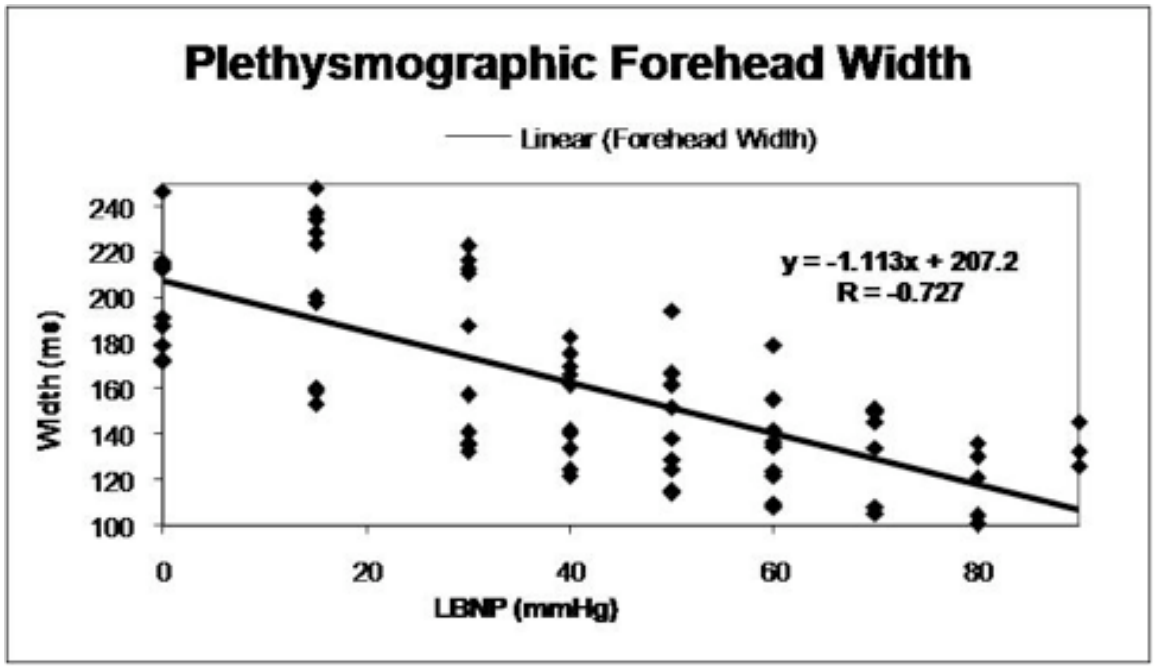

Figure 13. There was a steady decrease in forehead PPG width. Linear regression analysis revealed a slope of -1.113 (r = -0.727).

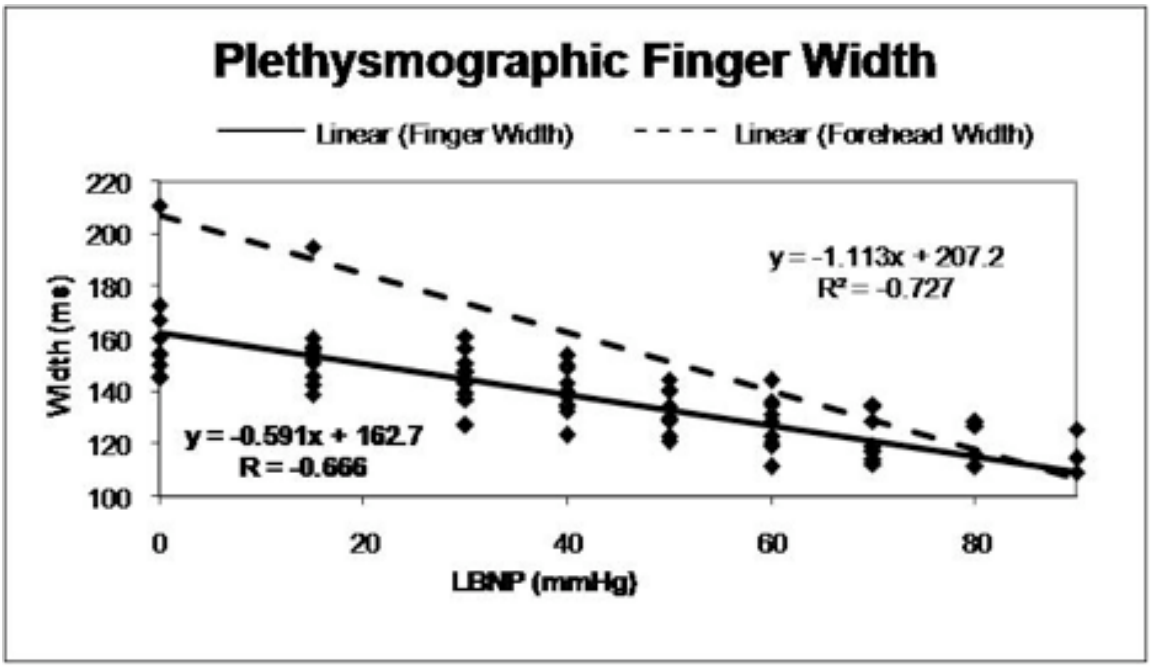

Figure 14. Linear regression analysis of forehead (dashed line) and finger (solid line) PPG. The forehead width decreased more rapidly than that of the finger and to a greater overall extent.

RESPIRATORY MODULATION

Amplitude density increased in the respiratory frequency band of the PPG waveform during the progressive hypovolemic challenge. Overall, the respiratory modulation of the ear PPG increased ($p=0.021$, Wilcoxon) from baseline to the symptomatic phase (150%) as measured within the respiratory frequency band (0.19-0.3) (Figure 15).

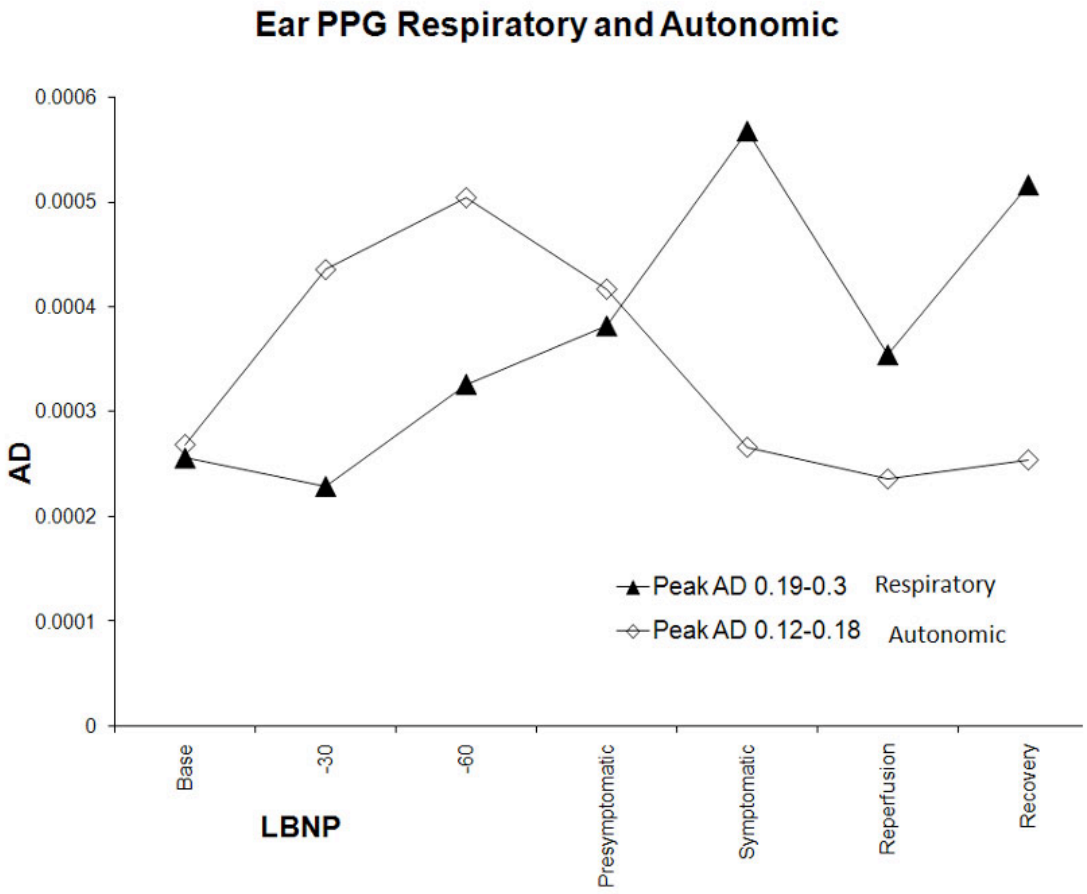

Figure 15. Displays the overall trend in amplitude density for the ear PPG at both the respiratory and autonomic frequency bands.

With the application of progressively greater levels of negative pressure, the ear PPG waveform was modulated in part by the autonomic nervous system, in addition to the respiratory system. A representative example of this phenomenon can be seen in Figure 16, which shows respiration occurring at a rate of 0.4Hz, where as the PPG is oscillating at 0.17 Hz, a known parasympathetic frequency. Within the ear PPG autonomic frequency (0.12-0.18) there was first an increase (87%), during the early stages of LBNP, followed by a decrease (return to baseline), as the subjects became symptomatic ($p=0.036$, Wilcoxon, Figure 16).

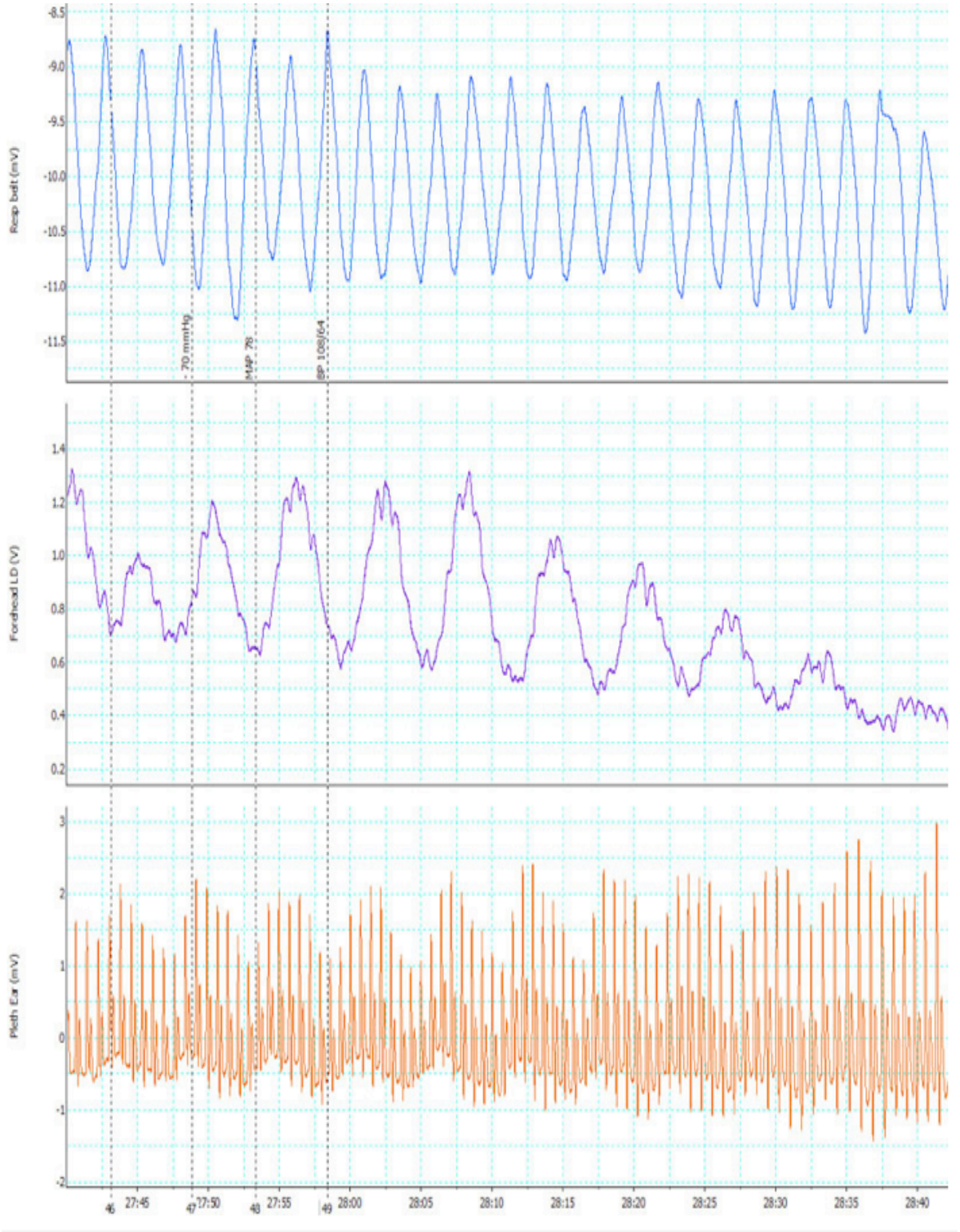

Figure 16. This tracing was taken at -70mmHg, during the transition from PRESYMP to SYMP. The ear PPG waveform (bottom) was modulated in part by the autonomic nervous system, and not by the respiratory system (top). A laser Doppler flowmeter tracing is shown for comparison also oscillating at the parasympathetic frequency (middle).

The finger PPG demonstrated less than a 10% fluctuation at either frequency during the LBNP runs (p=NS).

LASER DOPPLER FLOWMETRY

During the period when subjects experienced symptoms of hypovolemic shock, finger flow decreased by 52.2 +/- 12.3% between BASE and PRESYMPT (Figure 17) (p 0.01). In contrast, forehead flow remained within 9.5 +/- 53.1% of BASE (Figure 12); resulting in skin blood flow differences between the forehead and finger (p = 0.03). The maintenance of forehead flow was associated with oscillatory activity consistent with a parasympathetic frequency band (0.12 – 0.20 Hz, Figure 16. At SYMPT, finger flow decreased by 57.4 +/- 19.8% (p = 0.048) and forehead flow decreased by 37.2 +/-14.0% (p = 0.048) from PRESYMPT. Both sites increased promptly upon release of LBNP.

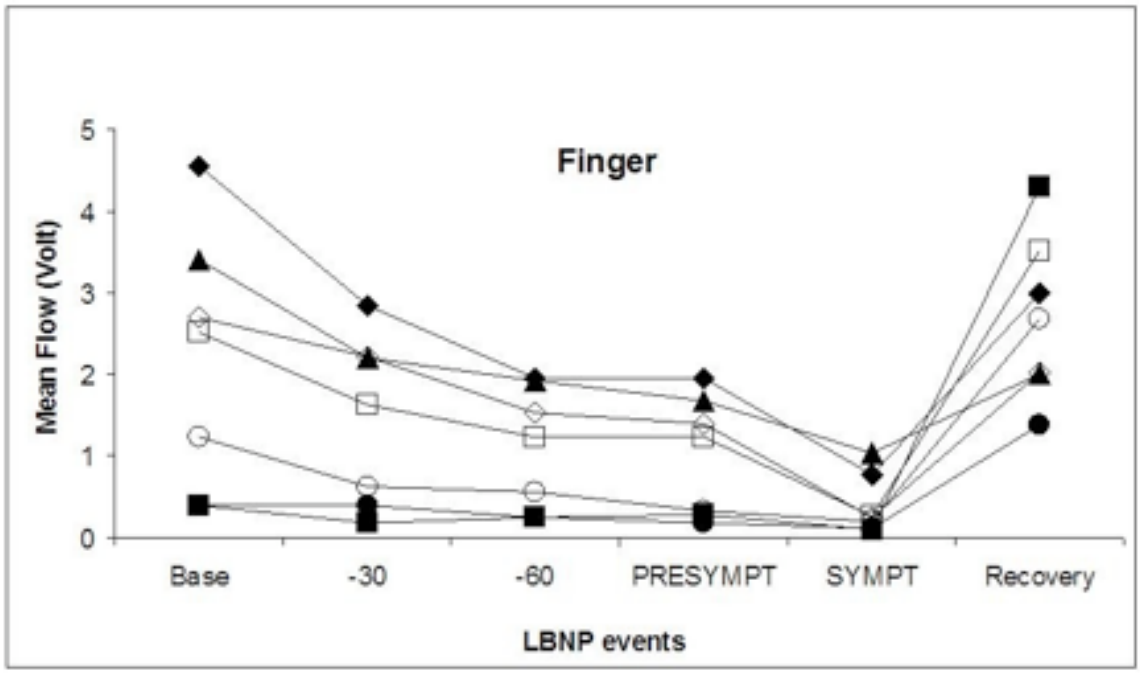

Figure 17. Finger blood flow shown to progressively and steadily decrease during LBNP as measured by LD.

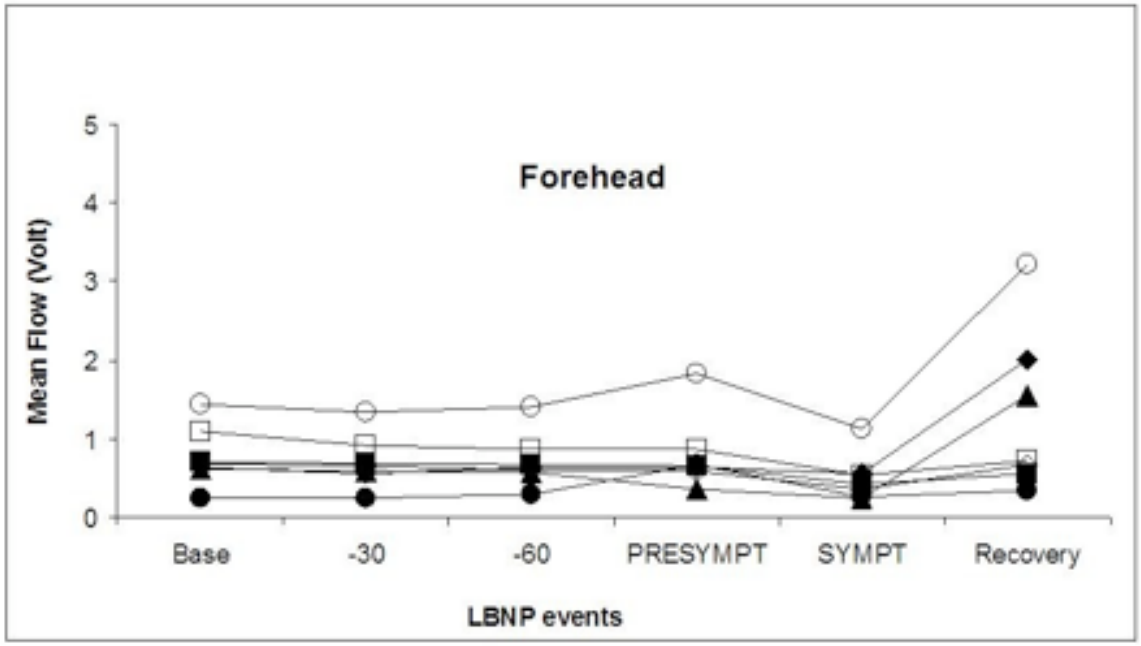

Figure 18. Forehead blood flow shown to be maintained within approximately 10% of baseline until the onset of symptoms during LBNP as measured by LD.

DISCUSSION

The most intriguing and exciting finding in our study was the relative sparing of central cutaneous blood flow when compared to a peripheral site. This was indicated by observable and quantifiable changes in the PPG waveform amplitude, width, and autonomic induced oscillations. To our knowledge, we are the first group to report such autonomic modulations in the PPG waveform, and thus to document these changes at the level of the terminal arterioles.

Our study sought to determine the effectiveness of the PPG waveform as a tool to distinguish regional differences in regulation of the microvasculature in the setting of the removal of between 1.5 and 2 liters of blood, via LBNP simulated hypovolemia, in healthy volunteers. This analysis focused on changes in PPG amplitude and width between the microvasculature of the finger, a surrogate for peripheral effects, and the forehead and ear, surrogates for central effects. Our aim was to provide greater insight into the interplay between the respiratory, local microvascular, and autonomic nervous system in the differing regulation of central and peripheral microvascular blood flow. This is important, as monitoring from central sites may be indicative of underlying circulatory effects at critical locations such as the brain. Additional analysis was sought to quantify the respiratory system's effect on the PPG waveform with the intention that this might serve as a sensitive monitor of volume status.

In order to appreciate the results and implications of this study, it is necessary to discuss what was observed, consider the relevance of the results, set forth possible explanations for the data, detail the limitations of the study, and suggest additional areas of exploration.

VITAL SIGN CHANGES

The observed changes in vital signs, hypotension and tachycardia, were consistent with the well-established clinical model of hypovolemic shock. The American College of Surgeons Committee on Trauma defines four stages of shock: Loss of up to 15% of the circulating volume (750 mL) may produce little in terms of obvious symptoms, while loss of up to 30% of the circulating volume (1.5 L) may result in mild tachycardia, tachypnea, and anxiety. Hypotension, marked tachycardia [i.e., pulse >110 to 120 beats per minute (bpm)], and confusion may not be evident until more than 30% of the blood volume has been lost; loss of 40% of circulating volume (2 L) is immediately life threatening, and generally requires operative control of bleeding.[80]

Using the fundamental equation of cardiovascular physiology, MAP = CO (HR x SV) x SVR, a decrease in circulating volume causes MAP to fall. This results in a compensatory increase in both CO and SVR in attempt to maintain organ perfusion. In our study blood pressure was well preserved, showing a less than 10% decrease from baseline, until the final moments of the study, at which time reperfusion was initiated. This reperfusion was accompanied by a decrease in pulse pressure, manifested as a decrease in systolic blood pressure and an increase in diastolic blood pressure, which is representative of compensatory sympathetic activation and resultant elevations in vascular tone. Additionally, there was continuous and increasing baroreceptors activation, causing a steady increase in heart rate.

Our results with regard to vital sign changes demonstrate that we were able to take our subjects to a significant level of hypovolemia. This validates our experimental model and gives us a way to standardize the estimated blood sequestered between subjects based on vital sign changes.

AMPLITUDE ANALYSIS

As expected, the finger PPG amplitude tracing declined over the course of the progressive LBNP challenge. This challenge is associated with a pronounced decrease in stroke volume and compensatory upper extremity vasoconstriction[77]. It is the increase in sympathetic tone, with its resulting vasoconstriction, which causes a decrease in microvascular compliance and distensability. Thus with each systole there is smaller overall volume of blood pumped into the microvasculature, the percentage of absorbed light emitted by the pulse oximeter decreases, and the amplitude voltage is lessened.

A less pronounced response in the forehead PPG tracing likewise was consistent with our hypothesis that this region of the body would be protected during hypovolemia, in view of its previously documented protection from systemic vasoconstrictive challenges[8, 23]. However, the maintenance of forehead PPG amplitude in the presence of a decline in stroke volume suggests activation of added means to preserve blood flow to this region. The potential for two such processes -- local vasodilatation that may be attributable to recently documented cholinergic vasodilator mechanisms[78] and/or shunting from constricted regions in the periphery remain to be explored. The later mechanism is currently being investigated by other members of our team in conjunction with echocardiography.

WIDTH ANALYSIS

Our data show that overall PPG width decreases in the context of LBNP, regardless of monitoring site. There was a more rapid decrease in the plethysmographic width of the forehead than of the finger during progressive hypovolemia. Additionally, the overall decrease in forehead width was also greater than that of the finger. Both width durations concluded at approximately 120ms, despite the forehead starting at 210.4ms and the finger starting at 163.2ms. The decline in width and the difference between the forehead and finger

responses were noted despite only a slight change (7.4%) in mean BP, suggesting that there was a greater decrease in relative intravascular volume than suggested by blood pressure.

Looking at the contribution of hypovolemia and vasoconstriction in isolation would suggest that hypovolemia should decrease the time during which a pulse of blood is detected by a sensor. If vessel caliber is constant, the width of a given waveform should decrease in proportion to the decline in stroke volume. The effect of hypovolemia is seen in or our result as a decrease in PPG width in both the finger and the forehead. The accompanying reflexive tachycardia seen with LBNP also serves to decrease stroke volume. Hence there would be less pulsatile flow with each cardiac cycle to the vasculature, and therefore a decreased width.

Vasoconstriction, as seen in the finger, reduces vessel diameter. In addition, vasoconstriction also reduces vessel compliance. Hence, with vasoconstriction, one would observe a decrease in the volume of blood that moves through the vessel in a given time. This becomes confusing, however, because the actual velocity of the blood likely increases in the context of vasoconstriction as predicted by the continuity equation. Additionally, with vasoconstriction there is potential for volume redistribution to more compliant parallel vascular beds. Overall then, the effect of vasoconstriction on the PPG width is difficult to predict.

In this context, the differing response of the finger and forehead widths becomes difficult to explain. In a region such as the forehead, which is known to be relatively immune to adrenergic influence, the width serves as a much more pure measurement, as drastic changes in vessel diameter are not occurring. Finger PPG width measurement, however, is much less useful as there are simply too many variables which confound the measurement. It seems most logical to assume that width may best serve as an indicator of forward flow (SV/ CO) rather than vessel compliance or caliber.

RESPIRATORY MODULATION ANALYSIS

Though there was an overall increase in the amplitude density at the respiratory frequency (0.2Hz) from baseline to the onset of symptoms, it was not the expected constant and steady increase at every negative pressure interval. Specifically, at -30 there was a decrease compared to baseline, from 2.5 $x10^{-4}$ $V/Hz^2$to 2.1 x 10^{-4} V/Hz^2. Additionally, though the overall trend was an increase in amplitude density at the respiratory frequency, this was not necessarily representative of individual subjects. Some subjects had greater levels of oscillations at baseline than others, and some showed more pronounced decreases in amplitude density upon application of negative pressure compared to baseline.

Based on the proposed mechanism for respiratory induced oscillations, this situation creates discordance. The reasoning behind respiratory induced oscillations of the PPG is that each breath sequesters a volume of blood, assumed to be approximately one quarter to one half a liter, in the pulmonary vasculature. Thus each inspiration represents a small hypovolemic challenge, and when superimposed on a large hypovolemic challenge, such as hemorrhage or LBNP, there should be a decrease in systolic pressure (an exaggeration of physiologic pulsus paradoxus) as well as a noticeable and quantifiable dip in both the AC and DC components of the PPG waveform. Since each negative pressure interval represents a greater degree of hypovolemia, it stands to reason that there should be a steady increase in respiratory induced oscillations, as measured by spectral domain analysis and amplitude density, at each pressure interval in our protocol.

A possible explanation for these results may lie in the newfound autonomic modulation of the ear PPG. To our knowledge, this is the first time that this phenomenon has been described. This modulation occurs at a frequency very close to the respiratory frequency, and the oscillations observed are thus likely not solely the result of respirations, but also the effect of the parasympathetic nervous

systems on the underlying microvasculature. We can then immediately conclude that the occurrence of autonomic modulation needs to be considered when studying signals that have their origins from central sites (ear). Additionally, the use of the PPG to monitor autonomic balance is intriguing and needs further investigation.

As a corollary, the same parasympathetic modulation was seen in the laser Doppler tracings, and is a well-documented effect in the setting of laser Doppler flowmetry. It is highly likely, then, that we were observing the same parasympathetic induced oscillations of the vasculature moving proximally up the microcirculation to the level of the terminal arterioles (the level measured by the PPG).

SPARING OF THE CENTRAL MICROVASCULATURE: AN EXPLANATION

Though this thesis has examined each component of the PPG waveform individually for analysis and discussion, it is paramount to remember that these changes happen in conjunction with one another. To truly begin to understand the underlying physiological processes occurring in the microcirculation during hypovolemia, we must look at the entirety of the PPG data. Our data have revealed the following: hypovolemia with lower body negative pressure induced virtually no change in the amplitude of the forehead PPG tracing despite causing a significant decline in the finger PPG amplitude; hypovolemia induces a greater decline in the width of the PPG tracing in the forehead than in the finger; the occurrence of autonomic modulation in the ear but not finer PPG waveform needs to be taken into account when studying signals that have their origins from central sites (e.g. ear & forehead); forehead flow as measured by laser Doppler flowmetry was maintained during LBNP until subjects became symptomatic, whereas finger flow progressively declines throughout. We speculate that these findings are due to local vasodilation that may be attributable to recently documented cholinergic mechanisms.[78]

Almost all blood vessels have stretch receptors. These receptors are most prominent at the carotid sinus, but are also apparent throughout the body. The development of autonomic oscillations in the PPG tracing could be due to activation of such receptors when there is significant systemic hypoperfusion. A marked hypovolemic state, as would be induced by severe blood loss or pronounced LBNP, decreases preload and likewise decreases stroke volume. At moderate degrees of hypovolemia, the parasympathetic compensatory response is probably limited to the microcirculation, with graded vasodilation and regional redistribution of blood. In more severe hypovolemia, major vessels may dilate, and there is preservation at the central microvascular level.

The proposed cholinergic mediated mechanism for terminal arteriolar and microvascular flow preservation is also supported by our laser Doppler flowmetry findings. A laser Doppler probe placed on the forehead adjacent to the PPG probe demonstrated oscillatory activity consistent with parasympathetic modulations of the microvasculature. It is worth noting that the laser Doppler measures flow distal to the PPG, at the level of the precapillaries and capillaries. The combination of preserved flow, as indicated by both a maintained forehead PPG amplitude and forehead laser Doppler flowmeters, and increased oscillations in the context of systemic vasoconstriction is consistent with microvascular oscillatory activity contributing to the discordance between large cerebral arterial blood flow changes and preservation of CNS blood flow.

The term cholinergic oscillatory control of the microvasculature (COCmicvasc) was coined by one of our members (DGS)[34] as a response of the body to optimize delivery of blood flow to the microvasculature in the context of decreased flow proximal to the precapillary sphincter. It also might serve to offset capillary congestion. In the context of hypoperfusion, the microvasculature of certain portions of the body (e.g., brain, forehead) develops what may be referred to as rhythmic inhibition of precapillary sphincters such that capillaries dilate in

synchrony and thereby provide transient decreases in local resistance (in proportion to the fourth power of the radius). This enables them to "do more with less".

Logically, the oscillations at the level of the microvasculature maintain autoregulation in the context of a manageable compromise of perfusion. The larger response that involves the plethysmograph is one where the body senses that, regardless of how efficiently blood is used when it gets to an area of the brain, there is not an adequate blood supply. Therefore the conduits going to the brain dilate to facilitate delivery to this region.

Vascular smooth muscle cells typically oscillate out-of-phase and thus may offset one another in the absence of extrinsic (e.g., neural) control. When a controlling force such as COCmicvasc is activated, it synchronizes the oscillations of capillaries of the same branching order[81] and thereby increases the summated amplitude of the oscillations under the laser Doppler probe. The resistance (impedance) of a vessel (or vascular bed) whose diameter varies sinusoidally is lower than that of a constant-caliber vessel with the same average diameter[82].

Further, if one views the microcirculation as a form of parallel circuitry, then, since the total resistance of a parallel circuit is less than that of any of its branches, the enhanced oscillations of the arteriolar– capillary networks would lower the circuit's resistance. The potential physiological impact of COCmicvasc is suggested by the evidence that oscillations of neighboring terminal vessels become synchronized during clamping of a regional feeding artery[83] and by the development of 0.13-Hz oscillations in laser Doppler flux in the rat cerebral cortex during norepinephrine infusion and hypoperfusion[84]. However, in neither of those studies was a cholinergic etiology sought or reported.

LIMITATIONS

Our study was subject to several limitations, which are worth mentioning, as they should be taken into account when interpreting our results. They also serve as points to address in any future experiments.

We had a relatively small number of subjects, hence our statistical analysis was not as strong as we would have liked, even though, prior power analysis indicated that six subjects would be required to detect a 50% intra-subject change in respiratory induced oscillations of the plethysmographic signal, with an alpha of 0.05 and a power of 0.90[38]. Another limiting factor of our study is the study population. These subjects were all young healthy individuals, and are not representative of the population as a whole. However, in trying to characterize these novel observable phenomena, healthy subjects are likely the best candidates to do so with.

Our use of spectral analysis to analyze the waveform from the entire case in the present study has the advantage being relatively immune to isolated artifacts such as motion.[38] It, therefore, was preferable for the overall assessment of the impact of ventilation on the plethysmographic signals. However, the integrated assessment of such long study intervals is not preferred for analysis of short-term events. In the final moments of the study, when subjects transition from a period of compensated to decompensated shock, there may be events that occur on such a timescale as to be missed by simple spectral domain analysis. For events happening on such a short time scale, wavelet analysis such as the Hilbert-Huang transform (HHT) are being looked into as alternative means of investigation. HHT looks to be the state-of-the-art method of energy-time-frequency representation of non-stationary and nonlinear signals, This signal processing technique allows greater resolution in both the time and frequency domain.

It is paramount to consistently quantify the characteristics of the PPG in such a

way as to allow the results from research efforts be translated into clinically useful devices.[4] At this time, no calibration procedure is known to standardize the PPG amplitude for comparing one patient waveform to another. The signal is therefore not given a unit designation. Similar to central venous pressure measurement, the value of the plethysmograph comes from an analysis over time, as opposed to any absolute number.[4]

The PPG width as a variable is a complex measurement, dependent upon heart rate, stroke volume, and also PPG amplitude. A decrease in stroke volume whether due to hypovolemia or an increase in heart rate will cause the width to decrease regardless of what is happening to vessel diameter. Also, as width is conventionally taken at the point of half the amplitude, as amplitude changes so does the point at which widths is measured. Thus width analysis is less robust than that of amplitude or respiratory/ autonomic induced oscillations,

The concept that LBNP may be an effective model to study cardiovascular responses to acute hemorrhage in humans is tenable from several perspectives. However, it should be emphasized that application of LBNP does not mimic all of the responses observed in traumatic hemorrhage, as LBNP clearly does not induce tissue trauma or subsequent metabolic responses (e.g., acidosis). Rather, data suggest that LBNP may be used as a model to study acute hemodynamic responses to the central hypovolemia associated with hemorrhage.[77]

Further, at a given level of negative pressure, each individual had a different amount of blood sequestered in his or her lower body. This makes data analysis difficult when attempting to compare responses at a particular level of negative pressure. Subjects can be compared at endpoints, as was done in our study, or indirectly with measures such as changes in vital signs.

Finally, enthusiasm for the method of assessment of PPG respiratory induced oscillations tested herein should be tempered by the realization that there is wide

inter-subject variability in baseline autonomic balance and thus in the degree of existing oscillations. This challenge will have to be overcome if the PPG is to be used commercially as a monitor of volume status or vascular tone.

FUTURE EXPERIEMENTS

We were able to demonstrate comparable preservation of flow in the forehead during lower body negative pressure, indicated by both PPG and laser Doppler data. It follows that we should determine if these changes are in any way reflective of what is occurring in a major vessel of the brain. A corollary study, which is presently being undertaken, involves transcranial Doppler measurements of the middle cerebral artery (MCA) flow in the context of lower body negative pressure. From the standpoint of cerebral autoregulation, we would expect that MCA flow falls progressively with LBNP and that maintenance is mostly at microvasculature.

Potential for further studies utilizing transcranial Doppler include a low-dose intravenous phenylephrine and/or hyperventilation to induce cerebral vasoconstriction and again comparing responses of the micro and macro vasculature. There also exist potential for measuring parenchymal blood flow and comparing that with blood flow in both the cutaneous microvasculature and/or MCA. Currently, members of our team are working to make laser Doppler embedded grids and strips to study epilepsy patients who are undergoing cortical resection for intractable seizure activity.

Two further areas that could be studied in attempt to help define the underlying physiology behind the observed effects on the PPG waveform during progressive hypovolemia are use of echocardiography to see if oscillations also occurring in stroke volume and eutectic mixture of local anesthetic (EMLA) under plethysmography, to investigate whether our observed oscillations are prevented by local anesthetic (equal quantities, by weight, of lidocaine and prilocaine), which

would support their autonomic nature. Members of our group are currently researching echocardiographic analysis during blood withdrawal.

SUMMARY

In summary we saw that during a hypovolemic challenge in healthy volunteers, despite minimal changes in blood pressure, appreciable changes in PPG amplitude, width, and respiratory induced oscillations could be detected and quantified. These changes differed based on the region being studied, and offer insight into contrasting regulation of central and peripheral terminal arteriolar and microvascular blood flow. The most intriguing and exciting finding is the relative sparing of central cutaneous blood flow when compared to a peripheral site as indicated by observable and quantifiable changes in the PPG waveform amplitude, width, and autonomic induced oscillations. At this stage our goal is still to elucidate the underlying physiology reflected in the PPG waveforms as opposed to guiding therapy. We hope that ultimately these noninvasively derived PPG parameters will give clinicians useful information about central cardiac function and its interplay with the respiratory and autonomic nervous systems and provide a reliable trend monitor for patients.

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