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Analysis of Pulse Transit Time with the Inclusion of a Microvascular Component in Head-up Tilt and Blood Withdrawal Induced Central Hypovolemia

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ANALYSIS OF PULSE TRANSIT TIME WITH THE INCLUSION OF A MICROVASCULAR COMPONENT IN HEAD-UP TILT AND BLOOD WITHDRAWAL INDUCED CENTRAL HYPOVOLEMIA

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ABSTRACT

ANALYSIS OF PULSE TRANSIT TIME WITH THE INCLUSION OF A MICROVASCULAR COMPONENT IN HEAD-UP TILT AND BLOOD WITHDRAWAL INDUCED CENTRAL HYPOVOLEMIA

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The pulse transit time (PTT) has been investigated as an early noninvasive marker of hypovolemia, reflecting a combination of changes in the pre-ejection period (PEP) and vascular transit time (VTT). The use of photoplethysmography (PPG) has limited the analysis to the macrovascular peripheral circulation by nature of the detection mechanism of the PPG itself. Excluded is the richly innervated microcirculation that may have a significant influence on the vasomotor response to a hypovolemic challenge. Therefore the time required for the blood to travel from the PPG to the microvasculature (VTT_m) under a laser Doppler flowmeter (LDF) would provide a more complete understanding of the physiologic response to hypovolemia. The present study sought to assess changes in the components of PTT, including VTT_m, in a head-up tilt (HUT) model and in a post hoc analysis of data recorded from a two-unit blood withdrawal (BW) experiment performed by members of this research team.

With IRB approval, 10 healthy volunteers were recruited for a 60^o HUT test to simulate mild-to-moderate hypovolemia. Monitoring included a 3-lead EKG, Finapres and a PPG and LDF applied to both the finger and ear. Measurements were taken during the pre-tilt phase while the subject was supine and again upon tilting. The data from the BW study were retrospectively analyzed (see Appendix for methodology). Paired t-tests were performed and p-values are given where $p < 0.05$.

During HUT and BW, PEP increased significantly ($p < 0.001$). While PTT also significantly lengthened with tilting ($p = 0.02$), no such change was observed with BW. There was no change in heart rate in either experiment. VTT also remained essentially the same after tilting and BW. As expected, VTT_m was a significant addition to VTT, however it did not exhibit any significant changes in any region with either hypovolemic challenge. VTT and VTT_m of the forehead were significantly different than the finger at baseline and after blood withdrawal. The component values of the ear and finger did not vary significantly.

Results confirm previous reports of an increase in PEP in response to a mild-to-moderate hypovolemic insult. Flow through the microcirculation is a significant component of PTT. However, the VTT_m did not exhibit a significant response to mild-to-moderate hypovolemia. VTT remained essentially the same in both conditions and PTT was only found to change significantly with tilting. It is interesting to note the lack of change in VTT_m with tilting in the finger as well as the ear, even though the arterial network of the finger is more densely innervated by α-adrenergic receptors than other parts of the body. In a comparison with the existing literature, timing of the measurements and testing conditions are believed to significantly influence the results. Though our early timing of the measurements after the hypovolemic challenge might veil the presence of a sympathetic hemodynamic response, it is believed that baseline hydration status of the subject and time spent in the supine position before tilting are the root causes of this discrepancy with other studies.

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INTRODUCTION

Perioperative early detection of progressive hypovolemia associated with internal bleeding is not a simple task. The bleeding site is often not identifiable and the vital sign measurements taken by the monitoring equipment, blood pressure (BP) and heart rate (HR), are not reliable early indicators of mild-to-moderate hypovolemia.[1] These physiologic indicators remain stable during the initial phase of central blood volume loss, masking the actual deficit because of the complex cardiovascular response. In the early stage, the baroreceptor reflex is triggered, maintaining the perfusing BP by increasing systemic vascular resistance (SVR), despite a decrease in stroke volume (SV) and cardiac output (CO). HR is also an insensitive marker of early hypovolemia because it changes in a complex manner as hypovolemia worsens.[2, 3] It is only in the later stages of hypovolemia, when about 30% or more of the total blood volume is lost, that the physiological regulatory mechanisms become ineffective and a noticeable change in BP is observed. Hypotension and tachycardia do not indicate "the beginning of circulatory failure, but rather represent the beginning of decompensation."[4]

The hemodynamic response to acute hemorrhage can be divided into three stages.[3, 5] The first stage encompasses a blood loss up to 15% and, despite this reduction, arterial BP is maintained because of an increase in total peripheral resistance from baroreceptor-reflex compensated vasoconstriction. HR does increase, but only modestly to less than 100 bpm.[5, 6] The second stage can be characterized by either a CO below 50-60% of the resting CO or a 30% loss in blood volume. Compensatory mechanisms begin to fail leading to bradycardia and hypotension.[7, 8] If hemorrhaging

continues, the third stage manifests as worsening hypotension and tachycardia greater than 120 bpm. This stage transitions to irreversible decompensation, with ischemia, cellular dysfunction and eventually death.[5, 9]

By the time hypotension and tachycardia manifest, there is a greater risk of cardiovascular collapse secondary to severe hemorrhagic shock.[1, 2, 10, 11] Hemorrhagic shock, defined by a systolic blood pressure less than or equal to 90 mmHg, is also associated with organ failure.[12] Delayed hemorrhage control has been identified as a major contributor to preventable trauma deaths and often, late detection is to blame.[13, 14] The unreliability of these vital signs as early markers of volume status demonstrates the importance of a timely and accurate indicator to diagnosis early stage hypovolemia. To decrease barriers of adoption and wider and quicker utilization, it is ideal that this marker be derived from common, currently used patient monitoring equipment.

Head-up tilting (HUT),[2, 15-18] blood withdrawal (BW)[19-23] and lower body negative pressure (LBNP)[10, 23-30] are all acceptable and well-established functional models of blood loss. HUT simulates mild and moderate hypovolemia only, while the latter two have the potential, depending on the degree to which the model is implemented, to represent all stages of hypovolemia. A head-up tilt of 60 degrees approximates moderate blood loss, about 10-20% of total central blood volume. This is equivalent to 550-1,000 mL of blood withdrawal or 20-40 mmHg LBNP.[10, 17]

In an unpublished study by members of our research team (Wardhan et al.),[31] withdrawal of two units of blood was protocoled to discover a signal of moderate blood loss. Subjects were monitored with EKG and noninvasive continuous finger arterial blood pressure as about 900 mL of blood was removed at a rate of 1-unit/10-minutes, after which the two units were reinfused along with 200 mL of saline. Despite this moderate hypovolemic insult, BP and HR remained stable within 5% of baseline. This is consistent with other findings and can be explained by the fact that BP and HR, because of their sensitivity to the reflex response, are secondary responses to blood loss and are therefore insensitive to moderate changes in volume status. Primary responses, such as central blood volume, diminished venous return, ventricular end diastolic volume (or preload) and reduced stroke volume, would be a more direct and appropriate target from which to glean volume status information.

Much of these primary events are accounted for in the pulse transit time (PTT), which in its technical sense is the time it takes the pulse wave to travel between two arterial sites.[32-35] PTT is a function of the physical characteristics of the blood vessel and blood, as well as the intravascular pressure. The speed at which the arterial pulse wave travels is therefore proportional to blood pressure. Vascular tone rises with BP, resulting in a constricted and stiffer arterial wall which causes PTT to shorten.[36] Multiple studies have attempted to use PTT as a surrogate for beat-to-beat blood pressure, but results have demonstrated that PTT lacks the clinical accuracy to be substituted for continuous BP measurements.[37-41] PTT also has had mixed success when applied to gain further insight into other conditions, such as diagnosing and monitoring sleep apnea,[42]

assessing cardiovascular reactivity[43] and estimating arterial stiffness.[44, 45] But, studies have been successful in demonstrating that PTT can detect hypotension caused by central hypovolemia,[46] and even holds promise in signaling the early stage of hypovolemia before hypotension manifests.[47, 48]

Practically, it is too difficult to continuously monitor artifact-free pulses from two peripheral sites. Instead, PTT can be observed between the ventricular electrical activity and a point on the systolic upstroke of the corresponding peripheral pulse wave. This has the advantage of using a proximal timing point that is simple to detect and tolerant of motion artifact. This interval can be functionally measured from the peak of the R-wave on an electrocardiogram (EKG)[35, 49-51] to a reference point on the periphery detected by pressure monitors, either a photoplethysmograph (PPG) or laser Doppler flowmeter (LDF), marking the arrival of the pulse wave.

The PPG is a noninvasive probe that utilizes infrared optics to produce a signal that reflects the minutely changing volume of red blood cells in the microvascular bed during a cardiac cycle.[52, 53] Light is transmitted through the tissue and sensed by a photodetector, which then amplifies and processes the emitted signal.[54] As the BP wave propagates along the arteries of the skin, the tissue blood volume increases and decreases with the periodicity of the heartbeats. The dynamics of this change depend on many factors, including heart function, neural processes and size and elasticity of the local vessels themselves.[55] PPG signals can be best obtained from the tissue pads of the fingers, toes and ears, because these are sites with adequate cutaneous perfusion and

good contact can be made between the skin and sensor.[56] Signal quality and accuracy is therefore dependent on sensor placement and contact,[57] peripheral temperature,[58] degree of perfusion[59, 60] and motion artifact.[61] The resulting pulse oximeter waveform carries a significant amount of information, however besides for measuring arterial blood oxygen saturation, most of its utility has not been clinically realized.[62-64]

From a clinical perspective, the ease of deriving PTT from monitors already routinely required for measuring vital signs makes any application of PTT more likely to be adopted in patient care. The literature calculates PTT as the sum of two distinct components: the pre-ejection period (PEP), which corresponds to the time from the start of ventricular depolarization to the onset of ventricular ejection (Figure 1), and the vascular transit time (VTT), which is the time it takes the arterial pulse wave to travel from the aortic valve to a point in the peripheral arteries. In other words, PTT consists of a central component, PEP, and a peripheral component, VTT (Figure 2). VTT and its inverse, the pulse wave velocity (PWV), have been used to evaluate the physical characteristics of peripheral arteries in atherosclerotics[45, 65-68] and to ascertain the autonomic cardiovascular response in diabetics.[32, 69]

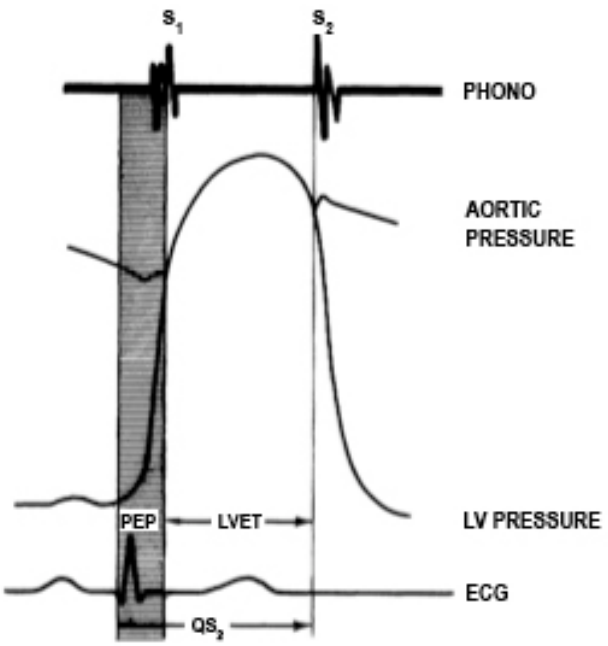

Figure 1. Relation of the measured systolic time intervals to the cardiac cycle. Abbreviations: LVET = left ventricular ejection time; QS_2 = electromechanical systole. [Reprinted from Stafford et al.[70]]

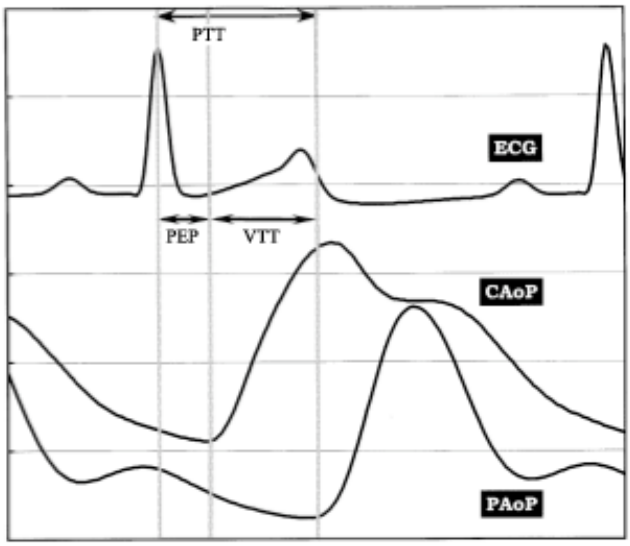

Figure 2. Representative drawing of electrocardiogram (EKG), central aortic pressure (CAoP), and peripheral aortic pressure (PAoP). The interval between the R-wave of EKG and pulse arrival of AoP is PEP. The interval between CAoP and PAoP is the VTT. The sum of PEP and VTT, which is the interval between the R-wave and the peripheral pulse arrival, is the PTT. [Adapted from Ochai et al.[39]]

Studies on the physiology of animals and man show that PEP is prolonged when either stroke volume or ventricular filling is reduced.[71, 72] The duration of the PEP also depends on the isovolumetric pressure gradient difference between the afterload and the end diastolic pressure, with a larger gradient correlating to a longer PEP.[70] In the setting of hypovolemia, the compensatory baroreceptor-mediated reflex, which increases peripheral vasoconstriction, elevates the aortic diastolic pressure, thereby widening the pressure gradient and consequently lengthening PEP. However in opposition to this effect, the reflex also sympathetically stimulates the myocardium,[15] quickening the isovolumetric rise of left ventricular pressure[73] and thereby shortening PEP. The observed lengthening of PEP in studies is instead explained by a widening of the pressure gradient due to the slower rise of left ventricular pressure during isovolumetric contraction secondary to shortened myocardial fiber length from the hypovolemic-reduction in left ventricular end-diastolic volume.[74, 75]

PWV, as described by the Moens-Korteweg formula,[76, 77] depends on the distensibility and the dimensions of the vessel, such that:

$$v = \sqrt{\frac{gEa}{\rho d}}$$

where v is the PWV, g is the gravitational constant, E is the elastic modulus, a is the vessel wall thickness, d is the interior diameter and ρ is the density of the blood. When BP increases, vessel diameter widens and the wall thickness thins. However, these adjustments are more than offset by the change in the elastic modulus, which increases

exponentially with the increasing BP.[78-80] This is why PWV increases with a rising BP. The VTT is inversely proportional to the velocity v, expressed as:

$$v = \frac{K}{T}$$

where T is the VTT and K is a proportional coefficient indicating the distance the pulse wave must travel.

Studies have verified this, showing VTT to have a negatively linear correlation with BP. However, the relationship between PEP and BP was inconsistent,[39] varying with cardiac preload[70, 81, 82] and sometimes contributing as much as 35% of PTT.[34] Several other reports also reveal this larger role of PEP on PTT when the cardiovascular response alters the myocardial contractility.[40, 41] This confirms that PEP is a major factor in modifying the relationship between PTT and BP and explains why studies that have only measured PTT alone have had trouble showing accurate estimations of BP.

It is our contention that the PTT should actually consist of three components. The peripheral VTT component can be separated between the macrocirculation and the microcirculation. After all, the microcirculation contains the major resistance vessels in the body and is the primary site of regulation.[83] The richly innervated microcirculation may have a significant influence on the vasomotor response to a hypovolemic challenge and this unmeasured section of the vascular circuit could very well provide a fuller understanding of the hemodynamic response to hypovolemia. It could also potentially serve as an early signal of volume loss in its own right. However, experiments to date have limited the pulse transit time to the major vessels only, because the PPG signal has

been ubiquitously used to measure the pulse arrival in the periphery. One study did use LDF to measure the PTT through the microvasculature, however there was no evaluation of PEP and VTT nor were any components tested under hypovolemic conditions.[84] The PPG is most sensitive to the larger peripheral arteries due to a reduction in the pulsation of the wave beyond the arterioles, which makes detection of the microvascular waveform unreliable. Though the PPG actually detects the blood at the level of the arterioles, it is its time-value component that serves as the endpoint in the VTT calculation, marking the arrival of the pulse from the macrovasculature of the peripheral circulation.

Therefore, a different measurement modality is needed to measure VTT through the microvascular (VTT_m). Laser Doppler flowmetry (LDF) has the capability to assess the microcirculation of the pulse wave at the level of the capillary network. The LDF is also a noninvasive probe, but it detects phase shifts of low-powered laser light to continuously measure microvascular perfusion.[85] LDF, like PPG, has been proven to satisfactorily detect pulse waveforms in the periphery for purpose of measuring PTT.[84] And just like the PPG, even though the LDF actually measures the blood flow directly beneath the probe in the capillary network, it is used to mark the endpoint of the wave propagation through the precapillary arteriolar network. Even though PPG and LDF measure different aspects of the blood, they can be compared on the common dimension of time. Any inherent differences in measurement due to the distortion between the mechanics of how the two probes actually measure – the PPG tracks blood volume, while the LDF relies on blood flow – are eliminated by the consistent

comparison of only the time values from each probe. The PPG clocks the wave propagation of the major vessels, while the time differential between the PPG and the LDF gauges it on the micro level. Because only the time differential is being analyzed, the arrival of the pulse at the PPG marks the new starting point and the VTT_m is therefore free from the influences of any proximal regulation in the major vascular network.

PTT is therefore the sum of not two, but three components. The PEP, which measures the cardiac portion, the VTT, which is the pulse travel time through the major arteries, and finally the VTT_m, which is the propagation of the wave in the microcirculation. Separate analysis of each component and its associated waveform can then be used to untangle and better understand the contributing factors and perhaps uncover a reliable pattern to serve as an early detection of diminishing volume status. In the analysis of PTT, it is also important to acknowledge that changes at one site in the peripheral arterial network might not be representative of changes at other sites.

Different locations vary in their sensitivity and responsiveness to stimuli because of the uneven presence and concentration of autonomic receptors.[54] Burton defines the role of the autonomic nervous system on the blood volume pulsations, such that:

$$\Delta V = \Delta P \times D$$

where ΔV is the volume pulsation, ΔP is the systemic intravascular pulse pressure and D is the distensibility of the vascular wall.[86] For instance, the vessel walls in the arterial network of the finger is more densely innervated by α-adrenergic receptors than other

parts of the body and is therefore more sensitive to sympathetic stimuli.[87] Since distensibility is chiefly dependent on vascular smooth muscle tone, it will be primarily altered by the autonomic nervous system.[88] Thus, vasoconstriction from an increase in sympathetic tone would create a vigorous decrease in vessel distensibility and, in turn, a sizeable reduction in volume pulsation. The waveform detected by a finger PPG therefore indicates local vascular tone and volume alterations.[89, 90]

This is opposed to the ear, which has a relatively less pronounced vasoconstrictive response to sympathetic stimuli.[89] These regional differences in microvasculature flow and regulation have been verified by a more profound decrease in amplitude in the finger PPG than the ear pinna PPG in response to cold immersion,[89] intubation and stressful surgical stimuli,[91, 92] as well as in numerous studies testing various pharmacological agents.[32, 93] Changes in volume detected at the ear would have to be mostly caused by changes in pulse pressure, inferring that the ear is a more fitting indicator of the systemic circulation and stroke volume.[88] Because the PEP reflects the contractility of the left ventricle, which is primarily controlled by β-sympathetic activity,[34, 35, 94, 95] the PTT to the ear is also a measure of β-sympathetic myocardial influences.

A PPG probe on the earlobe is a convenient and accurate surrogate to measure the central component of PTT,[34] even though the PTT to the ear technically incorporates the PEP and the VTT of the pulse originating in the aorta to the arrival at the probe. Anatomically, the blood supply of the lobule of the auricle mostly consists of the major

central arteries – the aorta, carotid and external carotid arteries. The remaining peripheral flow is from the posterior auricular and superior temporal branches, which directly feed from the external carotid artery.[96] The aorta is non-reactive to hypovolemia-induced sympathetic stimulation[97] and the remaining course of the blood supply to the ear is not sympathetically reactive enough to result in a significant difference between PEP and PTT.[34]

STATEMENT OF PURPOSE

A HUT model was used to investigate the multiple components of PTT, including the proposed VTT_m, and to determine if a change in any of these variables can signal early hypovolemia. LDF was added to the standard PTT measuring devices, so that the effects of the microcirculation on PTT could be quantified and analyzed.

Earlier studies have used a HUT model to measure the different factors of PTT.[47, 70] Head-up tilt testing repositions a subject from supine to reverse Trendelenburg, in which the feet are below the head. The sine of the tilt angle has a linear relationship with the hydrostatic effects of a decreasing thoracic volume.[18, 98] Although HUT is not identical to blood loss because the blood volume is only redistributed to the lower body under gravity's hydrostatic influence as opposed to permanently lost from circulation, it nonetheless still closely simulates the physiological responses to a compounding deficit in central blood volume.[2, 7, 11, 99] The resulting venous pooling reduces ventricular filling, end-diastolic volume, SV and CO, while increasing total peripheral resistance through baroreceptor-mediated reflexes.[70, 100, 101]

A post hoc analysis of the data collected from the research team's unpublished BW study was also done in hopes of confirming and supporting any findings from the current HUT trial. While BW cannot exactly reproduce the effects of progressive, uncontrolled hemorrhage,[102] it still is able to closely simulate the physiological response to a reduction in blood volume. The HUT model was used as the primary investigation because HUT, as compared to BW, more acutely simulates hypovolemia. The full physiologic simulation of hypovolemia in HUT occurs within a minute of tilting, whereas the hypovolemic effects of BW gradually occur, as the blood is removed overtime, in this case over twenty minutes at a rate of 1-unit/10-minutes. Also, while the data from the BW study showed no change in HR or BP, which is congruent with some of the blood withdrawal literature investigating hypovolemia, studies that used a head-up tilting model reported a change in HR and BP. It was therefore decided to measure the components of PTT using a model that has elicited a substantive sympathetic response and that could easily be applied to identify early stage acute hemorrhage in trauma settings.

Based on the literature, we hypothesized that a hypovolemic challenge will lengthen PEP and slow PTT. The increase in PTT, which is mostly attributed to the prolonged PEP, will be partially offset by a decrease in both VTT and VTT_m due to a sympathetic-mediated peripheral vasoconstriction. We anticipated the acceleration in VTT_m to have a greater contribution to the change in PTT than VTT, because the microcirculation

comprises the major resistance vessels, which are highly innervated and sensitive to sympathetic stimuli.

METHODS

BLOOD WITHDRAWAL

The data from the blood withdrawal study were retrospectively analyzed and the methodology protocol implemented in that study can be found in the Appendix. It should be noted that though the blood withdrawal study had a PPG monitor on the ear, it lacked an ear LDF. Without a measurement of the microcirculation in the ear, VTT_m cannot be directly compared between studies. However, the study does have a forehead PPG and LDF, allowing for other comparative analyses.

HEAD-UP TILTING

Subject

With IRB approval, 10 healthy volunteers were recruited for a 60° HUT test to simulate mild-to-moderate hypovolemia. All subjects were healthy and had no history of cardiovascular or respiratory disease.

Measurement devices

Unfiltered PPG (ADInstruments finger clip) and LDF (Perimed, Sweden) waveforms were measured from the tip of the right index and middle fingers, respectively, and from the right ear lobule. Data were recorded at 200 Hz and digitized to a computer using commercially available data acquisition software (PowerLab, ADInstruments).

Monitoring also included a lead III EKG configuration, with sensors placed on each wrist and the left shin. To measure continuous noninvasive finger arterial BP, a Finapres (Finapres, Ohmeda, Boulder, CO) was applied to the left middle finger between the proximal inter-phalangeal and the metacarpo-phalangeal joints.

Measurement protocol

The subjects rested on the tilt table in the supine position while the monitors were attached. Data recording was performed with the subjects positioned comfortably on the tilt table. Subjects were instructed to breath in synchronization with an audio metronome that was set to a respiratory rate of 12 breaths per minute. After a short period confirming satisfactory device signals with minimal artifact and with the subject resting comfortably, baseline measurements were recorded with the volunteer supine in the "pretilt" phase. The subject was then quickly tilted directly to 60° without pause and with a footboard supporting the subject's feet. The monitored (right) arm was passively maintained at heart level by one of the investigators. Measurements were recorded again in this half-minute "tilt" phase. The subject was then returned to the supine position for the monitors to be removed, remaining there for a minute to reach homeostatic balance before standing.

BLOOD WITHDRAWAL AND HEAD-UP TILTING

Signal processing and data analysis

Signals were processed and analyzed in LabChart (ADInstruments). A low-pass digital filter with a cut-off frequency of 5 Hz was applied to all signals except the EKG. On a per subject basis, a sequence of 11-13 consecutive beats with few or no artifacts in each of

the pre-challenge and challenge phases was chosen for evaluation of the parameters under investigation. The tilt phase data were collected as close to the act of tilting as allowed to us by artifact-free beats. PEP, VTT, VTT_m, PTT and HR were all derived from the EKG, PPG and LDF recordings. For the LDF and PPG tracings, the arrival of the pulse was measured at the trough preceding each upstroke of the PPG curve. This foot of the systolic upstroke is the ideal point of measurement because this initial portion of the signal rise is dependent only on pulse propagation, while the rest of the pressure pulse is determined by blood flow velocity and wave reflection from the periphery in addition to the pulse propagation.[39] The foot was identified from the plot of the first derivative by finding the time value where the derivative equaled 0, or failing that, the closest point greater than 0. The HR was calculated from dividing 60 by the R-R interval on the EKG tracing. PEP was determined by the time interval between the peak of the R-wave and the arrival of the subsequent pulse at the ear PPG. The time interval between this pulse arrival at the ear and the arrival of the pulse on the peripheral PPG constituted the VTT. The VTT_m is the measured time difference between the PPG and the LDF. PTT is the mathematical addition of PEP, VTT and the peripheral VTT_m. The mean and standard deviation for each parameter are reported. Paired t-tests were performed and p-values are given where $p < 0.05$.

RESULTS

The HUT experiment included finger and ear PPG and LDF monitors. PEP and VTT_m were measured at the ear. VTT, VTT_m and PTT were measured at the finger. The BW experiment included forehead PPG and LDF monitors in addition, but lacked an ear LDF.

Only PEP was measured at the ear. VTT, VTT_m and PTT were measured at the forehead and the finger.

HEAD-UP TILTING

In the HUT trial, both PEP and PTT significantly increased with tilting, $p < 0.001$ and $p = 0.02$ respectively. No significant change was seen with either VTT or VTT_m. The effects of tilting on the macrovasculature and microvasculature are summarized in Table 1.

Table 1. Effects of HUT on the components of PTT

	Macrocirculation			
Subject	*PEP*		*VTT*	
	Pretilt	**Tilt**	**Pretilt**	**Tilt**
1	0.118	0.132	0.077	0.076
2	0.112	0.120	0.063	0.067
3	0.083	0.097	0.077	0.079
4	0.098	0.105	0.062	0.071
5	0.092	0.109	0.069	0.076
6	0.097	0.112	0.057	0.057
7	0.112	0.131	0.080	0.074
8	0.124	0.126	0.040	0.059
9	0.079	0.096	0.055	0.052
10	0.112	0.135	0.070	0.075
Mean ± SD	0.103 ± 0.015	0.116 ± 0.015***	0.065 ± 0.012	0.068 ± 0.009

	Microcirculation				*Overall*	
Subject	*VTT_m Ear*		*VTT_m Finger*		*PTT*	
	Pretilt	**Tilt**	**Pretilt**	**Tilt**	**Pretilt**	**Tilt**
1	0.128	0.118	0.118	0.129	0.313	0.337
2	0.138	0.130	0.117	0.117	0.292	0.304
3	0.132	0.124	0.076	0.063	0.237	0.238
4	0.144	0.148	0.109	0.123	0.268	0.299
5	0.135	0.133	0.081	0.098	0.242	0.283
6	0.100	0.087	0.083	0.083	0.237	0.252
7	0.132	0.120	0.125	0.108	0.317	0.313
8	0.093	0.108	0.114	0.112	0.278	0.288
9	0.102	0.103	0.150	0.128	0.284	0.276
10	0.116	0.122	0.103	0.101	0.284	0.311
Mean ± SD	0.122 ± 0.018	0.119 ± 0.017	0.108 ± 0.023	0.106 ± 0.021	0.275 ± 0.029	0.290 ± 0.030*

Values reported in seconds. * $p < 0.05$, *** $p < 0.001$ for change from pretilt value.

During pretilt, the mean VTT_m was 0.122 and 0.108 seconds at the ear and finger respectively and during tilt it was 0.119 and 0.106 seconds (Table 2). As expected, in both the pretilt and tilt phases, the VTT_m component was found to be a significant addition ($p < 0.001$) to PEP at the ear and VTT at the finger, as shown in Table 2. VTT_m also constituted a significant addition ($p < 0.001$) to PTT between the R-wave of the

EKG and the PPG, which was 0.168 and 0.148 seconds during pretilt and tilting respectively (Table 3).

Table 2. The inclusion of VTT_m to PEP and VTT

Subject	*Ear*			
	Pretilt		*Tilt*	
	PEP	**PEP + VTT_m**	**PEP**	**PEP + VTT_m**
1	0.118	0.247	0.132	0.250
2	0.112	0.251	0.120	0.250
3	0.083	0.215	0.097	0.221
4	0.098	0.241	0.105	0.253
5	0.092	0.227	0.109	0.242
6	0.097	0.197	0.112	0.199
7	0.112	0.244	0.131	0.251
8	0.124	0.217	0.126	0.233
9	0.079	0.180	0.096	0.198
10	0.112	0.228	0.135	0.258
Mean ± SD	0.103 ± 0.015	0.225 ± 0.023***	0.116 ± 0.015	0.236 ± 0.022***

Subject	*Finger*			
	Pretilt		*Tilt*	
	VTT	**VTT + VTT_m**	**VTT**	**VTT + VTT_m**
1	0.077	0.195	0.076	0.205
2	0.063	0.180	0.067	0.184
3	0.077	0.153	0.079	0.141
4	0.062	0.171	0.071	0.194
5	0.069	0.150	0.076	0.174
6	0.057	0.140	0.057	0.139
7	0.080	0.205	0.074	0.182
8	0.040	0.154	0.059	0.171
9	0.055	0.205	0.052	0.180
10	0.070	0.172	0.075	0.176
Mean ± SD	0.065 ± 0.012	0.173 ± 0.023***	0.068 ± 0.009	0.175 ± 0.021***

Values reported in seconds. *** $p < 0.001$ for difference with addition of VTT_m.

Table 3. The inclusion of VTT_m to the traditional PTT (PEP+VTT) of the finger

Subject	*Pretilt*		*Tilt*	
	PTT (traditional)	**PTT (with VTT_m)**	**PTT (traditional)**	**PTT (with VTT_m)**
1	0.195	0.313	0.207	0.337
2	0.175	0.292	0.187	0.304
3	0.161	0.237	0.175	0.238
4	0.160	0.268	0.176	0.299
5	0.161	0.242	0.185	0.283
6	0.153	0.237	0.169	0.252
7	0.192	0.317	0.205	0.313
8	0.165	0.278	0.176	0.288
9	0.134	0.284	0.147	0.276
10	0.182	0.284	0.210	0.311
Mean ± SD	0.168 ± 0.019	0.275 ± 0.029***	0.184 ± 0.020	0.290 ± 0.030***

Values reported in seconds. *** $p < 0.001$ for difference with addition of VTT_m.

VTT_m of the ear and the finger did not differ significantly during pretilt, averaging 0.122 and 0.108 seconds respectively, despite the ear VTT_m being greater than the finger in 8 out of 10 subjects. The same was true during tilting, with a VTT_m of 0.119 seconds for the ear and 0.106 seconds for the finger. Furthermore, the changes in VTT_m with tilting at both the ear and at the finger were not significantly different between the two sites. It should be noted that these changes in VTT_m showed no consistent trend of either lengthening or shortening among subjects. These results are summarized in Table 4.

Table 4. Comparison of VTT_m at the ear and finger

Subject	*Pretilt VTT_m*		*Tilt VTT_m*		*Pretilt-Tilt VTT_m Change*	
	Ear	**Finger**	**Ear**	**Finger**	**Ear**	**Finger**
1	0.128	0.118	0.118	0.129	0.011	-0.011
2	0.138	0.117	0.130	0.117	0.008	0.000
3	0.132	0.076	0.124	0.063	0.007	0.014
4	0.144	0.109	0.148	0.123	-0.004	-0.014
5	0.135	0.081	0.133	0.098	0.002	-0.017
6	0.100	0.083	0.087	0.083	0.013	0.000
7	0.132	0.125	0.120	0.108	0.012	0.017
8	0.093	0.114	0.108	0.112	-0.015	0.002
9	0.102	0.150	0.103	0.128	-0.001	0.022
10	0.116	0.103	0.122	0.101	-0.007	0.002
Mean ± SD	0.122 ± 0.018	0.108 ± 0.023	0.119 ± 0.017	0.106 ± 0.021	0.003 ± 0.009	0.002 ± 0.013

Values reported in seconds.

Similar to what was found in the previous analysis of the BW study,[31] Table 5 shows there was no significant change in HR with the moderate hypovolemic challenge induced by tilting. The HR was 72.9 bpm during pretilt and 73.7 bpm during tilting.

Table 5. Changes in HR with tilting

Subject	*HR*	
	Pretilt	**Tilt**
1	65.40	65.84
2	82.70	84.49
3	71.18	66.09
4	67.94	61.18
5	87.07	89.10
6	72.35	75.24
7	59.39	57.03
8	68.64	69.61
9	77.67	80.79
10	76.71	87.48
Mean ± SD	72.91 ± 8.29	73.68 ± 11.39

Values reported in bpm.

BLOOD WITHDRAWAL

PEP significantly increased from baseline (p = 0.001) and PTT did not significantly change with the withdrawal of two units of blood. VTT and VTT_m to the forehead and to the finger also did not change significantly. These results are summarized in Table 6.

Table 6. Effects of BW on the components of PTT

	Macrocirculation					
Subject	*PEP*		*VTT - Forehead*		*VTT - Finger*	
	Baseline	**Blood Out**	**Baseline**	**Blood Out**	**Baseline**	**Blood Out**
1	0.087	0.098	0.027	0.020	0.221	0.213
2	0.086	0.097	0.042	0.035	0.186	0.201
3	0.119	0.128	0.002	0.004	0.199	0.206
4	0.136	0.139	0.047	0.047	0.217	0.237
5	0.105	0.118	0.035	0.032	0.217	0.221
6	0.123	0.141	0.030	0.018	0.198	0.210
7	0.125	0.146	0.023	0.013	0.203	0.198
8	0.104	0.123	0.000[§]	0.000[§]	0.203	0.221
9	0.133	0.134	0.014	0.016	0.183	0.194
Mean ± SD	0.113 ± 0.019	0.125 ± 0.018[***]	0.024 ± 0.016	0.021 ± 0.015	0.090 ± 0.025	0.086 ± 0.022

	Microcirculation			
Subject	*VTT_m Forehead*		*VTT_m Finger*	
	Baseline	**Blood Out**	**Baseline**	**Blood Out**
1	0.193	0.157	0.076	0.052
2	0.141	0.099	0.090	0.081
3	0.155	0.128	0.038	0.044
4	0.088	0.124	0.041	0.087
5	0.152	0.144	0.055	0.103
6	0.120	0.124	0.132	0.104
7	0.132	0.111	0.091	0.062
8	0.144	0.140	0.105	0.070
9	0.141	0.129	0.123	0.153
Mean ± SD	0.141 ± 0.028	0.128 ± 0.017	0.082 ± 0.035	0.084 ± 0.033

	Overall			
Subject	*PTT - Forehead*		*PTT - Finger*	
	Baseline	**Blood Out**	**Baseline**	**Blood Out**
1	0.306	0.275	0.297	0.264
2	0.268	0.231	0.223	0.281
3	0.276	0.260	0.240	0.251
4	0.271	0.310	0.262	0.324
5	0.292	0.294	0.263	0.324
6	0.273	0.284	0.330	0.314
7	0.280	0.270	0.294	0.259
8	0.246	0.260	0.307	0.291
9	0.289	0.279	0.306	0.346
Mean ± SD	0.278 ± 0.017	0.274 ± 0.023	0.280 ± 0.035	0.295 ± 0.034

Values reported in seconds. * $p < 0.05$, *** $p < 0.001$ for change from baseline value. § Values treated as 0 for calculations, because resolution did not enable a distinction between these two readings, causing the PPG to measure the pulse arrival at the forehead before the pulse arrival at the ear.

As expected, during baseline the mean VTT_m was 0.141 and 0.082 seconds at the forehead and finger respectively and after the blood was withdrawn it was 0.128 and 0.084 seconds (Table 6). Though VTT_m was essentially the same during the hypovolemic challenge, the VTT_m was a significant addition ($p < 0.001$) to VTT at both the forehead and finger in both baseline and blood out conditions (Table 7).

Table 7. The inclusion of VTT_m to VTT

Subject	*Forehead*			
	Baseline		*Blood Out*	
	VTT	**VTT + VTT_m**	**VTT**	**VTT + VTT_m**
1	0.027	0.220	0.020	0.177
2	0.042	0.182	0.035	0.134
3	0.002	0.157	0.004	0.132
4	0.047	0.135	0.047	0.171
5	0.035	0.187	0.032	0.177
6	0.030	0.150	0.018	0.142
7	0.023	0.155	0.013	0.124
8	0.000	0.144	0.000	0.140
9	0.014	0.156	0.016	0.145
Mean ± SD	0.024 ± 0.016	0.165 ± 0.026***	0.021 ± 0.015	0.149 ± 0.020***

Subject	*Finger*			
	Baseline		*Blood Out*	
	VTT	**VTT + VTT_m**	**VTT**	**VTT + VTT_m**
1	0.135	0.210	0.114	0.166
2	0.100	0.190	0.104	0.184
3	0.080	0.118	0.079	0.123
4	0.081	0.122	0.099	0.186
5	0.112	0.157	0.103	0.207
6	0.074	0.207	0.069	0.173
7	0.078	0.169	0.052	0.113
8	0.099	0.204	0.099	0.169
9	0.050	0.173	0.060	0.213
Mean ± SD	0.203 ± 0.013	0.285 ± 0.029***	0.211 ± 0.014	0.295 ± 0.034***

Values reported in seconds. *** $p < 0.001$ for difference with addition of VTT_m.

In the baseline and blood out conditions, VTT of the forehead was different than the finger ($p < 0.001$). VTT_m was also different for each region at both conditions, $p = 0.005$ and $p = 0.008$ respectively. VTT to the finger was greater than to the forehead in both conditions, while the VTT_m was shorter. PTT to the forehead was also significantly less than to the finger, but only in the blood out condition ($p = 0.05$). At baseline, they were statistically equivalent. Furthermore, the changes in PTT, VTT and VTT_m during blood out at both the forehead and the finger were not significantly different between the two locations. It should be noted that the changes in VTT and VTT_m showed no consistent

trend of either lengthening or shortening among subjects. Changes in PTT were almost uniformly negative. These findings are listed in Table 8.

Table 8. Comparison of PTT, VTT and VTT_m at the forehead and finger

Subject	*Baseline VTT*		*Blood Out VTT*	
	Forehead	**Finger**	**Forehead**	**Finger**
1	0.027	0.135	0.020	0.114
2	0.042	0.100	0.035	0.104
3	0.002	0.080	0.004	0.079
4	0.047	0.081	0.047	0.099
5	0.035	0.112	0.032	0.103
6	0.030	0.074	0.018	0.069
7	0.023	0.078	0.013	0.052
8	0.000	0.099	0.000	0.099
9	0.014	0.050	0.016	0.060
Mean ± SD	0.024 ± 0.016	0.090 ± 0.025***	0.021 ± 0.015	0.086 ± 0.022***

Subject	*Baseline VTT_m*		*Blood Out VTT_m*	
	Forehead	**Finger**	**Forehead**	**Finger**
1	0.193	0.076	0.157	0.052
2	0.141	0.090	0.099	0.081
3	0.155	0.038	0.128	0.044
4	0.088	0.041	0.124	0.087
5	0.152	0.046	0.144	0.103
6	0.120	0.132	0.124	0.104
7	0.132	0.091	0.111	0.062
8	0.144	0.105	0.140	0.070
9	0.141	0.123	0.129	0.153
Mean ± SD	0.141 ± 0.028	0.082 ± 0.035**	0.128 ± 0.017	0.084 ± 0.033**

Subject	*Baseline PTT*		*Blood Out PTT*	
	Forehead	**Finger**	**Forehead**	**Finger**
1	0.306	0.297	0.275	0.264
2	0.268	0.223	0.231	0.281
3	0.276	0.240	0.260	0.251
4	0.271	0.262	0.310	0.324
5	0.292	0.263	0.294	0.324
6	0.273	0.330	0.284	0.314
7	0.280	0.294	0.270	0.259
8	0.246	0.307	0.260	0.291
9	0.289	0.306	0.279	0.346
Mean ± SD	0.278 ± 0.017	0.280 ± 0.035	0.274 ± 0.023	0.295 ± 0.034*

Subject	*Baseline-Blood Out VTT_m Change*		*Baseline-Blood Out VTT Change*		*Baseline-Blood Out PTT Change*	
	Forehead	**Finger**	**Forehead**	**Finger**	**Forehead**	**Finger**
1	0.036	0.024	0.007	0.020	-0.005	0.008
2	0.041	0.009	0.007	-0.004	-0.004	-0.015
3	0.027	-0.006	-0.002	0.001	-0.011	-0.007
4	-0.036	-0.046	0.000	-0.018	-0.003	-0.020
5	0.007	-0.057	0.003	0.008	-0.010	-0.004
6	-0.004	0.028	0.012	0.006	-0.006	-0.012
7	0.021	0.030	0.010	0.026	-0.011	0.005
8	0.004	0.035	0.000	0.000	-0.017	-0.019
9	0.013	-0.030	-0.002	-0.010	-0.003	-0.011
Mean ± SD	0.012 ± 0.023	-0.001 ± 0.035	0.004 ± 0.005	0.003 ± 0.014	-0.008 ± 0.005	-0.008 ± 0.010

Values reported in seconds. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for difference between forehead and finger values.

Comparing information from the forehead in the BW study to findings from the ear in the HUT trial, Table 9 shows that the time interval between the R-wave on the EKG and the pulse arrival at each site is statistically different. This was true at both baseline and

during the hypovolemic challenge, $p = 0.002$ and $p = 0.004$ respectively. The time to the forehead was found to be longer than to the ear in both conditions.

Table 9. Comparison of PTT at the forehead and PEP at the ear

Subject	*PTT/PEP*			
	Baseline		*Challenge*	
	Forehead	**Ear**	**Forehead**	**Ear**
1	0.114	0.087	0.118	0.098
2	0.127	0.086	0.132	0.097
3	0.121	0.119	0.132	0.128
4	0.182	0.136	0.186	0.139
5	0.140	0.105	0.150	0.118
6	0.153	0.123	0.159	0.141
7	0.148	0.125	0.159	0.146
8	0.102	0.104	0.120	0.123
9	0.147	0.133	0.150	0.134
Mean ± SD	0.137± 0.024	0.113 ± 0.019**	0.145 ± 0.022	0.125 ± 0.018**

Values reported in seconds. ** $p < 0.0$ for difference between forehead and ear values.

A summary of the changes from baseline observed in each of the major components of PTT from both HUT and BW is depicted in Table 10.

Table 10. Summary of PTT components from HUT and BW

Component	*Challenge vs. Baseline*	
	HUT	**BW**
PEP	↑*	↑***
VTT Forehead	-	↓
VTT Finger	↑	↓
VTT_m Forehead	-	↓
VTT_m Finger	↓	↑
VTT_m Ear	↓	-
PTT Forehead	-	↓
PTT Finger	↑*	↑

* $p < 0.05$, *** $p < 0.001$ for difference between post-challenge and pre-challenge values.

DISCUSSION

In search for a noninvasive beat-by-beat BP monitor and an early detector of mild hypovolemia, investigators have examined PTT and its components for a potential solution. Their results have been mixed and less than promising. The current study was

undertaken in hopes of reviving the potential usefulness of measuring the PTT by including the overlooked peripheral microvasculature, with the understanding that the microvasculature is the primary site of vascular autoregulation. Our results confirm some of the previous outcomes, but not all.

Consistent with other experiments,[47, 70] PEP significantly increased during mild hypovolemia in both HUT and BW. In an effort to generate an effective SV, an elongated PEP maximizes ventricular filling while the left ventricular ejection time decreases[16, 48] to circulate the blood to the organs more effectively. PTT also significantly lengthened in HUT, though it was statistically unchanged in BW. Since both VTT and VTT_m remained essentially unchanged in both challenges, PEP must have contributed a majority portion to the change of PTT. This is consistent with other findings that show PEP to contribute as much as 35% of PTT.[34]

The lack of significant change in VTT does not necessarily rule out a vasoconstrictive hemodynamic response. It in fact may reflect a minor degree of constriction that serves only to maintain the baseline VTT, as opposed to hastening it to compensate for the increase in PEP. Also, the decrease in blood volume from the declining SV, which is independent from the increase in PEP, could have offset any observed effect of peripheral vasoconstriction as well.

The responses of each of the components of PTT of the forehead were compared to the finger and the ear using a retrospective analysis of the BW data. Results show that at

both baseline and blood out, the time for the pulse to travel to the forehead is not equivalent to the time it takes to reach the ear. The forehead is therefore not as good a surrogate for measuring the central component of PTT. This is not surprising, because the blood flow to the forehead involves a greater percentage of peripheral arteries than does the path to the ear lobule and therefore includes a partial VTT component in its timing. Furthermore, it has been shown in unpublished research by this team that the forehead vasculature distinctively reacts to preserve blood flow during decreased perfusion due to a mixture of sympathetic and parasympathetic activation. In fact, the dilation of the microvasculature from the vagal stimulation explains why the forehead VTT_m is significantly slower than the finger VTT_m at both baseline and blood out. This is contrasted to VTT_m of the ear and finger, which were not significantly different in both pretilt and tilt conditions. Though there was no significant difference between the two, the VTT_m of the ear was longer than of the finger in all but two subjects, which might be due to a higher basal level of vasoconstriction in those two subjects. The reason why the VTT to the forehead is significantly quicker than to the finger is because of the shorter path length the pulse travels.

The PTT to the forehead and to the finger are statistically equivalent at baseline, but become significantly different when the subject becomes hypovolemic after two units of blood withdrawal. The forehead microvasculature is less vasoconstricted at baseline than the finger, but the decrease in VTT_m with the hypovolemic challenge implies at least some vasoconstriction. Though there was no significant difference in the change in VTT_m between the forehead and finger, there likely is some disparate sympathetic

activity, because of the 0.012-second decrease in forehead VTT_m compared to the 0.001-second increase in finger VTT_m. This slightly asymmetric observation can be explained by either sympathetic activity or parasympathetic withdrawal. More thought should be given to this question, as well as to the significantly different basal tone of the forehead in future studies. Observed differences in the absolute values of the finger VTT_m among the HUT and BW studies (0.108 and 0.082 seconds at baseline and 0.106 and 0.084 seconds post-challenge) might be explained by the different PPG probes used in each experiment.

The gestalt of the results from this study seems to suggest that mild hypovolemia does not initiate a vigorous sympathomimetic response. Chan et al. reported that PTT increased significantly with up to 50˚ of tilt, but then becomes insignificant at higher degrees because of variations in VTT from sympathetic activation.[47] This has been the pervasive understanding – that α-sympathetic-mediated peripheral vasoconstriction shortens VTT, partially compensating for the increase in PEP. However, results from this current study show a significant change in PTT at 60˚ of tilt. It should also be noted that Chen et al., despite their explanation of the loss of a significant PTT change above 50˚, failed to show a significant difference in VTT at all tilt angles save one at 60˚.[47]

In additional support of a sympathetic absence, VTT_m remained essentially unchanged. Even though flow through the microcirculation turned out to be a significant component of PTT, no change in VTT_m indicates a minimal microvascular response to the hypovolemic challenge. Since sympathetic-mediated vasoconstriction affects the

microcirculation more than the major vessels due to its rich sympathetic innervation, and this study failed to show any consistent and significant decrease in either VTT or VTT_m, the lack of vasoconstriction hints that there was no sympathetic activation. Furthermore, it is interesting to note that VTT_m did not change significantly in the ear or in the finger, even though the arterial network of the finger is more densely innervated by α-adrenergic receptors than other parts of the body. A similar finding was discovered between the forehead and the finger in the BW study. If tilting activated the sympathetic nervous system, then we would expect a variation in the vascular response of different anatomical sites.

In opposition to results from previous studies, including Chen et al,[47] the current study also failed to appreciate a significant change in HR with tilting. Even though they similarly did not find a significant alteration of VTT, they did record a significant change in HR at tilt angles greater than 40˚. Chan et al. replicated their findings on HR in another study[16] and Stafford et al. likewise found HR to significantly change at tilting of 25˚ and higher.[70]

The current BW study also yielded some results that are incongruous with Middleton et al.'s study, which used a blood donation model to investigate PTT. Though the amount of blood withdrawn was half of the amount removed in our model, they showed a significant increase in PTT from baseline ($p < 0.01$), as well as a change in HR ($p < 0.01$),[48] while both these variables were found to insignificantly change in the present experiment. However, other blood donation studies confirm our HR findings[20, 21] and

another shows only a change of about 2 beats per minute.[23] The lack of change in VTT, VTT_m and HR in the current experiment suggests that there is no significant sympathetic response to hypovolemia induced by a two-unit blood withdrawal.

Yet another clue to this sympathetic absence is the different results yielded from the simulation of blood loss through HUT and BW than from experiments that administered drugs to mimic physiological responses to hypovolemia. In these studies, significant changes were observed for all variables – PTT, VTT, PEP and HR.[32, 40, 93] Payne et al. specifically showed significant changes in VTT from baseline after separate administration of glyceryl trinitrate, angiotensin II, norepinephrine and salbutamol.[40] The clear vascular response to these agents are not reproduced in our HUT and BW trials, which failed to show any significant decrease in VTT.

A possible explanation of the apparent absence of a sympathomimetic response is that the response to mild volume loss does not engage the sympathetic nervous system. In this early stage, there is no need to redirect blood to the muscles for fight or flight or to the brain for a mental challenge, so instead the response to this level of hypovolemia might be more of a neurohumoral modulation. The HUT study would be the ideal model to evaluate this claim, because there is no inherent stressful or painful stimulus to invoke an inadvertent sympathetic response that would confound the findings. BW and LBNP on the other hand, can create anxiety among subjects.

Some findings in the literature do acknowledge an endocrine response, however not this early. In the prehypotensive phase of hypovolemia, before about 30% of the blood volume is lost, sympathetic-mediated vasoconstriction and tachycardia are in fact the predominate mechanisms for matching the decrease in CO. Beyond this point when hypotension appears, there is a withdrawal of the sympathetic response and a rise in catecholamines, plasma renin and vasopressin in a final effort to countervail the progressing volume loss.[6] This second phase hormonal response has been shown to occur with 60˚ of tilt[103] and with 85˚ of tilt.[104] Concurrent with the precipitous decline in BP and HR, a definitive rise in epinephrine, vasopressin, aldosterone and angiotensin II was observed.[103]

In the current study, all subjects remained in the normotensive phase throughout the experiment. Tilting lasted less than half a minute and data from the first stretch of 11-13 artifact-free beats were analyzed. Data were collected close to the act of tilting because it was expected that this would be the window of greatest change, similar to the lightheadedness and tachycardia immediately experienced upon sudden standing by those with orthostatic intolerance. However, other studies that used a HUT model, including those by Chan et al., implemented a protocol with at least a 1.5-minute adaption period after tilting before gathering any data for analysis. A deeper review of the literature was performed to clarify the importance of measurement timing in HUT.

A study by Toska and Walløe investigated the time course of the hemodynamic response to HUT and found that, even though baseline values varied among subjects,

the time course of the cardiovascular response to tilting was fairly uniform.[105] It required about 30 seconds after tilting and 10 seconds after returning to supine for the measured parameters to respond and stabilize at their new compensatory levels. Though it did take about 30 seconds to reach the final levels, significant changes and trends were seen as early as 5 seconds after tilt. However, both CO and total peripheral capacitance (TPC) rose slightly over the first 5 seconds before falling to stable levels well below baseline. SV remained at pretilt levels for 4-6 seconds before steadily decreasing. HR, which also exhibited an almost immediate rise, was the only variable that did not reach a stable level.

In the current study, HR remained unchanged after tilting and so did VTT, which implies that there was no significant vasoconstriction or change in SVR. PEP did increase as expected, which means SV must have been threatened. However, the timing of the analysis most likely compromised the degree to which it actually changed. There is a rapid decline in blood volume within the first 30 seconds with a majority being lost within the first 10[106] and although our first beat was measured about 2 seconds on average after the tilting was completed, according to the literature, this is before almost all physiologic responses to the hypovolemic challenge would be expected to peak. Though the span of data collection over the 11-13 consecutive beats were equivalent to about 9-12 seconds, depending on the HR, this range overlaps with data values that are more representative of baseline and values that have yet to reach stable levels. Moreover, some of the values, notably CO and TPC, peaked before resolving to levels

below the baseline. This conceivably could have artificially skewed the means, hiding the expression of true significant changes.

The reasoning for our measurement timing was based on our collective experiential knowledge of the immediacy of symptoms upon standing by those with orthostatic hypotension. We aimed to capture the pure autonomic response, which would likely occur acutely after the challenge, before any neurohumoral modulation could occur. Review of the orthostatic hypotension literature reveals that there are actually conflicting reports as to the proper timing of the measurements because of the varying delay of symptoms. Symptoms accompanying an orthostatic drop in BP can appear as soon as 30 seconds,[107] but can sometimes take up to 30 minutes.[108] Most subjects reached the minimum BP within 2 minutes of standing.[109] Because none of these studies attempted to assess the emergence of symptoms earlier than 30 seconds, it seems to reason that our assumption of the immediacy of the response to a postural change is flawed.

However, a study on young normal subjects found that after a lying-to-standing maneuver, the HR immediately and rapidly increased and then about 15 seconds later rebounded to be bradycardic.[110] The same study also compared the responses from free-standing to tilting, each at fast (2-3 seconds) and slow (12 seconds) speeds, to determine if there exists a fundamental difference in the degree and timing of the physiological responses to standing as opposed to tilting. They found that tilting lacked this rebound bradycardia, which they attributed to the muscular activity of standing,

and that tilt speed was an important factor in determining the hemodynamic response. The blunted effect from slower tilting can explain the results from a study by Spranglers et al. that demonstrated a significant difference between a slow 6-second tilt and quick 3-second stand.[111]

Fast tilting was demonstrated to mimic the immediate response to postural change seen with standing,[106] except with regard to BP. After free-standing, BP noticeably dropped before recovering to above baseline, but only slowly and gradually increased after tilting.[105, 111] The rapid hypotension upon standing confirms our basis for the limited elapsed time after tilting before measurements began, however the hemodynamic responses to tilting and free-standing are too dissimilar to justify our underlying assumption.

Though, it could still be argued that because Toska and Walløe's results are from only 30˚ of tilt it took longer for the values to change than would be expected from a steeper 60˚ of tilt, as was used in the current experiment, Toska and Walløe's outcomes were confirmed in study with a protocol of a 70˚ tilt.[106] Furthermore, both of these studies used a fast tilt speed, reaching maximum tilt within 2.2 seconds. The same cannot be said for Sander-Jensen et al.'s experiment on endocrine mechanisms and Chan et el.'s study on PTT. Yet, both of these studies revealed an immediate and significant change in HR, while the current study with a fast tilt speed failed to do so.

A finer reading into the various protocols helps resolve this apparent disparity. All experiments except this current one had much tighter restrictions on the testing conditions and pre-experiment subject behavior. Many ensured a constant ambient temperature and room lighting. Precise time spent in the supine position and the tilt speed were also more rigidly controlled. Most required that subjects abstain from exercise, caffeine, alcohol, food and water for at least 2 hours prior. Some even went as far as requiring an overnight fast. In the current study, none of these factors were standardized. It was our belief that it was primarily the act of tilting that determined the hemodynamic response. However, assessing our data in the context of further literature review clearly indicates that other factors may be predominant. In addition to the importance of tilt speed as mentioned above, it was found that the preceding time spent in the supine position also influences the physiologic response.[112]

Since some of the subjects in these experiments refrained from food and drink, they began the study somewhere between slightly dehydrated and fully hydropenic. This can potentially explain the significant hemodynamic responses reported in the literature, including some extreme responses. In the aforementioned study by Sander-Jensen et al. on the endocrine response to tilting, all subjects exposed to a 60˚ tilt experienced presyncopal symptoms before the allotted 60 minutes was reached.[103] The same vaso-vagal outcome was even observed within 4 minutes after 85˚ of tilt.[104] The baseline hydration status of the subject seems to be a critical factor in eliciting a significant hemodynamic response to a hypovolemic challenge.

These results are interesting given that much of the literature describes HUT as a suitable model for simulating at most a moderate degree of volume loss. Yet these studies prove that HUT could even reproduce a second-stage functional hypovolemia in normal young subjects. With such variation in the responses among subjects in different HUT experiments, and with varying levels of control over the numerous contextual variables built into the methodology, a reevaluation of the application of the HUT model is called for. It seems that the simulation of hypovolemia is not just attributable to the redistribution of the blood volume resulting from the act of tilting itself, but to a variety of other variables that can extend hours before the tilting even occurs.

Conclusion

This study investigated the different components of PTT, including the new addition of the microvasculature transit time (VTT_m). Analyzing data from both HUT and BW models, results confirm previous reports of an increase in PEP in response to a mild-to-moderate hypovolemic insult. Changes in PEP have been the most consistent across all studies and it therefore seems to be the most promising early detection indicator to pursue. It should be noted that an absolute value in PEP has no inherent clinical value; rather it is the relative change in PEP, specific to each individual subject that can potentially be used as a signal of early stage hypovolemia. Neither VTT nor VTT_m exhibited a significant response, and PTT was only found to change significantly during tilting. While a lack of a significant increase in HR during BW is congruent with some of the literature, it is surprising that our tilting experiment failed to reveal this change.

Though the early timing of the measurements after the hypovolemic challenge might veil the presence of a sympathetic hemodynamic response, it is believed that the baseline hydration status of the subject and the time spent in the supine position before tilting are the root causes of this discrepancy with other studies. Further HUT studies with delayed measurement collection can confirm this. Regardless, the finding that VTT_m of the ear and finger did not significantly differ in their response to the volume loss, despite an anatomical difference in the vascular sensitivity to sympathetic stimuli, should be investigated further.

APPENDIX

Methodology of blood withdrawal study [Adapted from manuscript draft[31]]

With IRB approval, eight healthy volunteers (25-32 years of age) were recruited and instructed to abstain from caffeine and other known vasoconstrictive compounds for a minimum of four hours prior to testing. With the subject lying supine and the head propped up on two pillows, a 16-gauge intravenous catheter was inserted into an antecubital vein after local anesthetic to the skin.

After confirmation of a hematocrit of at least 36, the following noninvasive monitors were applied: limb electrodes for electrocardiogram (EKG), intermittent cuff BP, continuous noninvasive finger arterial BP (Finapres, Ohmeda, Boulder, CO) unfiltered photoplethysmograph from the finger, ear, and forehead (Modified Model 520A Oxypleth- Novametrix/Respironics, Wallingford, CT), laser Doppler flowmetry probes on the finger and forehead (Perimed, Sweden), and a Piezo Respiratory Belt Transducer around the chest (Model #MLT1132 ADInstruments, Castle Hill, Australia). The data were recorded at 400 Hz with a microprocessor-based data acquisition system (PowerLab 16 – ADInstruments, Colorado Springs, CO) and analyzed with commercially available software (Chart 5.02 – ADInstruments; SPSS v 14.0.2 – SPSS Inc.). This sampling rate was selected to enable effective identification of each R-wave for assessment of HR and HR variability.

In addition to the aforementioned continuous noninvasive monitoring, the venous peripheral pressure waveform was obtained from a 20-gauge IV catheter in an

antecubital vein. After baseline readings were obtained under resting conditions *("baseline" phase)* and with the subject breathing at a rate of 12 breaths/minute (0.20 Hz) in response to a metronome, two units of blood were withdrawn continuously over the course of approximately 25 minutes (range 12-40 minutes). Resting and metronome measurements were repeated at this time *("blood out" phase)*. Subsequent reinfusion was accomplished in 20 minutes (facilitated by the addition of 200 mL of normal saline); and measurements were obtained in within 5 minutes of reinfusion. Blood was taken for HCT readings at baseline, immediately after withdrawal and immediately after reinfusion. In addition, in six of the subject, transthoracic echocardiographic assessments were performed at selected time points. At the end of each phase, measurements also were obtained while the subject breathed against an expiratory retard.

Safety cutoffs included changes in HR or BP exceeding 15% of baseline and/or the development of any signs or symptoms of hypovolemia.

ABBREVIATIONS

BP	=	Blood pressure
BW	=	Blood withdrawal
CO	=	Cardiac output
EKG	=	Electrocardiogram
HR	=	Heart rate
HUT	=	Head-up tilt
LBNP	=	Lower body negative pressure
LDF	=	Laser Doppler flowmeter/flowmetry
PEP	=	Pre-ejection period
PPG	=	Photoplethysmogram/photoplethysmography
PTT	=	Pulse transit time
SV	=	Stroke volume
SVR	=	Systemic vascular resistance
TPC	=	Total peripheral capacitance
VTT	=	Vascular transit time
VTT_m	=	Microvascular transit time

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